

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

The Effect of the Timing of Leaf Removal on Berry Ripening, Flavour and Aroma Compounds in Pinot Noir Wine.

A thesis
submitted in partial fulfilment
of the requirements for the Degree
of
Doctor of Philosophy
at
Lincoln University

by

Belinda Kemp

Lincoln University

Declaration

Parts of this thesis have been submitted and accepted for publication and/or presented in advance of submission of the thesis.

Publications

- ❖ Kemp. B. Harrison. R. Creasy. G. L. 2010. The effect of timing of mechanical leaf removal on Pinot noir berry and wine composition. Proceedings of 7th International Cool Climate Symposium, Seattle, Washington, USA.
- ❖ Kemp. B. Harrison. R. Creasy. G. L. 2009. The timing of leaf removal and tannin concentrations in Pinot noir. New Zealand Winegrowers Magazine, August/September 2009. 13:1:45-146.
- ❖ Kemp. B. Creasy, G. L. Harrison. R. 2008. Timing of leaf removal affects Pinot noir fruiting zone, leaf layer number and bunch exposure. Proceedings of 14th Annual Technical Conference. Romeo Bragato Conference August 2008. Proceedings of 14th Annual Technical Conference, Christchurch, New Zealand.
- ❖ Belinda Kemp. 2010. Timing of leaf removal on Pinot noir. Invited contribution to “More Sunlight into Wine” by Dr. Richard Smart, Australia. (In publication).

Presentations

- ❖ Measuring tannins. Romeo Bragato Conference August 2008. Proceedings of 14th Annual Technical Conference, Christchurch, New Zealand.
- ❖ The timing of leaf removal affects Pinot noir wines. Romeo Bragato Conference August 2009. Proceedings of 15th Annual Technical Conference, Napier, New Zealand.
- ❖ Are you exposing yours early enough? Family of Twelve, Viticulture/Viniculture Training Day, November 2009. Pegasus Bay Winery, Waipara, New Zealand (unpublished).
- ❖ Belinda Kemp, Roland Harrison, Glen L. Creasy. 2010. “The timing of leaf removal impacts flavour and aroma of Pinot noir wine”. Pinot noir workshop, 14th Australian Wine Industry Technical Conference, July 2010, Adelaide, Australia (unpublished).

Abstract of a thesis submitted in partial fulfilment of the requirements
for the degree of Ph.D.

**The Effect of the Timing of Leaf Removal on Berry Ripening, Flavour and
Aroma Compounds in Pinot Noir Wines**

by

Belinda Sarah Kemp

In the 2007-2008 and 2008-2009 growing seasons mechanical leaf removal was performed on Pinot noir grapevines at different stages of berry development. Treatments consisted of: no leaf removal (NLR) (control), leaf removal seven days after flowering (LR7), leaf removal thirty days after flowering (LR30) and leaf removal at veraison (LRV). Partial cane removal was performed on all treatment vines in 2008-2009 to reduce crop load variability. The result was a decrease in leaf layer number and interior clusters while an increase in exterior clusters and canopy gaps occurred compared to 2007-2008. In 2008, LR7 resulted in vegetative re-growth. In 2008-2009 all treatments had lower yields per vine compared to 2007-2008 and cluster weight decreased in all treatments in 2008-2009. No significant difference was observed in °Brix, titratable acidity (TA g/L) or pH at maturity, but a significant difference in °Brix between years is reported.

Following the investigation into the reliability and variability of two tannin precipitation assays, the methylcellulose (MCP) assay was performed to determine tannin concentration in the microvinification wines produced from each treatment. Berries from each treatment at harvest were analysed for total tannin concentrations and wines were analysed throughout winemaking including to skins and seeds post fermentation. Tannin concentration in berries at harvest showed no statistical difference between treatments in either year but a statistically significant difference was observed during winemaking and in the bottled wine in both years. LR7 had the highest tannin concentration in the bottled wine in 2007-2008 but LR30 had the highest concentration in 2008-2009 whilst NLR had the lowest in both years. Acid catalysis in the presence of phloroglucinol using liquid chromatography mass spectrometry (LC-MS) showed no statistical difference in the mean degree of polymerisation (mDP) between treatment wines but there was a significant statistical difference between years. The 2009

wines had increased tannin concentrations compared to 2008 and a decrease in mDP of all wines in 2009 was found. Reversed Phase-High Performance Liquid chromatography (RP-HPLC) analysis of flavan-3-ols showed the highest concentration in LR7 wines and a shift from 2,3-*cis* to 2,3-*trans* was observed with increased leaf removal.

An aroma method for the identification and quantification of specific “fruity” and “green” volatile aroma compounds was developed using Headspace-Solid Phase Microextraction coupled with Gas-Chromatography Mass-Spectrometry Stable Isotope Dilution Analysis (HS-SPME-GC-MS-SIDA). An increase in both “green” and “fruity” volatile aroma compound concentrations in the 2009 wines occurred compared to 2008. However, “fruity” aromas were above their odour threshold in both years but “green” compounds were below. Sensory analysis consisted of a modified version of free choice profiling (FCP) carried out by a panel of Waipara winemakers. Sensory analysis revealed that NLR wines in 2008 were described as “vegetal” and leaf removal treatments resulted in wines with a higher intensity of “dark fruit aroma and palate” than wines from non-defoliated vines.

Results suggest that the timing of mechanical leaf removal increases tannin concentration but has no influence on the mean degree of polymerisation (mDP). Increased severity of defoliation and/or higher alcohol levels in the 2008-2009 season were responsible for the statistically significant differences in total tannin concentration compared to the previous year.

Keywords: Pinot Noir, leaf removal, tannin, aroma, green tannins.

Dedication

Dedicated to my wonderful son, Toby Kemp and my amazing family, my parents Barrie and Jennifer King plus my sisters, brothers and their partners Johanna, Jocelyne, Bernice, Bethany, Tim, Bruce, Jeffrey and Karin. My nieces: Hannah, Naomi, Bella, Agnes, Edith, Ingrid, Annie and nephews: Alfie and Archie.

Acknowledgements

The author would like to extend her sincere appreciation and gratitude to Supervisors **Dr. Roland Harrison and Dr. Glen Creasy** for their patience, advice, guidance and unwavering support during the course of this research. The author acknowledges Dr. Rob Sherlock for his expertise in aroma compounds, Jason Breitmeyer for technical assistance and Dr. Richard Sedcole for assistance with statistical analysis.

The author would like to acknowledge the funding from Lincoln University and New Zealand Winegrowers and contributions in kind from Pegasus Bay Winery.

The author wishes to thank Lincoln University for her International Doctoral Scholarship without which this research would not have been possible.

The author wishes to thank Ivan Donaldson, Christine Donaldson, Greg Miller, Ryan Collins, Lynnette Hudson and Matt Donaldson for their support and assistance

The author wishes to thank the lecturers at Plumpton College, UK for sparking my interest in viticulture and oenology and Nyetimber Ltd, UK

Grateful thanks is extended to all those who have assisted me in the vineyard, winery or laboratory over the three years; Gilbert Wells, Emma Rice, Wade Sowman, Jocelyne King, Nicky Wakeford, Kirsten Creasy, Elizabeth Tomasino, Olivia Ross, Regon Hung, Sue Blackmore, Richard Hider, Toby Kemp, Khiry Hewitt and Sam Bromfield.

Contribution of Authors

Dr. Roland Harrison and Dr. Glen Creasy supervised the entire research project. Dr. Rob Sherlock and Jason Brietmeyer assisted with the technical and statistical analysis of wine aroma compounds. Dr. Richard Sedcole assisted with sensory data and statistical analysis. Richard Hider assisted with protein and polysaccharide precipitation assays.

Table of contents

Declaration	ii
Abstract	iii
Dedication	v
Acknowledgements	vi
Contribution of Authors	vii
Table of Contents	viii
List of Tables	xiii
List of Figures	xv
Chapter 1 Introduction and literature Review	1
1.1 Introduction	1
1.2 Literature review	3
1.2.1 The effect of leaf removal on grapevine photosynthesis and growth	3
1.2.2 The effects of leaf removal on berry development and composition	4
1.3 Phenolic compounds	6
1.3.1 Nonflavonoids	6
1.3.2 Flavonoids	7
1.3.3 The function of flavonoids in plants	9
1.3.4 Proanthocyanidins	9
1.3.5 Biosynthesis of proanthocyanidins	11
1.3.6 Factors affecting proanthocyanidin biosynthesis	13
1.3.7 Proanthocyanidins in grape berry skins and seeds	13
1.3.8 Proanthocyanidins in red wine and the effects on wine flavour and colour	15
1.3.9 Oenological influences on extraction and accumulation of proanthocyanidins	17

1.4	Aroma compounds	18
1.4.1	Pinot noir aroma compounds	19
1.4.2	The effect of leaf removal on aroma compounds	21
1.4.3	Interactions between aroma compounds and tannins	22
1.5	Sensory evaluation by free choice profiling (FCP)	24
Chapter 2	Objectives and methodology	25
2.1	Research objectives	25
2.2	Vineyard design	26
2.2.1	Vineyard measurements	27
2.3	Winemaking	29
2.4	Tannin analysis	30
2.4.1	Protein and polysaccharide precipitation assays	30
2.4.2	Mean degree of polymerisation (mDP) by acid catalysis in the presence of phloroglucinol	30
2.4.3	Identification and quantification of flavan-3-ols and quercetin	32
2.4.4	Spectral analysis	33
2.5	Aroma analysis	33
2.5.1	HS-SPME-GC-MS-SIDA	34
2.6	Sensory evaluation	35
2.6.1	Participants	35
2.6.2	Sensory procedures	35
2.6.3	Modified free choice profiling	36
2.7	Statistical analysis	37
2.8	Summary	38

Chapter 3 **39**

Effect of timing of mechanical leaf removal on *Vitis vinifera* L. Pinot noir grapevine growth and berry composition

3.1	Abstract	40
3.2	Introduction	41
3.3	Materials and methods	42
3.4	Results	45
3.5	Discussion	47
3.6	Conclusion	51
3.7	Acknowledgements	51
3.8	References	52
3.9	Tables and figures	57

Chapter 4 **64**

Effect of protein-tannin ratio and protein concentration on the bovine serum albumin (BSA)-based precipitation method to determine wine tannin

4.1	Abstract	65
4.2	Introduction	66
4.3	Materials and methods	68
4.4	Results and Discussion	70
4.5	Acknowledgements	73
4.6	References	74
4.7	Tables and figures	77

Chapter 5 **81**

Effect of mechanical leaf removal and timing on the concentrations and composition of phenolics in *Vitis vinifera* L. Pinot noir wines.

5.1	Abstract	82
5.2	Introduction	84

5.3	Materials and methods	85
5.4	Results	90
5.5	Discussion	93
5.6	Conclusion	100
5.7	Acknowledgements	101
5.8	References	103
5.9	Tables and figures	109
Chapter 6		118

Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry stable isotope dilution analysis technique to identify “green” and “fruity” volatile aroma compounds in Pinot noir wine.

6.1	Abstract	119
6.2	Introduction	120
6.3	Materials and methods	122
	Results and discussion	125
6.5	Conclusion	132
6.6	Acknowledgements	133
6.7	References	134
6.8	Tables and figures	137
Chapter 7		146

The effect of the timing of leaf removal on “green” and “fruity” aroma concentrations and sensory attributes of (*Vitis vinifera* L. cv.) Pinot noir wine by a modified-free choice profiling technique.

7.1	Abstract	147
7.2	Introduction	148
7.3	Materials and methods	150
7.4	Results	153

7.5	Discussion	157
7.6	Conclusion	163
7.6	Acknowledgements	164
7.7	References	165
7.8	Tables and figures	172
Chapter 8	General discussion and conclusion	187
8.1	Sensory analysis by modified-free choice profiling	187
8.2	“Green” and “fruity” aroma compounds	188
8.3	Wine phenolic composition	189
8.4	Grapevine growth and berry ripening	191
8.5	Conclusion	192
Chapter 9	References	193

List of tables

Table 2.1	Aroma compounds identified in Pinot noir wines	34
Table 3.1	Canopy characteristics from Point Quadrat data in 2007-2008 and 2008-2009	55
Table 3.2	Average leaf area per meter of row, leaf number and leaf area to fruit weight ratio in 2007-2008 and 2008-2009	56
Table 3.3	Mean seed number, mean seed weight and percent of seeded berries	57
Table 3.4	Clusters per vine, berries per cluster and cluster weight	58
Table 3.5	The effect of leaf removal on berry ripeness parameters in 2008 and 2009	59
Table 4.1	Volumes used to prepare mixtures ranging in wine/protein concentrations at different final BSA concentrations	77
Table 5.1	Wine analyses post bottling in 2008 and 2009	108
Table 5.2	Spectral analysis of Pinot noir wines from 2008 and 2009	109
Table 5.3	Total tannin concentrations during the winemaking process by the methylcellulose precipitation assay in 2008 and 2009 wines	110
Table 5.4	Total tannin concentrations in Pinot noir post fermentation skins and seeds in 2008 and 2009	111
Table 5.5	Concentrations of terminal and extension units in Pinot noir wines in 2008 and 2009	112
Table 5.6	Proanthocyanidin composition and mean degree of polymerisation (mDP) in Pinot noir wines in 2008 and 2009	113
Table 5.7	Flavan-3-ol composition in Pinot noir wines in 2008 and 2009	114
Table 5.8	Quercetin concentrations in Pinot noir wines in 2008 and 2009	115
Table 6.1	Volatile aroma compounds in Pinot noir wines	137
Table 6.2	Internal standard information	138
Table 6.3	Quantification parameters for fourteen analytes	139
Table 6.4	Limits of detection (LODs) and quantification (LOQs)	140
Table 6.5	Mean concentrations of the aroma compounds in four Pinot noir wines	141

Table 7.1	Descriptors generated at the preliminary sensory session for the modified-free choice profiling sensory analysis	172
Table 7.2	Descriptors generated by an expert panel of winemakers for free choice profiling	173
Table 7.3	Scaling factors residual variances for each panellist	174
Table 7.4	Correlations between descriptors by Spearman rank correlation	175
Table 7.5	The concentration ($\mu\text{g/L}$) of selected “green” and “fruity” aroma compounds in the treatment wines	177
Table 7.6	The detection and quantification of “fruity” and “green” aroma compounds in Pinot noir wines by different analytical methods	178
Table 7.7	Relationship between treatment wine descriptors and wine compositional data.	179

List of figures

Figure 1.1	The basic chemical structures of flavonoids	8
Figure 1.2	Proanthocyanidin chemical structures	10
Figure 1.3	The biosynthetic flavonoids pathways in grapevines	12
Figure 1.4	The biosynthesis of C ₁₃ norisoprenoids	22
Figure 2.1	Experimental research vines at Pegasus Bay vineyard, Waipara	26
Figure 2.2	A schematic representation of the experimental vineyard design	26
Figure 2.3	Photographs of shaded and exposed fruit on the control and leaf removed vines	27
Figure 3.1	The percentage of exposed clusters per treatment in 2007-2008	60
Figure 3.2	Fruit weight per vine removed at cluster thinning in 2007-2008	61
Figure 3.3	The effect of the timing of leaf removal on yield per vine 2008 and 2009	62
Figure 3.4	Mean berry weight at harvest in 2008 and 2009	63
Figure 4.1	The effect of wine/protein ratio on tannin concentration using a range of BSA concentrations for a 2004 New Zealand Syrah wine	78
Figure 4.2	The effect of wine/protein ratio on tannin concentration determined using the BSA precipitation assay for a range of New Zealand red wines	79
Figure 4.3	The effect of protein concentration on tannin concentration determined using the BSA precipitation assay for a range of New Zealand red wines	80
Figure 4.4	Plot of tannin precipitated using a range of BSA concentrations for five New Zealand red wines	81
Figure 5.1	Total proanthocyanidins in Pinot noir wines in 2008 and 2009 by acid hydrolysis in the presence of phloroglucinol	116
Figure 5.2	Total flavan-3-ol concentration in Pinot noir wines in 2008 and 2009	117
Figure 6.1	The effect of increasing the adsorption extraction temperature on peak areas with an extraction time of 60 minutes	142
Figure 6.2	The effect of increasing the adsorption extraction time on peak areas with an extraction temperature of 60 °C	143

Figure 6.3	Calibration curve for cis-3-hexen-1-ol	144
Figure 6.4	The degradation of two deuterated internal standards	145
Figure 7.1	Scree plot from PCA of generic descriptors	180
Figure 7.2	Scree plot from PCA of generic and panellist-generated descriptors	181
Figure 7.3	Panellist wine ratings using genric descriptors	182
Figure 7.4	Panellist wine ratings using genric plus expert derived descriptors	183
Figure 7.5	Consensus location of wines using the first two dimensions of PCA of the generic descriptors dataset	184
Figure 7.5a	Consensus locations of generic descriptors using the first two dimensions of PCA	185
Figure 7.6	Consensus location of wines using the first two dimensions of PCA of generic plus panellist-generated descriptors dataset	190

Chapter 1

Introduction and Literature Review

1.1 Introduction

One of the most important and commonly applied summer canopy management operations in viticulture for Pinot noir and other *Vitis vinifera* varieties is the removal of leaves in the fruit zone (Poni et al. 2006). Leaf removal is performed on grapevines to increase air circulation, light exposure, penetration of fungicide sprays and decrease disease incidence, especially rot. Smart and Robinson (1991) and Chellemi et al. (1992) state that leaf removal is performed at veraison in Europe with the objective being to decrease the incidence of *Botrytis cinerea* and *Unicinula necator* (Zoecklein et al. 1992). However, New World wine regions often perform it at fruitset to increase wine colour, flavour and aroma (Percival et al. 1994). Currently leaf removal can be carried out either manually or mechanically and personal observation has shown that a variety of defoliation techniques have been adopted by vineyards worldwide. These range from one or two-sided leaf removal, varying the timing and severity or combining defoliation with shoot thinning. In cool climate winegrowing regions ripening can be hastened, colour enhanced and aroma compounds increased. Pinot noir grapes are thin-skinned and therefore, susceptible to sunburn, disease, and temperature increase. Understanding the impact of the timing of leaf removal on New Zealand's premium red wine variety, Pinot noir, is crucial for vineyard managers and winemakers.

An important quality parameter for Pinot noir wines is mouthfeel. It is recognised that tannins contribute significantly to perceptions of astringency, weight and body (Gawel 1998). Tannins are located in the seeds, skins and stems of grapes, although hydrolysable tannins are imparted from oak barrels to wine. The primary aim of this study was to establish the effect of viticultural practice, specifically leaf removal and its timing on grape tannins. Leaf removal timings were chosen to coincide with the commencement of tannin synthesis between flowering and veraison and change during fruit ripening. Research was conducted at Pegasus Bay Vineyard, Waipara over two years. Mechanical leaf removal was used because an important consideration was to ensure customary vineyard practices were carried out with the only exception being changes to leaf removal timing. Grape berry ripening was monitored six weeks prior to harvest in both years. Grapes at harvest were divided by treatment and

small scale winemaking was conducted, in triplicate, to emulate commercial scale winemaking.

Tannin concentration in the wine was monitored during the winemaking process as well as the final wine colour and tannin concentration in all treatment wines. Tannin concentration and composition by reverse phase-high performance liquid chromatography (RP-HPLC) and liquid chromatography-mass spectrometry) LC-MS was undertaken.

In addition to monitoring berry ripeness parameters (i.e. °Brix, pH and titratable acidity) to determine harvest dates, viticulturists and winemakers also taste berries to monitor tannin ripeness. Unripe berries have seeds that are often found by winemakers to be harsh, bitter and have a “green” flavour on the palate and are referred to as “unripe, green tannins”. Anecdotal evidence from viticulturists and winemakers suggests that a decrease in the perception of “green” characteristics in red wine such as “green” and “unripe” flavours occurs when there is an increase of “fruity” notes. Additionally, Herderich et al (2004) suggest that one explanation for “green tannins” could be that leaf aldehydes i.e. hexanal, contribute to the “green” perception described by wine writers and winemakers. Therefore, further investigations were conducted into the concentrations of “green” and “fruity” aroma compounds in the wines to ascertain whether concentration differences existed amongst treatments. All wines were chemically analysed following the development of a new headspace–solid phase microextraction-gas chromatography-mass spectrometry-stable isotope dilution analysis (HS-SPME-GC-MS-SIDA) method to determine a range of “green” and “fruity” volatile compounds previously identified in Pinot noir.

Furthermore it was essential to explore whether wine chemical analysis correlated with wine sensory analysis. The ability to allow sensory panellists, specifically Waipara winemakers familiar with local Pinot noir wines during winemaking, the opportunity to use their own vocabulary to describe the wines was explored. Wines lacked barrel aging to ensure that viticulture treatment effects from leaf removal could be analysed without interference from hydrolysable tannins. Consequently, it was also important to use sensory panellists who recognised, and were familiar with, unfinished Pinot noir wines. A modified version of “free choice profiling” (FCP) was used for the first time in wine sensory analysis (Perrin et al.

2007). This technique enabled winemakers to rate wines using descriptors provided to them, alongside their own descriptors.

In summary, the purpose of this research was to determine how the timing of leaf removal during the growing season affected vegetative growth, yield and fruit composition in addition to the flavour and aroma both chemically and sensorially of the resultant wine.

1.2 Literature review

1.2.1 The effect of leaf removal on grapevine photosynthesis and growth

Grapevine leaves are net importers of carbohydrates until they reach 50% to 80% of their final size (Koblet 1969, Yang and Hori 1980, as cited in Vasconcelos and Castagnoli 2000). The photosynthetic rate increases until approximately 40 days after unfolding when they reach full size, and decreases steadily after that. However, a study by Petrie et al. (2000) reports that the decline in leaf photosynthesis previously associated with advanced leaf age (Poni et al. 1994, 2006) is caused by an increase in leaf area to fruit weight (source to sink) ratio, as new leaves grow. The impact of defoliation on grapevine photosynthesis performance was demonstrated by Petrie et al. (2003). The study on Sauvignon blanc vines found leaf removal from the lower quarter of the canopy during the lag phase of berry growth caused a significant decrease of whole-vine photosynthesis. Grapevine leaf areas were reduced by 14% and the authors suggest that in their research the lower portion of the canopy contributed more than the upper portion of the canopy to the whole-vine carbon budget (Petrie et al. 2003). Poni et al. (2006) explain that the more mature basal leaves are the largest on the shoot and when removed by defoliation whole vine photosynthetic rates are lowered. Nevertheless, Hunter and Visser (1990) reported that leaf removal can result in more active photosynthesis in the remaining leaves. The removal of leaves receiving low photosynthetic photon flux rate (PPFR) increased sugar levels and reduced titratable acidity, malate, pH and K^+ levels in must (Hunter and Visser 1990).

Regarding vegetative growth, Intrieri et al. (2008) compared post flowering mechanical and manual leaf removal of Sangiovese vines. It was observed that manually defoliated vines

compensated for early leaf removal by vegetative growth. Kliewer and Fuller (1973), Hunter (2000), Candolfi-Vasconcelos and Koblet (1990), and Hunter and Visser (1990) found that leaf removal stimulated lateral shoot growth. Additionally, early defoliation of grapevines post bloom resulted in increased lateral shoot length and growth (Jackson and Lombard 1993). These studies suggest that the timing of leaf removal may affect Pinot noir grapevine growth, the average leaf age within the canopy, and whole vine photosynthesis. Excessive regrowth may result in vines that require further leaf removal during the growing season and leaf age may affect photosynthesis thereby delaying ripening in early leaf removal treatments.

1.2.2 The effects of leaf removal on berry development and composition

In cool climates leaf removal can lead to increased levels of anthocyanins and the decomposition of malic acid which improves the sugar: acid ratio of the resulting must (Petrie et al. 2003, Dokoozlian and Kliewer 1996, Phelps et al. 1999, Koblet et al. 1994 and Poni et al. 2006). Spayd et al. (2002) separated light and temperature by heating and cooling clusters and found that temperature had little or no effect on flavonol concentrations. However, the same study reported that light increased monomeric anthocyanins and flavonols in Merlot berry skins. A study by Downey et al. (2004) established that tannin accumulation in Shiraz grapes, analysed by HPLC, were largely unaffected by artificial bunch shading. Downey et al. (2004) applied light exclusion boxes to Shiraz clusters at different times in the growing season, although unfortunately treatments did not occur in the same year. Artificial shading of grape clusters does not mimic clusters shaded by leaves as non-defoliated vines still allow light to pass through leaves to clusters. Smart (1985) reported that 8-10% of photosynthetically active radiation (PAR) passes through a single leaf layer. Additionally, the ratio of red to far-red light decreases in the canopy interior and it is this ratio that regulates the photo-equilibrium of the photoreceptors (phytochromes), pigments responsible for light detection in plants (Dokoozlian and Kliewer 1995). Phytochromes have been implicated in grape berry growth regulation and composition (Dokoozlian and Kliewer 1995). Therefore, trials using artificial shade and natural shade are difficult to compare due to the differences in the moderation of sunlight. Complete light exclusion late in the growing season (Downey et al. 2004) had no effect on tannin accumulation. If light exclusion had occurred at the stage of

tannin synthesis then results may have been different as clusters exposed to light have shown increased tannin concentrations (Ristic et al. 2004).

Research by Joscelyne et al. (2007) in the warm climate of Sunraysia, Australia with Cabernet Sauvignon involved leaf removal four weeks post fruitset. The study dealt with severity of leaf removal but not the timing. No difference in tannin using a protein precipitation assay or total phenolics was observed between treatments. The wines were analysed two years after production so no immediate treatment effect could be observed. Additionally, no attempt was made to investigate tannin composition as opposed to concentration. Joscelyne et al. (2007) suggest that increased exposure in a warm climate may have resulted in an excessive heat-load within the fruit which could have inhibited some metabolic processes or initiated degradation of metabolites. Ristic et al. (2007) established that wines made from artificially shaded Shiraz berries had lower tannin concentration, particularly larger molecular weight skin tannins, and lower total phenolics than wine made from exposed berries. Downey et al. (2004) did not produce wines from the artificially shaded clusters and only analysed the Shiraz skins and seeds for tannin concentration and composition. Cortell et al. (2006a) in Oregon, used HPLC analysis and light exclusion boxes on Pinot noir grapes to ascertain the effect on flavanols, but did not apply leaf removal to the vines. In accordance with Ristic et al. (2007) results indicated lower concentrations of skin proanthocyanidins in artificially shaded clusters. A recent study regarding naturally shaded clusters reported that low light intensity reduced accumulation of Shiraz skin tannins by more than 30% compared to clusters exposed to higher light levels (Ristic et al. 2010). Pereira et al. (2006) established that greater leaf removal in the cluster zone resulted in berries with higher concentrations of flavonols in agreement with Price et al. (1995).

Previous studies regarding leaf removal and yield have provided varying results. Bledsoe et al. (1988) found that yield and yield components were not significantly affected by the timing or severity of leaf removal in Sauvignon Blanc grapes. However, Hunter and Visser (1990) found that 33% defoliation prior to berries reaching pea size reduced berry size and yield, but had no effect when applied at veraison.

Leaf removal and its effects on berry ripeness parameters have also been studied and Petrie et al. (2003), Ollat et al. (1998), Morrison et al. (1990) and Bergqvist et al. (2001) found that titratable acidity, sugar per berry and cluster weight were significantly reduced by leaf removal, while must pH increased in accordance with previous studies (Bledsoe et al. 1988). Keller (2009) explains that the early season carbon limitation imposed by defoliation may restrict berry number and size but does not usually impair ripening. Caspari et al. (1998) and Poni et al. (2006) state that leaf removal at flowering should be avoided as it reduces fruitset and berry size but increases colour, aroma and astringency.

1.3 Phenolic compounds

The term “phenolic” encompasses approximately 8000 naturally occurring compounds, all of which possess one common structure, a hydroxy-substituted benzene ring (Manach 2004, Svobodova et al. 2003, Kennedy 2006). Phenolics are secondary plant metabolites common in fruit, tea, vegetables and coffee (Svobodova et al. 2003). They have relatively high acidity due to the aromatic ring tightly coupling with the oxygen and a relatively weak bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is intermediate between that of aliphatic alcohols and carboxylic acids (Soleas et al. 1997). The unconjugated hydroxy-phenolic groups on tannins give them their distinctive protein-binding property (Soleas et al. 1997). Phenolic compounds are classified based on the nature of the flavonoid monomers, their bonding, esterification to other compounds, or functional properties (Soleas et al. 1997). The different groups of phenolic compounds found in wine include phenolic acids, cinnamic acid esters, flavonols, flavan-3-ols and anthocyanins and their contribution to wine includes colour, flavour, astringency and bitterness (Sun et al. 2007).

1.3.1 Nonflavonoids

Nonflavonoids are predominantly represented by the phenolic acids and their esters and are found at low concentrations in grape pulp and wine, with the exception being hydroxycinnamic acids (Kennedy et al. 2006). Flamini (2003) explains the important hydroxycinnamates in grapes are the tartaric esters of caffeic, *p*-coumaric and ferulic acids, namely caftaric, coutaric and fertaric acid. The acids are differentiated by the substitution of their benzene ring and possess C₆-C₃ carbon skeletons. Post harvest hydrolysis, especially by

pectin esterase, frees at least part of the hydroxycinnamates of grapes from their tartrate moiety. Concentrations as high as 200 mg/L have been found in grapes, with the compounds being released during pressing (Noble 1999). In red wines the concentration of cinnamic acid derivatives is lower than that of flavonoids, yet Gawel (1998) suggests that they may contribute to bitter nuances in red wine. The hydroxybenzoic acids are primarily degradation products with the most important being gallic acid. Hydroxycinnamic acids are the dominant UV-B protective compounds in *V. vinifera* leaves but not berries. This function is replaced by epidermal flavonoids during leaf development (Kolb et al. 2003). This is because grape berries exhibit a multi layer skin with phenolics spread across the entire skin region, but grapevine leaves possess a single layered outer skin with phenolics confined to the chlorophyll free epidermis (Kolb et al. 2003). However, gallic acid is important to grape seed tannins as they contain a higher percentage of subunits comprising of gallic acid esters (degree of galloylation) than grape skins (Herderich and Smith 2005).

1.3.2 Flavonoids

Flavonoids consist of a C₁₅ (C₆-C₃-C₆) three-ring system with a central oxygen-containing pyran ring with different oxidation states (Waterhouse 2002). This benzopyrano moiety is also referred to as a chroman ring and typically bears an aromatic ring at C-2, C-3 or C-4. The fused ring is referred to as the “A” ring while the phenyl constituent is known as the “B” ring and the benzopyrano heterocycle as the “C” ring (Figure 1). Flavonoids divide further into eight groups based on saturation and oxidation status of the C-ring: flavan, flavanone, flavone, flavonol, dihydroflavonol, flavan-3-ol, flavan-4-ol, and flavan-3,4-diol and a sub family called the flavenes (Aron et al. 2007, Downey et al. 2006). The flavan-3-ols polymerise to form the flavonoids subclass proanthocyanidins (Manach et al. 2004, Aron et al. 2007). Botha et al. (1981) and Delcour et al. (1983) state that the electrophilic C4 position of the extension unit (flavan-3,4-diol) condenses with the nucleophilic C8 or C6 position of the terminal unit (flavan-3-ol) to form proanthocyanidins. However, these models do not take into account that the enzymatic formation of flavan-3,4-diol is specific to 2,3-*trans* and many extension units in plants are 2,3-*cis* (Petit et al. 2007).

Figure removed due to copyright regulations.

Figure 1. Basic structures of flavonoids consisting of two benzene rings connected by an oxygen containing pyrene ring.

1.3.3 The function of flavonoids in plants

Physiological roles attributed to proanthocyanidins in plants include facilitating male fertility in pollen, controlling seed permeability and dormancy, antifungal activity and the establishment of symbioses with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria in the rhizosphere (Treutter 2006, Downey et al. 2006, Manach et al. 2004). Flavan-3-ol monomers and proanthocyanidins also act as deterrents to herbivores and increase disease resistance (Dixon et al. 2004, Downey et al. 2006, Roberts et al. 2006, Paolucci et al. 2005).

1.3.4 Proanthocyanidins

Proanthocyanidins (Figure 2) are often referred to as condensed tannins and are formed by polymerisation of flavan-3-ol monomers. Subunit composition varies amongst grape skin, stalk and seed tannins. Epicatechin occurs mainly in the extension units (i.e. C-4 position involved in inter-flavan bonding) while catechin is encountered in the terminal units (Fulcrand et al. 2005). Proanthocyanidins mainly consist of subunits of (-) epicatechin, but also significant amounts of epigallocatechin, (+) catechin and epicatechin 3-O-gallate (Fournand et al. 2006, Cheynier 2006, Manach et al. 2004). Using high performance liquid chromatography-diode array detection/mass spectrometry (HPLC-DAD/MS) on whole skins and seeds, Mattivi et al. (2009) found that Pinot noir berries had the greatest proportion of catechin terminal units compared to Syrah, Carmenere, Cabernet Sauvignon, Teroldego, Marzemino, and Merlot.

Figure removed due to copyright regulations

Figure 2. Proanthocyanidin chemical structure.

Amongst grape-based proanthocyanidins two subclasses exist. Firstly, procyanidins found in skins and seeds, consisting of (epi) catechin units which possess dihydroxylated extension units and release cyanidin upon oxidative heating in concentrated acidic media. Secondly, prodelphinidins found only in the skins deriving from (epi) gallo catechin which possess trihydroxylated extension units that release delphinidin (He et al. 2008). However, proanthocyanidins exist that have both di- and trihydroxylated units (Cheynier 2005, Herderich et al. 2005, Broussaud et al. 2001, Pascual-Teresa et al. 2000, Dixon et al. 2005).

Different -H and -OH group substitutions on the B and C rings lead to different stereoisomers i.e. (+)-gallo catechin, (-)-epigallo catechin, (+)-catechin and (-)-epicatechin, with the latter two occurring in concentrations of up to 200 mg/L in red wine (Du Toit et al. 2006). These molecules can associate through C4-C6 and C4-C8 bonds to form dimers, trimers and oligomers (Du Toit et al. 2006, Lazarus et al. 1999, Fournand et al. 2006, Broussaud et al. 2001). Dimeric proanthocyanidins can be divided into types A and B. Type A has interflavan C4-C6 and C4-C8 bonds, and a bond between the C-5 or C-7 carbon units of the terminal unit

and the C-2 carbon of the upper unit but are not frequently encountered in nature (Peleg et al. 1999). Although Passos et al. (2007) using ESI-MS, ESI-MS/MS and LC-MS discovered type-A galloylated procyanidins in Chardonnay, Touriga Nacional, Tinta Roriz and Touriga Francesa seeds. Koerner et al. (2009) isolated A-type proanthocyanidins using phloroglucinol from cranberry juice and peanuts by RP-HPLC. Type B dimeric procyanidins are characterised by C4-6 and/or C4-C8 interflavan bonds (Du Toit et al. 2006). Trimeric procyanidins are divided into Types C and D. Type C has two type B interflavan bonds, and type D has a type A and a type B bond (Du Toit et al. 2006). Trimers or C-type proanthocyanidins such as C1 consist of three flavanol units linked by C4-C8 interflavan bonds (Peleg et al. 1999, Lazarus et al. 1999).

1.3.5 Biosynthesis of proanthocyanidins

The C₆-C₃-C₆ flavonoid structure is the culmination of several pathways. The bridge (C-ring) and the aromatic B-ring constitutes a phenylpropanoid unit synthesised from *p*-coumaroyl-CoA (Crozier et al. 2006) via the shikimate and phenylpropanoid pathways (Figure 3). The six carbons of the A-ring originate from condensation of three acetate units via the malonic acid pathway that is also referred to as the acetate/malonate pathway or the polyketide pathway (Dey and Harbourne 1997, Crozier et al. 2006, Bogs et al. 2005 and 2006, Deluc et al. 2006, Herrmann et al. 1995). Significant progress has been made concerning monomer production and the role of leucocyanidin reductase (LAR) in (-)-epicatechin and (+)-catechin synthesis (Gagne et al. 2009). Proanthocyanidin biosynthesis shares common steps with anthocyanin biosynthesis but branches off from this after the reduction of leucoanthocyanidin (or cyanidin) to catechin (or epicatechin by LAR or anthocyanidin reductase (ANR) (Braidot et al. 2008). Research by Lacampagne et al. (2009) indicated that abscisic acid, the plant growth regulator, is a grape berry tannin biosynthesis regulator. Expression of genes *VvLARI*, *VvLAR2* and *VvANR* in red grape skins and seeds show a specific temporal regulation of proanthocyanidin synthesis, starting early in berry development until veraison (Braidot et al. 2008). Higher values of gene expression were found in grape seeds than in grape skins and Bogs et al. (2005) confirm the different patterns of gene expression in seeds and skins affect the concentration and composition of proanthocyanidins (Braidot et al. 2008, Petitt et al. 2007, Downey et al. 2003a). The proanthocyanidin polymerisation pathway has not yet been

elucidated, although quinone methides or carbocations derived from leucoanthocyanidins have been generally accepted as playing a role in polymerisation (Gagne et al. 2009). Some researchers propose that a series of enzyme-driven reactions which have been carried out *in vitro* are responsible (Paolucci et al. 2005, Fujita et al. 2005, 2006).

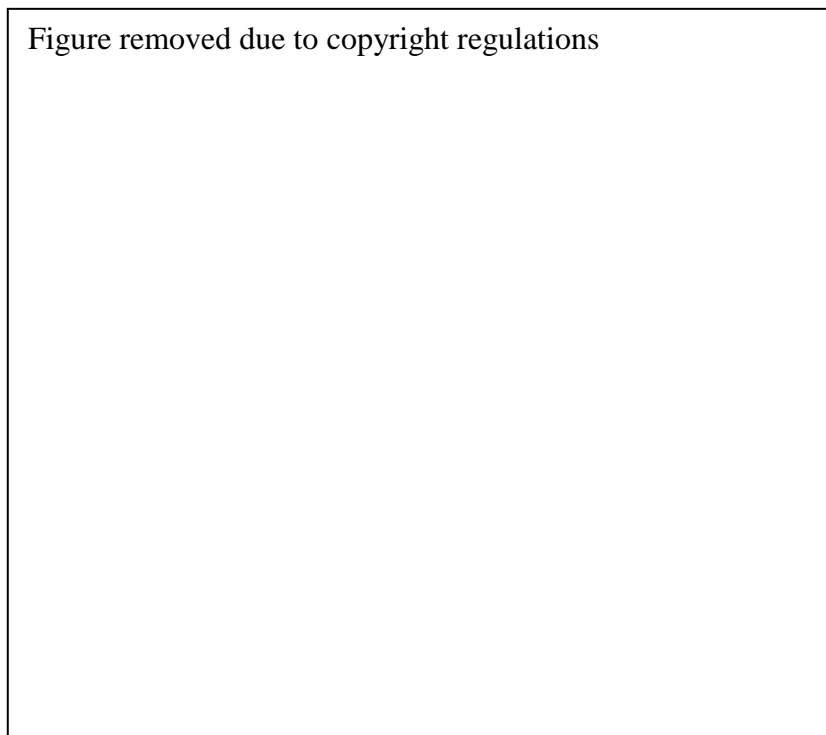


Figure 3. The biosynthetic pathways of flavanoids in grapevines.

1.3.6 Factors affecting proanthocyanidin biosynthesis

The emphasis in studies investigating factors affecting proanthocyanidin biosynthesis has been on vine water status and sun exposure (Price 1995, Ristic et al. 2005). A study of Cabernet Franc in ten vineyards in the Loire planted in different soils showed no difference in skin and seed tannin levels between the 1995 and 1996 vintages, but significant differences in tannin composition due to soil type were reported (Broussaud et al. 1999). Viticultural treatment effects are less clear, but Cortell et al. (2005) reported that the concentrations of skin proanthocyanidins were higher in low vigour vines. In another study, decreased vine vigour resulted in an increase in epigallocatechin extension subunits and an increase in the average size of polymers (Cortell et al. 2006b, Downey et al. 2006). Cohen et al. (2008) state that there is an increase in accumulation of extension units as opposed to subunits at veraison due to increased heat within the cluster microclimate. Those results indicate that heat in the cluster zone at veraison increases proanthocyanidin polymer length. Cohen et al. (2008) suggests that cooler fruit results in more rapid berry ripening and a shift in metabolism away from proanthocyanidin synthesis to accumulation of anthocyanins. These results suggest that high vine water status increases vigour but high vigour vines have low fruit exposure due to increased shade. Therefore, increasing cluster exposure will improve light penetration and increase temperature thereby increasing tannin concentration in grapes. Studies regarding trellis system, clones, rootstock, plant hormones, growth regulators, and pruning style have yet to be published.

1.3.7 Proanthocyanidins in grape berry skins and seeds

Soluble polymeric flavan-3-ols are located in the hypodermal layers of the berry skin and the soft parenchyma of the seed between the cuticle and the hard seed coat (Adams 2006). The average size of skin tannins (expressed as mean degree of polymerisation: mDP) is much larger than seed tannins and skin tannins contain epigallocatechin subunits which seed tannins generally lack (Adams 2006, Cheynier 2005). Grape seed tannins consist of partly galloylated procyanidins whereas skins also contain prodelpinidins (Cadot et al. 2006). Price et al. (1995) did not detect catechin in chromatograms of Pinot noir grape skin extracts yet Goldberg et al. (1998) found high levels of catechin in Pinot noir wines from all regions. Additionally, Thorngate et al. (1994) reported that Pinot noir seeds contain six times more

monomeric flavan-3-ols than Cabernet Sauvignon seeds. It is evident from studies by Aron et al. (2007), Des Gachons et al. (2003), Pastor de Rio et al. (2006) and Harbertson et al. (2002) that Pinot noir skins possess one flavan-3-ol, (+) catechin. (-) Epigallocatechin and (-) epicatechin exist as extension subunits which accounted for the majority of the skin proanthocyanidins in the study regarding Pinot noir by Pastor del Rio et al. (2006). Pinot noir skin proanthocyanidin concentration declined during veraison due to stable associations with polysaccharides or proteins resulting in less extractable compounds (Pastor del Rio et al. 2006). According to Bindon et al. (2010a & b) reduced extraction of tannin from berries is a result of tannin and cell wall material interactions and polymerisation as longer chain tannins are more difficult to extract. These are driven mainly by hydrogen bonding and hydrophobic reactions, presumably via the A and B ring or on the gallic acid moieties of epicatechin esters (Bindon et al. 2010a & b, Hanlin et al. 2009). The strength of cell wall binding is influenced by the structure and composition of both interacting partners (Bindon et al. 2010a & b). Indeed, Huang et al. (2005) established that phenolic cross-linkage formation became active in the walls of the epidermis and subepidermis cells of Golden Muscat berries post veraison. It is this ability to bind with proteins and polysaccharides that formed the basis for two tannin assays to examine tannin in grapes seeds, skins and wine (Harbertson et al. 2002, Sarneckis et al. 2006).

Fournand et al. (2006) state that whether the total amount or/and composition of tannins change in skins during ripening remains unclear. Two separate studies on Shiraz grape skins during development resulted in contrasting results: Kennedy et al. (2001) found an increase in mDP but Downey et al. (2003b) reported a decrease of mDP. Both studies used phloroglucinolysis analysis but sample preparation and storage differed, which may have affected proanthocyanidin extraction and/or results of composition and concentration determination.

While the total number of flavanol units in extractable grape seed tannins ranges from 2 to 17, much higher degrees of polymerisation may exist, but large polymers tend to be insoluble and therefore difficult to study (Schofield et al. 2001). In contrast to this view, Cheynier (2005) and Vidal et al. (2003) explain that studies have shown that higher molecular weight proanthocyanidins are both soluble and more astringent on a weight basis than the oligomeric

proanthocyanidins. Ristic and Iland (2005) established that seed tannin concentration declines during ripening and accompanied colour change; Kennedy (2000) suggests that this is due to oxidation, but the resultant chemical structures have yet to be determined (Adams 2006). Downey et al. (2003b) suggest that the decline in seed tannin during ripening may reflect their covalent attachment to the insoluble matrix of the seed resulting in tannins which are more difficult to extract (Adams 2006).

According to Downey et al. (2006) research has not revealed a correlation between skin and seed tannins in berries and the tannin composition in the corresponding wine. Ristic et al. (2004) reported that tannin concentration in finished wine was related to exposure of Shiraz grapes, but only a small amount of the total grape tannin could be detected in the finished wine. Bindon et al. (2010a & b) suggests that this difference could be the result of either tannin extraction differences or modifications to tannin structure and composition during winemaking. Tannins are also involved in reactions with anthocyanins but can be absorbed in yeast lees which would account for a percentage of the "missing" tannin from final wines.

1.3.8 Proanthocyanidins in red wine and the effects on wine flavour and colour

Grape proanthocyanidins are responsible for wine astringency, which is a result of interactions of proanthocyanidins with salivary proteins (Fulcrand et al. 2005, Guadalupe et al. 2006). Red wine may contain up to 800 mg/L of flavan-3-ol monomers (Noble 1999). Bitterness decreases and astringency increases from monomeric flavanols to trimeric flavanols (Noble 1999, Kielhorn et al. 1999). On a weight basis, astringency of proanthocyanidins with mDP of 3 to 20 also increases with the molecular weight and the percentage of galloylation, as their ability to complex with peptides and proteins increases (Cheynier et al. 2005). Studies on taste properties of tannins are scarce, but epicatechin has a higher maximum intensity and longer persistence of bitterness than catechin in red wine (Perret et al. 2003, Kielhorn et al. 1999, Waterhouse 2002). Goldberg et al. (1998) confirmed that from 800 red wines, Pinot noir from all wine regions contained higher catechin levels and higher catechin:epicatechin ratio than Cabernet Sauvignon, Pinotage, Malbec, Merlot, Bordeaux blends and Shiraz. Pinot noir wines from Canada had the highest levels of catechin

followed by Burgundy then South Africa. Pinot noir from Australia had the lowest levels while New Zealand wines were not included in the study (Goldberg et al. 1998). Unfortunately, no mention of the effect on red wine flavour was explored. Oligomers arising from catechin oxidation are likely to contribute astringency, like their procyanidin isomers, and interact similarly with proteins (McRae et al. 2010). Vidal et al. (2004) established that ethyl-linked catechin oligomers were as astringent and bitter as procyanidins. Broussard et al. (2001) state that astringency of wine extracts corresponded with the concentration of proanthocyanidin units, but the analytical method could not distinguish between subunits and polymers.

Higher molecular weight polymers are also formed in red wine by oxidation, polymerisation, cleavage reactions and interactions between anthocyanins and tannins (Bindon et al. 2010a). Salas et al. (2003) explain that there are two proposed mechanisms for the production of anthocyanin – tannin (A - T) and tannin - anthocyanin (T-A) adducts. In the formation of A-T adducts the anthocyanin is in the flavylum form and acts as an electrophile. The hydroxyl groups at C5 and C7 have a mesomer effect and confer on the flavanol a nucleophilic character at C6 and C8. The addition of the flavanol onto the flavylum cation leads to the colourless flavene (A-T), which is either oxidized to the red flavylum and onto a xanthylum salt or proceeds to a colourless cyclic condensation product with an A type bond. In the formation of T-A adducts, proanthocyanidins undergo acid-catalysed cleavage of their inter-flavanic bond, releasing the intermediate carbocation T⁺, which acts as an electrophile, while the anthocyanin in its hydrated hemiketal form acts as a nucleophile. This reaction yields a colourless dimer which dehydrates to the red flavylum form. Both these reactions have been observed in model solutions, and red wine, and have an important role to play in red wine aging (Salas et al. 2003). During storage and aging, wine colour changes from a bright red to a reddish-brown hue and this is attributed to the formation of these A-T and T-A adducts which are more stable than smaller anthocyanins (Salas et al. 2003, Remy et al. 2000)

1.3.9 Oenological influences on extraction and accumulation of proanthocyanidins

Although tannins are extracted from skins and seeds the degree of extraction is hindered by their low solubility in water, but this increases with higher alcohol content, pH, total acidity, exogenous enzymes, temperature, extended post fermentation skin contact and lees aging (Busse-Valverde et al. 2010). Diffusion of skin proanthocyanidins into red fermenting wine occurs more rapidly than the extraction of seed proanthocyanidins (Canals et al. 2005) although this can be hastened with higher alcohol levels (Busse-Valverde et al. 2010). However, Busse-Valverde et al. (2010) reported increased seed tannins in Monastrell and Cabernet Sauvignon wines that underwent prefermentation cold soak at 10 °C for ten days. The use of polysaccharide degradative enzymes breaks down cell walls which improves the release of proanthocyanidin from berry skin cell walls (Bindon et al. 2010a & b, Busse-Valverde et al. (2010).

Further proanthocyanidin changes occur due to the presence of stalks, grape crushing and pumping over as these winemaking techniques cause the precipitation of the most condensed molecules (Ribereau-Gayon et al. 1986). The use of a rotary fermenter decreased proanthocyanidin extraction in the study by Zimman et al. (2002). Extended maceration and increased temperature at the end of fermentation increased total proanthocyanidins, both the lower and higher molecular weight tannins (Zimman et al. 2002). Similarly, Busse-Valverde et al. (2010) reported that proanthocyanidin concentration in Monastrell and Cabernet Sauvignon wines increased with cold soak but had no effect on Syrah wine. However, Cabernet Sauvignon which underwent ten days of cold soak prior to fermentation yielded the same concentration of proanthocyanidins as the wines without cold soak (Koyama et al. 2007). Barrel aging affects total tannins in red wine as hydrolysable tannins are extracted from oak and concentrations reported in wine range from 7-20 mg/L (Jackson 2008). Other oenological factors that affect proanthocyanidin extraction include fermentation temperature, destemming, whole cluster fermentation, saignée, carbonic maceration, delestage, sur lies, protein fining and filtration, (Busse-Valverde et al. 2010, Saachi et al. 2005). Castelão wines produced using stem contact, contained the highest concentration of total oligomeric and

polymeric proanthocyanidins compared to non-stem contact wines due to the high tannin concentration of grape stems (Spranger et al. 2004).

Tannins in red grape skins are extracted throughout fermentation whereas the more bitter seed tannins are extracted at the closing stages of alcoholic fermentation (Cerpa-Calderon and Kennedy 2008). During traditional winemaking only 30% of total phenolics are extracted and Bindon et al. (2010b) found that 25% of Shiraz grape proanthocyanidins were extracted into wine.

1.4 Aroma compounds

Grape aroma compounds form a large and complex group of chemicals that are found in the mesocarp vacuoles and in the pericarp immediately under the berry skin (Esti et al. 2006). Conde et al. (2007) state that wine aromas consist of several hundred volatile compounds at concentrations ranging from several mg/L to a few ng/L or even less. As Fischer (2007) and Ebeler (2001) explain there are four distinct classes of varietal aroma compounds; monoterpenes, C₁₃ norisoprenoids, methoxypyrazines and sulfur compounds with a thiol function. Most grape aroma compounds are present as free volatiles, which may contribute directly to odour, or as sugar conjugates, which are non-volatile (Zoecklein et al. 1998). The volatile metabolites produced by yeast fermentation are derived from sugar and amino acid metabolism and include esters, higher alcohols, carbonyls, volatile fatty acids, and sulfur compounds (Conde et al. 2007). Chemical analysis of aroma compounds in wines can generate data that provides an indication of the relative importance of the aroma compounds of interest (Francis and Newton 2005). Aroma threshold values for compounds vary in published literature due to their determination by different methods, differing degrees of rigour and their analysis in diverse matrices including air, water, model wines, red and white wines. The concept of “odour activity values” (OAVs), referred to as aroma or odour units, is a useful measure to assess the importance of individual aroma compounds present in a sample. The odour value is obtained in the wine by dividing the concentration of the odorant in the sample by the detection threshold concentration of that compound. However, although OAVs can provide valuable information they do not take into account the interactions

between volatile compounds and other matrix compounds which can enhance or suppress aromas (Francis and Newton 2005).

1.4.1 Pinot noir aroma compounds

Kilmartin and Nicolau (2007) suggest that C₁₃-norisoprenoids particularly β -damascenone and β -ionone as well as C₆ alcohols, higher fermentation alcohols, carboxylic acids and esters are important aroma compounds in Pinot noir wines. Fang and Qian (2005, 2006) propose that 2-phenylethanol, which gives rose and honey aromas, and 3-methylbutanol compounds are the most important Oregon Pinot noir aromas, while other important odour active compounds include short chain fatty acids, sulphur compounds, acetates, 3-ethylthio-1-propanol, methionol, methional, benzaldehyde, benzyl alcohol, linalool, *trans*-linalool oxide, γ -octalactone, γ -nonalactone, ethyl and methyl vanillate, aceto-vanillone, whisky lactone, β -damascenone and vanillan. Additionally, ethyl and methyl anthranilate, ethyl cinnamate and ethyl dihydroxycinnamate were identified in Burgundy Pinot noir, but later quantification proved that concentrations were below sensory thresholds. However, these compounds may act synergistically with each other or other compounds to contribute to Pinot noir aroma (Fang and Qian 2006). The total concentration of short chain fatty acids in wine decreased with Pinot noir grape maturity but there was no correlation between esters and grape maturity in Pinot noir wine (Fang and Qian 2006). Ethyl esters contribute “fruity” aromas to wine and are formed from ethanol and a medium chained fatty acid (Saerens et al. 2008). Aroma-active esters are formed intracellularly by fermenting yeast cells and, being lipid soluble, can diffuse through the cellular membrane into the fermenting medium (Saerens et al. 2008). Saerens et al. (2008) state that the transfer into the fermenting medium decreases with increasing chain length from 100% for ethyl hexanoate to 54-68% for ethyl octanoate. The rate of ethyl ester formation is dependent on two factors: the concentrations of the two co-substrates (the acyl coenzyme A and ethanol) and the activity of the enzymes involved in their synthesis and hydrolysis (Saerens et al. 2008).

C₁₃-norisoprenoids are the product of chemical and enzymatic reactions and these breakdown products of carotenoids (Figure 4) include compounds with the megastigmane structure i.e. the ionone and damascenone families, with oxygen at different positions as in β -ionone with

a keto group at C-9 and β -damascenone at C-7 (Mendes-Pinto 2009). Ebeler (2001) explains that β -damascenone is formed from the degradation of the allenic carotenoid neoxanthin and Mendes-Pinto (2009) states that β -carotene is the precursor carotenoid of β -ionone. Light promotes the increase of carotenoids in unripe grapes compared to shaded grapes and they are mainly synthesised in the first stage of fruit formation until veraison then degrade until the end of maturity (Mendes-Pinto 2009). β -Damascenone, which has the aroma of exotic flowers with a heavy fruit undertone, is a commonly identified norisoprenoid in Pinot noir, and has a floral/fruity odour with a reported aroma threshold in water of 2 ng/L (Fischer 2007, Ebeler 2001, Fang and Qian 2006). β -Ionone, has a distinct berry and violet aroma and exists in concentrations from 0.2 to 0.6 $\mu\text{g/L}$ in Burgundy Pinot noir (Kotseridis et al. 2000). In the study by Fang and Qian (2006) β -ionone had a low sensory threshold of 0.007 $\mu\text{g/L}$, which means this compound is an important Pinot noir aroma. However, odour threshold levels and odour activity of aroma compounds depends on the medium and the sensitivity of the taster.

The “green” compounds, *trans*-3-hexenol and *cis*-3-hexenol were also found to be important in Pinot noir aroma (Fang and Qian 2006). Alcohols and aldehydes associated with “green” aromas are C₆ compounds which are synthesised via the lipoxygenase pathway at harvest, during transportation, crushing, pressing, must heating and grape maceration (Oliveira et al. 2006). Linoleic and α -linolenic acids are produced by the action of an acyl-hydrolase, and then the corresponding 13-hydroperoxides are formed by the lipoxygenase activity, which requires oxygen (Oliveira et al. 2006). Addition of a hydroperoxide-lyase leads to the formation of hexanal from linoleic acid. (*Z*)-3-hexenal and (*E*)-3-hexenal are derived from α -linoleic acid and an isomerase can inter-convert the two hexenals. Finally, an alcohol dehydrogenase reduces the alcohols to the corresponding aldehydes i.e. 1-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol. Aparicio et al. (1998) characterised olive ripeness by “green” aroma compounds in virgin olive oil. Hexanal is a major contributor to the “green” unripe stage of olive development and its concentration decreases with increased ripeness level. C₆ aldehydes hexanal and (*E*)-2-hexenal, were abundant in less ripe musts from English white grapes and low relative abundances of these C₆ compounds could indicate an over-ripe stage in red grapes (Caven-Quantrill and Buglass 2008).

1.4.2 The effect of leaf removal on aroma compounds

Giovanelli and Brenna (2007) state that climatic conditions, temperatures during ripening and cluster sun exposure are all significant factors that affect levels of varietal aroma compounds in grape berries. Increased sun exposure enhanced glycosidic aroma precursors including monoterpenes and C₁₃ norisoprenoid aglycones and generally accelerates carotenoid breakdown, but can also cause sunburn (Fischer 2007). An increase in C₁₃ norisoprenoids has been reported in Riesling, Syrah, Chardonnay and Golden Muscat grapes with increased sun exposure (Reynolds et al. 2007, Marais et al. 1991, Fischer 2007, Bureau et al. 2000). In contrast, Lee et al. (2007) reported that Cabernet Sauvignon had the highest concentration of β -damascenone in non-defoliated grapes and subsequent wines, compared to grapes from vines which were defoliated at fruitset by either lateral or primary leaf removal or individually. Fischer (2007) and Keller (2009) state it is a common reaction of grapes to increase polyphenols and degrade carotenoids in berry skins in sun exposed grapes as increased UV-B reduces carotenoid concentrations at harvest. Research conducted by Ristic et al. (2007) established that bunch shading of Shiraz grapes decreased the levels of norisoprenoids in the wine and suggest that other compounds may be altered which influence wine aroma and flavour. The variation between C₁₃ norisoprenoid synthesis in Shiraz (Ristic et al. 2007) and Cabernet Sauvignon (Lee et al. 2007) may be due to microclimate, mesoclimate, canopy architecture, training system, light, temperature and varietal differences. Leaf removal in Sauvignon Blanc resulted in a greater reduction in vegetal aromas and flavours if undertaken at, or shortly after fruitset, compared to veraison (Arnold et al. 1990). Keller (2009) states that the influence of temperature on most grape aroma and flavour compounds are not presently very well understood.

Figure removed due to copyright regulations

Figure 4. The biosynthesis of norisoprenoids either via direct carotenoid degradation or via glycosylated intermediates.

1.4.3 Interactions between aroma compounds and tannins

Polyphenols have been known to interact non-covalently with wine aroma compounds and these interactions could affect the release of wine aroma compounds (Moreno-Arribas and Polo 2009). Dufour and Bayonove (1999) used nuclear magnetic resonance (NMR) to show that in hydroalcoholic solution, when the only polyphenol present was (+) catechin, ethyl hexanoate and isoamyl acetate were prevented from volatilising. However, no evidence of this effect was found when larger molecular weight proanthocyanidins were added to the hydroalcoholic solution. Another study showed that the addition of (+) catechin to red wine resulted in a decrease in volatility of hexanal and ethyl hexanoate by 10-20% and it was reported that catechin influenced the release behaviour of several wine volatiles (Jung and Ebeler 2003). In contrast, when (+) catechin was added to Bordeaux wines the aroma compound 3-mercaptohexanol disappeared but this mechanism was partially inhibited by sulfur dioxide addition (Blanchard et al. 2004). Aronson and Ebeler (2004) using HS-SPME-GC-MS, found that larger molecular weight aroma compounds with later retention times in Cabernet Sauvignon and Chardonnay, were affected by tannin levels to a greater extent than early eluting, smaller compounds. These results suggest that different volatile aroma compounds react differently to flavan-3-ols and proanthocyanidins.

Additionally, aroma compounds interact with each other and a strong earthy aroma from methoxypyrazines was more easily perceived when (*Z*)-3-hexenol and 1-hexanol was added to a Spanish wine (Escudero et al. 2007). The inference is that these aroma compounds may interact synergistically, but the authors suggest that further investigation is required regarding different levels of each compound. No studies to date have investigated the interaction

between Pinot noir aromas or the effect of viticultural treatments on aroma and tannin interactions.

Studies regarding tannin concentration and composition often refer to “green tannins” but as Herderich et al. (2004) states, the structure and biosynthesis of individual “green tannins” are unknown and no chemical measures are available. Anecdotal evidence relates the occurrence of “green tannins” to grapes from young vineyards, ripening difficulties in certain vintages and over extraction in the winery (Herderich et al. 2004). However, whilst the chemical structure remains unknown, the existence of these undesirable mouthfeel and taste properties of “green tannins” cannot be dismissed. A possible explanation proposed by Herderich et al. (2004) is that the concept of “green tannins” is a consequence of an overall enhanced concentration of tannins that contributes to a wine with an unbalanced astringency profile. No remedy is available to eliminate “green tannins” which suggests that a group of individual tannins is responsible. It has been suggested that “green tannins” are potent molecules effective at smaller concentrations than the average tannin from ripe grapes (Herderich et al. 2004). They could be more readily extracted and retained in wine than other tannins due to higher solubility and resistance to fining. However, evidence of actual tannins being responsible for “green tannins” remains elusive so studies into the interactions with other aroma and taste compounds in red wine are critical. Tannins are not the only molecules to elicit astringency in wine, organic acids, and quercetin glycosides are also astringent molecules (Noble 1999). A further explanation from Herderich et al (2004) is that the absence of pectins and/or other molecules from the red wine matrix can modulate astringency. Alternatively, grape aroma compounds such as leaf aldehydes i.e. hexanal contribute “green” aromas to wine, and in the absence of “fruity” aromas are more perceivable. No research studies to date could be found that investigated viticultural treatment effects on “green” or “fruity” volatile aroma concentrations in Pinot noir wine.

1.5 Sensory evaluation by free choice profiling (FCP)

Researchers use established sensory evaluation techniques to characterise wines from trials and the central principle is that the method should be matched to the objectives of the test (Perrin et al. 2007, Lawless and Heymann 1998). A sensory evaluation method known as free choice profiling (FCP), developed in the United Kingdom during the 1980s (Williams and Arnold 1985, Perrin et al. 2007), allows individual panellists to choose their own series of attributes with which to assess wines (Arnold et al. 2007). FCP assumes that panellists do not differ in the number and kind of sensory characteristics they perceive, but they do differ in the way they label them (Guy et al. 1989). The distinct advantage of FCP is the avoidance of panel training as panellists only need to be able to use a scale and be familiar with the product (Murray et al. 2001). However, Heymann (1994) suggested that FCP should be used with subjects with prior experience in sensory methods as “sensory naïve” subjects did not produce consistent results. In agreement with this view, Barcenas et al. (2003) found that panellists without sensory experience lacked consensus of attribute scoring even though the attributes were self-generated. Nevertheless, FCP has been used successfully in sensory studies to compare sensory characteristics of orange juices (Aparicio et al. (2007), dairy products (Kirkmeyer and Tepper 2003), inorganic and organic acids (Rubico and McDaniel 1992), Port (Williams and Langron 1984), dry-cured ham (Dolors Guàrdia et al. 2010) and wine (Perrin et al. 2007). Conventional sensory analysis using a trained panel was compared to FCP analysis carried out by professional wine tasters (Perrin et al. 2007). Results showed that primary characteristics found in wines by conventional sensory analysis were also found by FCP (Perrin et al. 2007). FCP is complemented by the statistical technique generalised procrustes analysis (GPA) (Gower 1975, Murray et al. 2001). Using GPA to analyse FCP data reduces the information from studies to two or three dimensions, which means FCP can reveal large differences between wines (Murray et al. 2001). Additionally, by allowing attributes to be generated by panellists wine characteristics were identified that may not have been considered using a traditional approach (Perrin et al. 2007). No studies have been undertaken that used FCP to identify and distinguish wines according to viticultural or oenological treatments.

Chapter 2

Objectives and methodology

2.1 Research Objectives

The primary aim of this research was to analyse wines produced from leaf removal trials carried out at different times during the growing season in two consecutive years. The main focus was on the resultant Pinot noir wines and whether the timing of leaf removal affected the final flavour, colour, aroma and structure of the wine. Additionally, the vineyard focus was to characterise grapevine canopies within each treatment and measure leaf area to fruit weight ratios, and berry ripening parameters. Following replicate winemaking each year, laboratory analysis was carried out with the objective being to ascertain the effect of the timing of leaf removal on wine colour, tannin concentration and composition. An investigation into the anecdotal suggestion that leaf removal increases “fruity” aromas in wine and decreases “green” aromas was carried out. To achieve this aim, a method was developed specifically to analyse a range of different volatile aroma compounds thought to be responsible for them. It was an important objective to establish whether chemical concentration, both aroma and phenolic differences, could be detected by sensory analysis. Therefore, a modified version of an existing sensory technique, free choice profiling, was developed to investigate whether the timing of leaf removal produced wines that could be identified according to specific traits, and whether these traits were related to the chemical composition of the wine.

Hypotheses

- ❖ Early leaf removal will result in significantly higher wine colour and tannin concentration.
- ❖ Leaf removal reduces the perception of "green" characteristics and increases “fruity” nuances.

2.2 Vineyard design

A commercial block of 10/5 clone of Pinot noir planted in Glasevin soil, grown on own roots and trained on a Vertical Shoot Positioned trellis system (VSP) was utilised for the study (Figure 1).



Figure 1. The Pinot noir experimental research vines at Pegasus Bay vineyard, Waipara.

The vines were pruned to four canes, each with eight buds and were approximately eighteen years old. The block consists of twelve rows of thirty seven bays with six vines between each bay totalling two hundred and twenty two vines per row. Treatments were applied per row according to a randomised block design (Figure 2).

North												
Block	A	A	A	A	B	B	B	B	C	C	C	C
Row	20	21	22	23	24	25	26	27	28	29	30	31
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X
Treatment	T2	T4	T1	T3	T4	T2	T1	T3	T1	T4	T3	T2
South												

Figure 2. A schematic representation of the experimental vineyard design in Pegasus Bay vineyard, Waipara.

There were four treatments and three rows of vines per treatment. The treatments were: **NLR**: no leaf removal (control), **LR7**: leaf removal from the fruiting zone 7 days after flowering, **LR30**: leaf removal from the fruiting zone 30 days after flowering, **LRV**: leaf removal at commencement of veraison (5% colour change by visual assessment) (Figure 3).

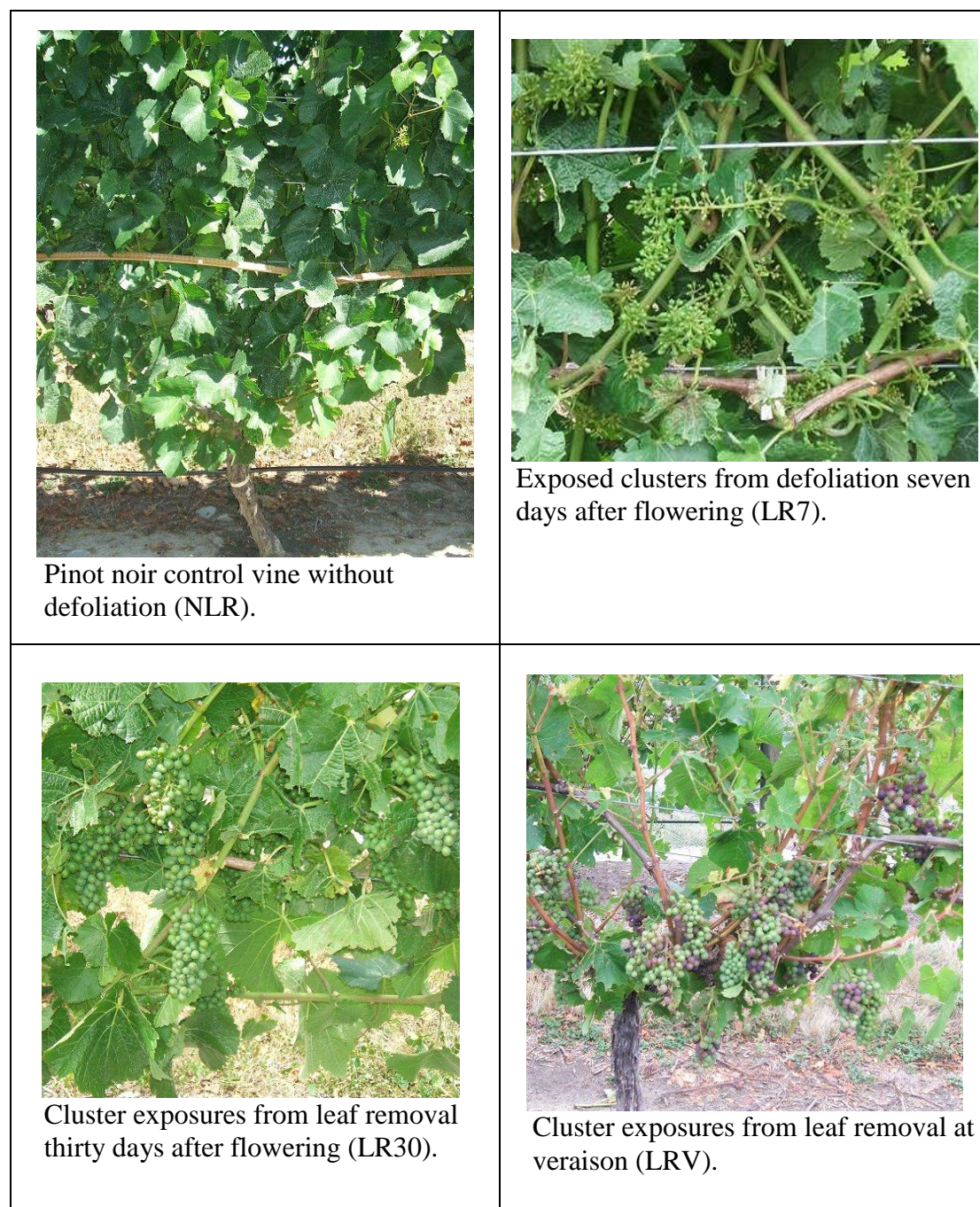


Figure 3. Photographs of shaded and exposed fruit on defoliated and non-defoliated grapevines.

2.2.1 Vineyard measurements

Monitor vines in each treatment row were used for berry ripening analysis, berry weight, cluster weight, seed data and leaf area to crop weight ratio data collection throughout the two year study. There were five monitor bays used per row containing six vines each, resulting in thirty vines per row and ninety vines per treatment, chosen on the basis of uniformity. To ensure uniformity of comparisons across treatments, vines of similar sizes were selected within each replicate row as monitor vines. This was achieved by measuring the trunk diameter of all vines prior to commencement of the trial (at approximately 50 cm from the ground) as this value can be used as an indicator of cumulative plant development (Strong and Azarenko 2000). No statistical difference ($P > 0.05$) was found between the monitor vines as mean trunk diameters were NLR: 14.9 cm, LR7: 14.6 cm, LR30: 14.8 cm and LRV 14.6 cm. Point Quadrat analysis (Smart and Robinson 1991) was carried out to characterise the vine canopies and fruit exposure prior to, and after each treatment application. In viticulture, a balance between grapevine vegetative growth and crop load is considered as essential to obtain a good fruit quality (Winkler et al. 1974, Nuzzo 2004). For a given cultivation system and cultivar/rootstocks combination this ‘balance point’ is largely determined by the ratio of total leaf area to crop load (Nuzzo 2004). Additionally, the ultimate source of sugar produced in grapevines is from leaf photosynthesis, which is dependent on the total amount of exposed leaf area (Kliewer and Dokoozlian 2005). Therefore, in this study, leaf area was determined destructively by removing one metre of leaves from two different vines per row in both years. Berry ripeness monitoring began 4–6 weeks prior to the predicted harvest date (based on data from previous years) and 200 berries per treatment, per row in triplicate were randomly sampled weekly for °Brix, pH, titratable acidity and berry weight according to standard industry protocols (Iland et al. 2000). From this information, harvest date was determined according to °Brix level; the aim was for 23 °Brix in 2008 although early ripening occurred in 2009 resulting in fruit reaching 25 °Brix). At harvest, 200 berries from the harvest bins of each replicate treatment were collected in triplicate and frozen at $-20\text{ }^{\circ}\text{C}$ then analysed at a later date to measure berry tannin concentration.

2.3 Winemaking

An addition of SO₂ at the rate of 50 ppm was added to the fruit at harvest to protect the grapes from microbial spoilage on the journey from the vineyard to the winery at Lincoln University (approximately 74 km). For wine production in 2008 and 2009, grapes from the replicates of each treatment were pooled and subsequently divided into three equal lots and placed in picking bins for 12 hours at 4 °C. Fruit was then de-stemmed, crushed and the must, skins and seeds were placed into three replicate 30 L fermentation vessels in 2008 and 10 L in 2009. Attention was given to making wines in a consistent manner so that differences in wine composition could be attributed to leaf removal treatments in the vineyard. The must was placed in a refrigerator and underwent a 24 hour cold-soak at 4 °C. The wines were placed in a 28 °C room. Inoculation with Elegance yeast (1 g/L), produced by the Institute of Burgundy, France, (AB Mauri, Sydney, Australia) occurred in two stages: half in the morning and the rest in the afternoon. All twelve fermentations were completed within five days in each year and were monitored twice daily for temperature and °Brix by hydrometer. Fermentation temperatures did not exceed 30 °C and five punch downs were conducted daily. Clinitest (Bayer New Zealand, Auckland) tablets were used to confirm the end of fermentation, at which point the wines were moved to an 18 °C room and punch downs continued until the caps remained submerged. Wines were pressed in a 40 kg capacity vertical hydraulic bladder press to 1.2 bars into demijohns and samples of the pressed wines, skins and seeds post-fermentation were removed and frozen at -20 °C for later analysis. Wines were inoculated with malolactic starter culture, placed in the 18 °C room and progress monitored by thin layer chromatography. Following malolactic fermentation, SO₂ was added at a rate of 40 ppm with subsequent further additions made periodically to maintain 20 ppm. Free-SO₂ analysis was carried out according to the method of Iland et al. (2000). Filtration and bottling occurred on 22nd September 2008 and 22nd June 2009, respectively, for the two harvests.

2.4 Tannin analysis

2.4.1 Protein and polysaccharide precipitation assays

A number of methods are available to determine tannin concentration in grape skins, seeds and wine. These include the method from Harbertson and Adams (2002) using bovine serum albumin (BSA) and the methylcellulose precipitation method by Sarneckis et al. (2006). Preliminary results showed that the methylcellulose (MCP) precipitation method resulted in strong repeatability (Chapter 4). Therefore, the MCP method was chosen in this research to analyse total tannin concentrations in berries at harvest, wine during winemaking and seeds and skins post fermentation. These experiments were carried out to investigate whether the timing of leaf removal affected tannin extractability from berries, tannin concentration in wine as well as examining tannin concentration remaining in skins and seeds post fermentation.

2.4.2 Mean degree of polymerization (mDP) by acid catalysis in the presence of excess phloroglucinol

A modified analytical method based on that by Kennedy and Jones (2001) was used to allow analysis to be carried out by LC-MS. Each wine sample (4 mL) was reduced to 0.2-0.3 mL by rotary evaporation at 40 °C and 3 mL of deionised water was added. A 0.36 g C₁₈ SEP-PAK cartridge (WAT051910, Global Science, Auckland, New Zealand) was activated using 5 mL methanol, 7.5 mL ethyl acetate (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand), then 7.5 mL deionised water. The sample was applied to the C₁₈ cartridge and washed with deionised water (7.5 mL) and allowed to dry with nitrogen gas at a flow rate of 1 L/min for 60 minutes. Ethyl acetate (5 mL) was added to the C₁₈ cartridge to ensure all monomeric material was completely discarded. To remove proanthocyanidins, the cartridge was eluted with 5 mL methanol (HPLC grade, Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand). The extracted solution then reduced by rotary evaporation using a bath temperature at 30 °C to less than 1 mL before being reconstituted with methanol to a final volume of 1 mL. The solution was transferred to a 1.5 mL eppendorf tube and stored at -20 °C until acid hydrolysis was performed.

For the blank reagent, 0.06 g ascorbic acid (Sigma-Aldrich New Zealand, Auckland, New Zealand) was added to 800 μL of HPLC grade methanol (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand) and 49.8 μL of concentrated hydrochloric acid (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand). The solution was sonicated for 20 minutes and once cooled HPLC grade methanol (2150.2 μL) was added. For the phloroglucinol reagent, the method was the same as the blank but instead of methanol addition after cooling, 0.301 g of phloroglucinol dissolved in 600 μL of HPLC grade methanol was added to the solution and HPLC methanol (1550.2 μL) was added. All reagents were prepared on the day of analysis to prevent reagent degradation.

The blank or phloroglucinol reagent (0.5 mL) was added to each sample (0.5 mL) and heated at 50 °C for 20 minutes. To stop the reaction 5 mL of 40 mM sodium acetate was added and left for 60 minutes at room temperature. The solution was mixed using a syringe and passed through a 13 mm 0.45 μm PTFE filter (Grace, 2165 catalogue, Taiwan) into an HPLC vial with the first few drops discarded.

Liquid chromatography was performed on a Shimadzu 2010 equipped with two binary LC-20AD pumps, SIL-20AC auto-sampler, SPD-M20A PDA detector and 2010EV mass spectrophotometer with ElectroSpray ionization (ESI) probe operated in negative ion mode. The mass spectrometer heat block temperature was 200 °C and the curved desolvation line (CDL) temperature was 250 °C. Both nebulizing and drying gas were nitrogen, the nebulizing gas flow at 1.5 L/min and drying gas pressure at 0.14 MPa. Detector voltage was 1.5 kV, interface voltage was 4.5 kV and CDL voltage was -45 V. The PDA detector wavelength collected data from 250 nm to 700 nm and quantification was performed at 280 nm. The mobile phase solvent A was 2% acetic acid in water and solvent B was 2% acetic acid in methanol (MeOH). The flow rate was 0.8 mL/min and a flow splitter (Upchurch, UK) was installed to direct 0.2 mL/min flow to the mass spectrophotometer. The linear solvent gradient was 5% B to start, raised to 10% B in 5 minutes and to 40% in 25 minutes and quickly to 100% in 0.5 minutes, maintained at 100% B for 5.5 minutes and reduced to 5% B in 0.5 minutes. The column was re-equilibrated at 5% solvent B for 5.5 minutes before the next injection. The column used was LUNA C-18, 250 x 4.6 mm, particle size 5 μm (Phenomenex, USA).

Catechin, epicatechin, epicatechin gallate and epigallocatechin were determined from their respective standard curves although the latter two were found to be not present in the samples analysed. The phloroglucinol adducts (-PG) were estimated as follows: concentrations of catechin-PG, epicatechin gallate-PG and epigallocatechin-PG were calculated using the standard curve of the flavan-3-ol monomer and corrected to allow for the difference in molar absorptivity according to Kennedy and Jones (2001). Catechin was not present as a phloroglucinol adduct. Concentrations were converted to catechin equivalents using the appropriate molecular weights. The mDP information was calculated by subtracting the phloroglucinol adducts from the corresponding blank.

2.4.3 Identification and quantification of flavan-3-ol monomers and quercetin

The method was modified from Meagher et al. (2004) and Ibern-Gomez et al. (2002). Wine samples were analysed by injection of 5.0 μL on to a HPLC system (Agilent 1100 series, Germany) equipped with a quaternary pump, a thermostat, an autosampler and a DAD detector. The column was a LUNA C-18, 250 x 4.6 mm, particle size 5 μm held at 25 $^{\circ}\text{C}$ (Phenomenex, USA). The elution solvents were: 2 % acetic acid in deionised water (A) and 2 % acetic acid in methanol (B). The linear pump gradient was 5% solvent B, increase to 10% B by 5 minutes, 40% B by 30 minutes, 100% B by 40 min and held until 45 min, then reduced to 5% B at 45.5 minutes and maintained until 51 minutes. The flow rate was constant at 1.0 mL/min. All wine samples were filtered using a 0.45 μm PTFE, 13 mm syringe filter from Grace Davison (Auckland, New Zealand). Catechin, epicatechin, epigallocatechin, galocatechin, epicatechin gallate and quercetin standards were purchased from Sigma Aldrich, Auckland, New Zealand, and prepared at concentrations of 0, 12.5, 25, 50 and 100 ppm. The detector scanned between 250 and 650 nm and quantification was carried out at 280 nm. ChemStation software (Agilent Technologies, Inc., California USA) was used to identify and quantify compounds.

2.4.4. Spectral analysis

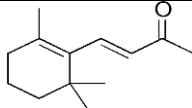
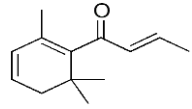
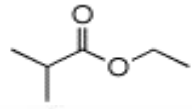
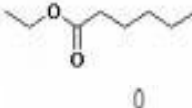

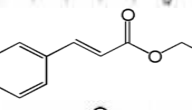
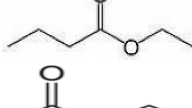
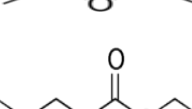
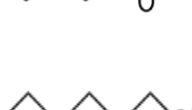
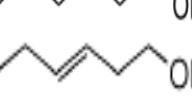
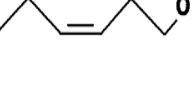



Colour analysis was carried out on the treatment wines in both years to investigate the effect of the timing of leaf removal on wine colour density, colour hue, total red pigments, SO₂-resistant pigments, anthocyanins and chemical age of colour compounds. Spectral colour analysis was performed according to Iland et al. (2000) using a UV-VIS Spectrophotometer (Helios, Spectronic *Unicam*, Cambridge, U.K).

2.5 Aroma analysis

2.5.1 HS-SPME-GC-MS-SIDA

A new method was developed to ascertain the effect of the timing of leaf removal on “fruity” and “green” aroma compounds and their contribution, if any, to the perception of “green tannins” in Pinot noir wine. This method was developed at Lincoln University using headspace solid-phase microextraction-gas chromatography-mass spectrometry-stable isotope dilution analysis (HS-SPME-GC-MS-SIDA). Due to the different molecular weight aroma compounds to be analysed (Table 1), it was necessary to develop a specific method that would allow identification and quantification of all compounds of interest. The complete method that was developed is described in detail in Chapter 6 entitled “Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry stable isotope dilution analysis technique to identify “green” and “fruity” volatile aroma compounds in Pinot noir wine”.

Table 1 Aroma compounds identified in Pinot noir wine in this research study. *Compiled using data from Fang et al. (2005), Escudero et al. (2007), Pineau et al. (2007) and Kotseridis et al. (1999).*

	Aroma	Structure
C13 norisoprenoids		
β -ionone	Berry/violet	
β -damascenone	Heavy fruity undertone/exotic flowers	
Esters		
Ethyl isobutyrate	Apple/fruity	
Ethyl hexanoate	Blackberry/fruity/ Strawberry	
Ethyl octanoate	Fruity/cooked aroma	
Ethyl decanoate	Fruity/grapey	
Ethyl cinnamate	Cherry/fruity	
Ethyl butanoate	Fruity/peach	
Ethyl acetate	Blackcurrant/ sweet fruity	
Ethyl pentanoate	Fruity/mint	
Alcohols		
Hexanol	Toasted/ Green/Dry grass	
Trans-3-hexen-1-ol	Green/bitter	
Cis-3-hexen-1-ol	Fresh cut grass/ Fruity/green	
Aldehyde		
Hexanal	Green	

2.6 Sensory evaluation

Free choice profiling (FCP) was used as the basis for sensory evaluation to enable Waipara winemakers, familiar with local Pinot noir wines, to use their own vocabulary to describe the research wines (Perrin et al. 2007). However, specific phenolic and aroma compounds were analysed in the treatment wines. Therefore to ensure that appropriate descriptors associated with these compounds were considered an expert panel of oenology lecturers and postgraduate students was convened to generate attributes. A tasting session of the wines in duplicate occurred followed by a discussion and a group consensus was reached of the thirteen generic descriptors that were used. This technique allowed an assessment of the wines that combined winemakers' attributes with those generated by an expert panel at Lincoln University.

2.6.1 Participants

Thirteen unpaid Waipara winemakers were recruited for the sensory analysis due to their availability and on the basis of extensive experience of tasting, and working with Pinot noir wines from the region. The eleven males and two females ranged between 25-60 years of age. All panelists were non-smokers and one male winemaker was registered blind. The blind panelist was seated in a private room for both sessions and accompanied by a reader/writer who did not taste the wines and had no influence over descriptors generated or ratings of the wines. In session two the blind panellist was asked to score each wine from 0-100 for each descriptor and their assistant placed a mark on the 100 mm line accordingly.

2.6.2 Sensory procedures

Following approval from the Human Ethics Committee at Lincoln University, all invited participants were provided with information about the imminent study and then completed and signed the consent forms. The study was conducted in one day at Pegasus Bay Winery, Waipara and the two sessions took place; one morning session of 60 minutes and an afternoon session of 90 minutes. A purpose built room was used with natural lighting and an absence of noise and other stimuli. The ambient temperature was regulated by an air conditioning unit and held constant at 20° C throughout both sessions. Panellists were seated in separate white booths and sixteen wines (20 mL) were poured into standard ISO (1977)

glasses with 3-digit random codes and protected with coverslips (Parr et al. 2007) for session one. There were six participants in one session, and seven in the other due to having eight individual tasting booths preventing all thirteen from being seated simultaneously.

2.6.3 Modified free choice profiling

The generic attributes provided to panelists were placed on the description generation sheet for each participant to ensure panelists chose alternative descriptors in session one. Panellists were invited to arrive thirty minutes prior to commencement of the sensory study to allow for a full briefing to address any queries that may have arisen.

Session one: The first session involved the panellists assessing the 16 wines in a randomised order for aroma and flavour. Panellists were supplied with the list of previously generated descriptors and asked to attach their own descriptors to the list. Sixteen 20 mL wine samples was provided in ISO glasses in a random order unique to each panellist and three-digit numbers were assigned to each wine. It was emphasised that descriptors provided by each participant had to be applicable to all wines to allow the rating system to be valid in session two. No discussion between participants regarding the wines or descriptors occurred during or between the sessions.

Session Two: Following the session for generating descriptors the panellists were provided with lunch and asked to return in the afternoon to rate the intensity of each descriptor on a 100 cm scale after expectoration. The afternoon session consisted of two rating sessions to ensure wines were tasted in duplicate but in unique random orders for each panellist. As in session one, 3-digit codes were assigned to the wine glasses and participants vacated the room while the second wines (20 mL) were poured. Using both the descriptors provided, and their own individual descriptors participants were asked to place a line on the 100 mm scale thereby rating each wine for that particular attribute.

2.7 Statistical analysis

Due to the wide variety of information to be interpreted ranging from vineyard vine measurements to modified-free choice profiling data, specific statistical analysis was required for each section of this research. Regarding vineyard data, analysis of variance (ANOVA) and Tukeys test were used to identify significant differences between treatments and year. This statistical investigation was carried out to analyse grapevine growth response, leaf area and fruit weight data and berry composition using GenStat 11, VSNi, Hemel Hempstead, UK. To analyse the protein-tannin ratio and protein concentration in wines a variable number of replicates (up to 6) were used in the determination of tannin by the BSA precipitation assay. Consequently, average standard errors were calculated as the harmonic mean of the pooled variance from replicate analyses using Excel 2003, Windows XP, Microsoft, USA and graphs were produced using SigmaPlot version 2002, Windows version 8.02 (SPSS Inc, Cranes Software International Pty Ltd, Melbourne, Australia). To analyse the phenolic data ANOVA with contrasts was carried out using GenStat 11. This approach enabled significant contrasts between non defoliated and defoliated treatments to be evaluated, as well as between defoliation treatment timings. Graphs were produced using SigmaPlot version 2002, Windows version 8.02 (SPSS Inc, Cranes Software International Pty Ltd, Melbourne, Australia). Method development data analysis required precise method validation testing so limits of detection (LODs), limits of quantification (LOQs), standard deviations, mean values and graphs were calculated and produced using Excel 2003, Windows XP, Microsoft, USA. Chemical aroma data was analysed using one and two-way ANOVA and Tukey's test to identify significant differences between treatments and year. Regarding sensory data, results from ANOVA and restricted maximum likelihood (REML) using GenStat 11, (VSNi, Hemel Hempstead, UK) showed no statistical significance between microvinification replicates and flights for panellist ratings. Therefore, generalised procrustes analysis (GPA), using the Commandeur algorithm to allow for zeros in the data matrix was carried out on sensory data. Principal component (PCA) analysis was carried out to ascertain the consensus regarding treatment wines from the sensory analysis and Spearman rank correlation was carried out to determine correlations between sensory and wine chemical analysis existed using XLSTAT version 2010.2, (Paris, France).

2.8 Summary

The research methodology used in this study was chosen to examine specific grapevine, berry and wine responses to the timings of leaf removal and compare results to the non-defoliated treatment. Results from leaf removal studies benefit grape growers and winemakers by increasing their understanding of the impacts that viticultural treatments have upon wine flavour and aroma. Additionally, the investigation into “green” and “fruity” aromas in the resultant treatment wines enabled research into the anecdotal suggestion that “green” aroma compounds could be responsible for the perception of “green tannins” in red wines.

Chapter 3

(A preparation for submission to American Journal of Enology and Viticulture)

Effect of mechanical leaf removal and timing on *Vitis vinifera* L. Pinot noir grapevine growth and berry composition.

B.S. Kemp ^a, G.L. Creasy ^{a *}, R. Harrison ^a

^a Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.

* Corresponding author. Fax: +64 3 3253850. Mail address: Centre for Viticulture and Oenology, Faculty of Agriculture & Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, Canterbury, New Zealand. Email: Glen.Creasy@lincoln.ac.nz (G. Creasy)

Short running title: Leaf removal effects on grapevine and berry growth

Abstract

Mechanical leaf removal is increasingly common in vineyards, but the effect that its timing has on Pinot noir fruit and wine qualities remains to be determined. Leaves were removed in a commercial vineyard in Waipara, New Zealand in the 2007-2008 and 2008-2009 seasons using a Collard pulsed-air machine. Fruit zone treatments consisted of **NLR**: no leaf removal (control), **LR7**: leaf removal from the fruiting zone 7 days after flowering, **LR30**: leaf removal from the fruiting zone 30 days after flowering, **LRV**: leaf removal at 5% berry colour change (by visual assessment). Partial cane removal was performed on all treatment vines in 2008-2009 to reduce crop load variability. The result was a decrease in leaf layer number and interior clusters while an increase in exterior clusters and canopy gaps occurred compared to 2007-2008. In 2008-2009 all treatments had lower cluster weights and yields per vine compared to 2007-2008. No significant difference was observed in Brix, titratable acidity or pH at maturity, but a significant difference in Brix between years is reported. Results indicate that early mechanical leaf removal of Pinot noir is appropriate for vigorous vines while not affecting berry ripening parameters.

Keywords: Berry growth, leaf removal, mechanical, Pinot noir.

Introduction

Leaf removal carried out between fruitset and veraison increases exposure to sunlight and temperature in the fruit zone. Additionally, defoliation decreases disease by aerating clusters resulting in improved fruit composition (Poni et al. 2006). In viticulture, the leaf area and its density and distribution within the canopy are fundamental parameters for characterizing the light microclimate and to understand the responses of vines to environment, training systems and canopy management (Lopes and Pinto 2005). Hunter (2000) explains that canopy management is aimed at optimizing carbon allocation to fruit sinks without disturbing grapevine growth and development.

The effects of leaf removal can be variable depending upon climate, grape variety and the technique employed i.e. manually or by a mechanical leaf suction device or compressed pulsed air blower (Intrieri et al. 2008, Poni et al. 2006). However, both Smith and Codrington (1988) in New Zealand and Gubler et al. (1991) in the USA found no significant difference in fruit yield between the compressed air and leaf suction devices compared to manual leaf removal. Smith and Codrington (1988) carried out leaf removal treatments on vines pre-veraison and on different vines post-veraison and found no difference in Brix, but exposed fruit had lower acidity compared to naturally shaded fruit. Percival et al. (1994) found that mechanical compared to manual leaf removal resulted in no statistical significance in yield, Brix, acidity or pH at harvest when performed on Riesling at pea size and at veraison.

Leaf removal studies conducted by Bledsoe et al (1988), Petrie et al. (2003), Ollat et al. (1998), and Morrison and Noble (1990) found that titratable acidity (TA g/L), sugar per berry, cluster weight and whole vine photosynthesis were significantly reduced by leaf removal, while juice pH increased. Additionally, defoliation studies by Poni et al. (2009) and Pereira et al. (2006) resulted in smaller berries, decreased acidity and higher sugar levels in Lambrusco, Barbera and Merlot berries in Italy and France, respectively.

With regards to leaf removal from Pinot noir grapevines, Koblet et al. (1994) performed defoliation when berries were 7 mm in diameter and both Brix and pH declined with increasing exposure compared to non-defoliated fruit. The defoliated vines had a lower yield and berry weight compared to non-defoliated vines. Vasconcelos and Castagnoli (2000) performed manual leaf removal at four weeks post bloom which decreased Brix, but did not affect acidity or pH compared to shaded fruit.

Cortell and Kennedy (2006) performed artificial shading when berries were 2 mm in diameter and exposed fruit resulted in both higher juice pH and acidity. No statistical difference was observed in Brix levels at harvest between artificially shaded and exposed fruit, but a higher seed weight occurred in exposed fruit. The artificial shading involved the attachment of light exclusion boxes to clusters. This artificial shading of grape clusters does not mimic clusters shaded by leaves as non-defoliated vines still allow light to pass through leaves to clusters. Smart (1985) reported that 8-10% of photosynthetically active radiation (PAR) striking a canopy passes through a leaf. Therefore, trials using artificial shade and natural shade are difficult to compare due to the differences in the moderation of sunlight. Regarding the timing and severity of leaf removal, Bledsoe et al. (1988) found that Sauvignon blanc yield and yield components were not significantly affected by either. However, Hunter and Visser (1990) found with Cabernet Sauvignon that 33% defoliation prior to berries reaching pea size reduced berry size and yield, but had no effect when applied at veraison. These variable leaf removal results suggest effects of defoliation can depend upon severity and timing of the treatment as well as the type of trellis system, the cultivar, the environment and the climate.

This field trial was established to investigate the impact of early mechanical leaf removal on Pinot noir grapevine growth and fruit composition at harvest. Manual leaf removal is time consuming, labour intensive and expensive while mechanical leaf removal offers a cheaper alternative. To our knowledge, no studies concerning the effect of the timing of mechanical leaf removal on Pinot noir grapes have been reported. Due to the increase in vineyard mechanization, the importance of Pinot noir to New Zealand and a need for research regarding the timing of leaf removal on Pinot noir fruit this trial was designed to evaluate grapevine and fruit response.

Materials and Methods

The trial was carried out on a commercial north facing block of 10/5 clone of Pinot noir planted in Typic Dystrustept soil, classified according to the soil classification system found on the United States Department of Agriculture website (www.soils.usda.gov). Vines were grown on own roots, irrigated and trained on a Vertical Shoot Positioned (VSP) trellis system. The vines were pruned to 4 canes, each with 8 buds and were approximately 18 years old. The block consisted of 12 rows of 37 bays with 6 vines in each bay, 222 vines per row spaced at 1.5 m apart, 2.7 m between rows and an historical yield of 2.5 kg of fruit per vine. Treatments were applied to both sides of the row and laid out in a completely randomized

block design incorporating rows as replicates, with three replicates per treatment. The four treatments were **NLR**: no leaf removal (control), **LR7**: leaf removal from the fruiting zone 7 days after flowering, **LR30**: leaf removal from the fruiting zone 30 days after flowering, **LRV**: leaf removal at 5% colour change at veraison (by visual assessment). LR7 was applied on 20th December 2008 and 12th December 2009, LR30 was applied on 15th January 2008 and 5th January 2009 the LRV was applied 11th February 2008 and 13th February 2009. Early flowering in the 2008-2009 season resulted in earlier treatment application in December and January compared to the previous season.

Monitor vines in each treatment row were used for berry ripening analysis, berry weight, cluster weight, seed data and leaf area to crop weight ratio data collection throughout the two year study. There were five monitor bays used per row containing six vines each, resulting in thirty vines per row and ninety vines per treatment, chosen on the basis of uniformity. To ensure uniformity of comparisons across treatments, contiguous vines of similar sizes were selected within each replicate row as monitor vines. This was achieved by measuring the trunk diameter of all vines prior to commencement of the trial (at approximately 50 cm from the ground) as this value can be used as an indicator of cumulative plant development (Strong and Azarenko 2000). No statistical difference ($P > 0.05$) was found between the monitor vines, and mean trunk diameters were NLR: 14.9 cm, LR7: 14.6 cm, LR30: 14.8 cm and LRV 14.6 cm.

Excessive fruitset in 2008-2009 resulted in a commercial decision to limit yields per vine in the second year by partial cane removal. The four cane system was reduced when the top two canes were cut in December 2009, removing three shoots from both sides of the cane. Canes were left to dry with the shoots attached and were removed post senescence of the leaves on the cut canes and shoots. Heavy hail on 3rd January 2009 resulted in damaged leaves and clusters.

The target for leaf removal was 80% cluster exposure in the fruiting zone by visual assessment in both years. Each row was mechanically leaf plucked using a Collard E2200 pulsed-air leaf removal machine (Wilyabrup Western Australia, Australia) at the designated stage of vine and berry development. The height was set at 850 mm from the ground and covered the fruiting zone from 850 mm-1250 mm. A second pass of both sides of LR7 and LR30 occurred when LRV was performed in 2008 to maintain bunch exposure and, remove

secondary growth in the fruit zone. However, due to partial cane removal and hail damage to the east side of the rows, only the west side of the rows of treatments LR7 and LR30 required a second pass when LRV was performed in 2009.

Crop thinning occurred as part of standard commercial yield management at veraison and all rows were cluster thinned in 2008 with a target yield of 2.4 kg of fruit per vine to remove slow ripening clusters. Clusters that were green or overlapped another were removed to remove less ripe clusters and reduce the chance of botrytis infection, respectively. Due to partial cane removal and hail damage in 2009 this practice was not conducted in the second year of the study. All pest and disease control was carried out in accordance with Sustainable Winegrowing New Zealand recommendations (www.nzwine.com/swnz).

Point Quadrat

Point Quadrat analysis was completed according to Smart and Robinson (1991) on all monitor vines in each treatment row. Insertions were made every 20 cm and all leaf and cluster contacts recorded to determine mean leaf layer number (LLN), percent of exterior clusters, percent of interior clusters and percent gaps using standard techniques. Data were first collected on 10th-13th December 2007 for the 30 monitor vines per row, but subsequent data obtained was from the first 3 m of vine canopy in each monitor bay, 7 days after each treatment was applied. Following results from 2008 Point Quadrat was carried out twice in 2009 after LR7 and LRV was performed.

Total leaf area and leaf area: crop weight ratio

Mean leaf area was determined using a destructive method in which leaves were removed from two 1.0 m sections of the treatment row, post-veraison, but three weeks prior to harvest. Leaves were removed from part of a vine that looked similar to the monitor vines to ensure total vine defoliation did not adversely affect growth in subsequent seasons. A 10% sub sample of the total leaf weight per meter of leaves was removed, their area determined with a LI-COR 3100 (LI-COR Inc, Lincoln, Nebraska, USA) leaf area meter, and the leaf area per weight of leaf value used to estimate the total area per meter of row (Johnson and Pierce 2004). Yield data to calculate the leaf area to crop weight ratio was obtained by weighing the fruit per vine in each monitor bay in the vineyard at final harvest and adjusting the values to the metre of row equivalent.

Berry sampling, harvest, and fruit composition

Total soluble solids (as Brix) were measured by digital refractometer (Model PR-100, Atago Co. Ltd, Tokyo, Japan). For six weeks prior to harvest, 100 randomly selected berries per row were sampled from the monitor vines every week for juice Brix, pH using a pH meter (Orion Model 21A, Thermo Fischer Scientific, Waltham, Massachusetts, USA) and titratable acidity by titration using 0.10 M NaOH (BDH/VWR International Ltd, Leicestershire, UK) to an end point of pH 8.2. At harvest, 20 randomly selected clusters from the monitor bays in each row were used for analyses. Fruit was weighed on digital platform scales (Model UWE AFS, GEC Avery Ltd, Walsall, West Midlands, UK) to calculate mean cluster weight. Berries were removed ($n = 20$) from clusters, counted and weighed to obtain mean berry weight on electronic scales (Model BL150, Sartorius, Goettingen Germany). The hand harvested fruit was picked on the same day, 20th April 2008, at 22-23 Brix and earlier the following year on 5th April 2009 at 25 Brix. The 20 clusters per row collected at harvest were stored at -20 °C until seed analysis was performed. The frozen skins and pulp from 200 randomly selected berries from the frozen clusters were removed with a scalpel and discarded. The seeds were separated, counted, weighed, and then re-weighted after being in an oven at 80 °C for 24 hours. The weights were expressed on a seed and per berry basis.

Statistical analyses

One and two - way ANOVA and Tukey's test for significant difference was carried out using GenStat 11, VSNi, Hemel Hempstead, UK.

Results

Point Quadrat analysis

The percentage of exterior clusters in 2008 increased as expected, post treatment application, with NLR possessing the least number of exposed clusters (Figure 1). The opposite pattern was observed for interior clusters (data not shown), leaf layer number (LLN) and gaps in all treatments which decreased after each treatment was applied. This established pattern was the basis for the decision in 2009 to gather Point Quadrat data twice, once after LR7 and again after LRV.

The final Point Quadrat data were obtained in both years one week post veraison and is summarized in Table 1. LLN substantially decreased in NLR in 2008-2009 compared to 2007-2008. LLN increased in the leaf removal treatments in the second year, but were not

statistically significantly different although there was a statistically significant difference with NLR ($P < 0.05$) compared to the defoliated vines. However, it should be noted that the second pass of leaf removal at veraison for LR7 and LR30 resulted in a further, but small, decrease in LLN (2008-2009 data not shown).

There was no statistical difference ($P > 0.05$) in cluster exposure between the defoliation treatments. It should be noted that although the aim was for 80% cluster exposure by visual assessment, Point Quadrat measurements revealed this was only achieved in 2009. The reduced foliage from partial cane removal and hail damage is evident in 2009 compared to 2008 from the higher percent of gaps ($P < 0.001$). In both years NLR had the lowest percentage gaps with LR30 possessing the highest value. LR7 retained the highest proportion of exterior clusters throughout the growing season (Figure 1).

Total leaf area and leaf area: crop weight ratio.

Several weeks from harvest in 2008 NLR and LRV had the highest leaf area per meter of row (Table 2). LR30 had the least leaf area and the fewest leaves per meter of row. Leaf area decreased across all treatments in 2009 compared with 2008 due to partial cane removal and hail damage. In 2009 the leaf area per meter of row was smaller yet leaf number per meter of row showed a statistically significant increase ($P < 0.05$) using two-way ANOVA with leaf removal and year as factors. This resulted from a smaller (almost 50%) mean leaf size. Both NLR and LR7 had a high leaf area per gram of fruit of 16 cm²/g and 14 cm²/g, respectively, in 2008 and 16 cm²/g and 18 cm²/g, respectively, in 2009. However, leaf area per gram of fruit (cm²/g) showed no statistically significant difference between treatments in either year. Cluster thinning was carried out in 2007-2008 (Figure 2) but due to partial cane removal it was not repeated in 2008-2009. Yield per vine decreased across all treatments in 2008-2009 compared to the previous season as illustrated in Figure 3.

Berry composition

Berry weight was affected by early leaf removal in both years ($P < 0.05$) (Figure 4). LR30 had the smallest mean berry weight in 2008 and LR7 the smallest in 2009. Additional data collected in 2009 showed no significant difference between average number of seeds per berry, the percent of seeded berries, clusters per vine, cluster weight or berries per cluster (Tables 3 and 4). In 2008-2009 all treatments decreased in cluster weight and yield per vine compared to the previous year, suggesting partial cane removal and hail damage decreased crop weight or

inflorescences were smaller and/or poor fruit set occurred. There was also no difference for titratable acidity (TA g/L), Brix or pH during ripening between treatments in either year as detailed in Table 5, but fruit was picked earlier and at a higher Brix level in 2009.

Discussion

Grapevine growth

Point Quadrat analyses show that leaf removal decreased LLN from 3.4 in 2007-2008 to 1.0 and 1.9 to 1.3 in 2008-2009. Smart and Robinson (1991) state a LLN value of 1-1.5 is optimum for berry ripening. Leaf area decreased from 2007-2008 to 2008-2009, most likely the effect of partial cane removal and hail damage. Leaf area was highest for NLR and LRV and lower in LR7 and LR30 (Table 2). Intrieri et al. (2008) observed no difference in the leaf area of non-defoliated and manually defoliated Sangiovese vines at harvest. Leaves in the study were removed at fruitset when ovary diameter was 2-4 mm, which was later than LR7 in our study (berries 2 mm in diameter). These results suggest that early mechanical leaf removal, when berries are less than 4 mm in diameter, encourages vegetative re-growth. Petrie et al. (2000a) did not observe increased leaf size or leaf growth in pot grown Pinot noir but berries were pea size when leaf removal occurred in line with LR30 in this study. LR7 maintained leaf remnants from early defoliation that were included in the leaf area measurements and could have contributed to high leaf area results. Personal observation was that vines with no leaf removal had canopies that were too dense.

The suggested optimum value for percent interior clusters is 40% and that for percent gaps is 20% to 40% (Smart and Robinson 1991). The quantity of canopy gaps was low in 2008 across all treatments but in 2009, LR30 was within the recommended range at 34%, while LR7 and LRV were just below the optimum value. The increase in gaps in the second year can be attributed to partial cane removal and hail damage. NLR maintained the highest percent interior clusters in both seasons compared to the other treatments (Table 1) which were less than half the recommended levels.

Leaf area to fruit weight

The increase in leaf area to fruit weight ratio of defoliated vines between 2008 and 2009 indicates that the temporary source limitation caused by leaf removal was offset by possible lateral re-growth (Poni et al. 2008, Intrieri et al. 2008). In the present study, an increase in node number, and consequently leaf number could have occurred which would explain the

lack of statistical difference of leaf number per meter of row. Previous research has shown an increase in leaf number occurred on fruitless Pinot noir vines after defoliation as a result of an increase in node number (Petrie et al. 2000b). Reviews demonstrate that between 7 and 15 cm² of leaf area is required to ripen one gram of fruit, but optimum leaf area to crop weight is varietal and climate dependent (Petrie et al. 2000b). In 2008, LR30 and LRV were within the recommended range as they both retained 8 cm² of leaf area per gram of fruit. The four cane VSP trellis system may have contributed to the relatively high leaf area due to the high shoot and leaf number compared to two cane systems. In a study of trellis systems, the Scott Henry four cane trellis system was shown to result in the highest leaf area/crop weight ratio with a value of 15.9 cm²/g (Kliewer and Dookoozlian 2005).

Yield components

Yield per vine

The variability of cluster thinning in 2007-2008 amongst treatments undoubtedly impacted on the final harvest yield per vine as more clusters per vine were removed from LR7 than the other treatments (Figure 3). Additionally, the reduced yield in 2008 of LR7, although not statistically significant, compared to NLR, LR30 and LRV, is also in agreement with the studies using manual leaf removal pre-bloom by Poni et al. (2006) with pot grown Trebbiano and Sangiovese in Italy. Furthermore, Hunter et al. (2004) with Sauvignon blanc in South Africa performed leaf removal at berry set and pea size, but combined this with suckering, shoot positioning and topping and found no statistical difference amongst treatment yields, which is in agreement with our 2009 results. In contrast, Intrieri et al. (2008) with Sangiovese in Italy performed basal defoliation when separate flower buttons were present on field grown vines in Italy. This study found that the early leaf removal decreased yield compared to the non-defoliated vines. Similarly, Vasconcelos and Castagnoli (2000) observed reduced fruit yield in Pinot noir which received manual leaf removal four weeks post bloom (LR30 in our study) in Oregon. Differences reported in studies regarding consequences to crop yield from early leaf removal could therefore be attributed to canopy differences as warm climate varieties require more shade and leaves to ripen than cool climates and to varietal variants.

Cluster weight

The lack of statistical difference amongst average cluster weights from non-defoliated vines compared to the defoliated treatments (Table 3) is in agreement with artificially shaded and exposed Pinot noir clusters in the study by Cortell and Kennedy (2006). Price et al. (1995)

found that exposed and naturally shaded Pinot noir berries had similar cluster weights, but the moderately exposed clusters were the heaviest. The lower weight of naturally shaded clusters in that study was primarily due to fewer berries per cluster compared to exposed clusters, which also had smaller berries. Additionally, a study on early leaf removal (four weeks post bloom) on New Zealand Chardonnay reported no impact on cluster weights (Bennett et al. 2005). In contrast to these results, non-defoliated pot grown Sangiovese, and field grown Barbera, Croatina, Malvasia di Candia and Sangiovese clusters had higher cluster weights than defoliated ones (Poni et al. 2006, Bavaresco et al. 2008, Intrieri et al. 2008). These contradictory results regarding cluster weights could be attributed to climate, variety, trellis system or a combination of all of these. Whilst early fruit exposure did not affect cluster weights, there were differences in berry weight (see below) indicating that another yield component i.e. berry skin weight or rachis weight was responsible for the similar cluster weights

Berries per cluster and cluster number per vine data were not collected in 2008, but no significant difference ($P > 0.05$) in 2009 was observed. This indicates that early mechanical leaf removal did not damage the inflorescences post flowering.

Berry weight

Berry weight at harvest in 2008 was highest in NLR and LR7 ($P < 0.01$) and lowest in LR30. However, in 2009 berry weight was similar for NLR, LR30 and LRV and the lowest was LR7 ($P < 0.01$). These results are in agreement with the study by Koblet et al. (1994) who reported smaller Pinot noir berry weights when manual leaf removal occurred when berries were pea size. Non-defoliated pot grown Trebbiano fruit had the highest berry weights compared to the early manual leaf removal crop indicating that climate and varietal variations are responsible for these contradictory results (Poni et al. 2006).

A decrease in berry size following leaf removal is likely to result from two sets of factors, lower cell numbers within each berry and a reduction in the final sizes of these cells (Petrie et al. 2000a). Ollat and Gaudillere (1998) and Keller (2009) reported that berry growth after flowering is highly dependent on assimilate supply. The removal of leaves early in the first stage of berry growth may have disrupted cell division and growth due to a reduction in assimilates. Keller (2009) explains that Hale and Buttrose (1974) state that environmental factors, especially heat stress seem to restrict berry size if it occurs before the lag phase of

growth. Sun exposed berries are heated by the incoming radiation and the temperature effect implies that in warm climates and possibly microclimates, early leaf removal can potentially limit berry size (Spayd et al. 2002, Keller 2009). Small berry size has important implications for winemaking as smaller berries increase the skin to pulp ratio.

Seed number and weight

No statistical significance was found between seed number, weight or the percent seeded berries between treatments in 2009 (Table 3) in agreement with Ebadi et al. (1996). Neither Chardonnay nor Shiraz vines grown in pots in growth chambers differed in seed number when light was reduced by 8%, 40% and 72% (Ebadi et al. 1996). However, these results are in contrast to Ristic et al. (2007) who reported reduced seed weight and seed number in exposed Shiraz fruit compared to artificially shaded fruit. Poni et al. (2006) performed early manual defoliation and also observed reduced seed weight, seed number and berry weight in pot-grown Sangiovese berries, but not Trebbiano, suggesting these results can be dependent upon variety. Hardie and Aggenbach (1996) state that seed number per berry is influenced by factors impinging on events related to pollen and ovule development and fertilization, such as low temperatures at flowering. However, the intensity of berry cell division is positively related to the number of seeds per berry, probably due to hormones synthesized in the seeds (Coombe 1960, Ristic and Iland 2005). Therefore reductions in assimilate supply early in berry growth in 2008-2009 did not affect seed growth in the present study.

Berry composition

The timing of leaf removal in our study had no effect on berry ripening parameters and fruit from each treatment did not differ in pH, TA (g/L) or Brix in either year. There was vintage variation as Brix levels were higher and TA (g/L) was lower in 2008-2009 than in 2007-2008, possibly due to decreased crop per vine and increased fruit exposure. Artificially shaded Pinot noir in a study by Cortell and Kennedy (2006) resulted in lower pH and lower TA (g/L), the latter being statistically significant, but Brix levels were similar to exposed fruit. Price et al. (1995) found that exposed Pinot noir berries had the highest Brix and lowest acidity, but there was no difference in pH compared to moderately exposed and naturally shaded fruit. The manual removal of four basal leaves four weeks post bloom reduced Brix in Pinot noir with statistically significant results (Vasconcelos and Castagnoli 2000). Merlot berries manually exposed at veraison had higher sugars compared to artificially shaded fruit in the study by Pereira et al. (2006). Early-season carbon supply limitations, whether imposed

by environmental stress or by cultural practices such as leaf removal may restrict berry size and/or number, but they do not usually impair berry ripening (Keller 2009), which has been clearly demonstrated in our study. However, early leaf removal affected berry weight in 2008 and 2009. It can be deduced from all these results that ripeness parameters at harvest from leaf removal trials vary with climate, variety, clone, trellis system, method, timing and severity of defoliation. In our study LR7 and LR30 were on four cane VSP systems and our Pinot noir vines experienced compensatory re-growth but the leaf area per gram of fruit (cm^2/g) was sufficient to ripen the fruit in this cool climate. That no sun burnt fruit was observed visually in either year suggests that grapes adapted to the UV radiation and temperature changes, quite possibly from increased levels of UV protectants such as phenolics (Price et al. 1995) (Chapter 5). New Zealand is exposed to high incidence of UV radiation in the growing season and further research is required to ascertain the effects of early mechanical leaf removal under these conditions on Pinot noir wine flavour and aroma.

Conclusion

Early mechanical leaf removal seven days after flowering (LR7) promoted vegetative re-growth and resulted in smaller berry weights in 2009 compared to later defoliation and no defoliation. Yield, seed weight, seed numbers, cluster weight, berries per cluster and ripeness parameters were not affected. Results indicate that early mechanical leaf removal could be suitable for high yielding Pinot noir vines. It reduced yield in 2008 and reduced berry size in 2009 which has implications for winemaking as investigated in Chapter 5. It has been emphasized that defoliation at or very near flowering should be avoided in low vigour vines due to yield and berry size reduction (Poni et al. 2006), as noted in this study. However, leaf removal results depend upon climate, grape variety, clone and trellis system, all of which affect the sunlight and temperature within the grapevine canopy.

Acknowledgements.

The authors wish to thank Lincoln University and New Zealand Winegrowers for financial assistance and Pegasus Bay Winery for assistance in kind. Grateful thanks are extended to Jocelyne King, Regon Hung, Olivia Ross, Elizabeth Tomasino, Emma Rice, Wade Sowman, Nicky Wakeford, Drew Kuchta, Greg Miller, Ryan Collins and Gilbert Wells for their assistance with vineyard data collection.

References

- Bavaresco, L., Gatti, M., Pezzutto, S.M., Fregoni, and F. Mattivi. 2008. Effect of leaf removal on grape yield, berry composition and stilbene concentration. *Am. J. Enol. Vitic.* 59:292–298.
- Bennett, J., Jarvis, P., Creasy, G.L. and M. Trought. 2005. Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. *Am. J. Enol. Vitic.* 56:4:386–393.
- Bledsoe, A., Kliewer, W., and J. Marois. 1988. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. *Am. J. Enol. Vitic.* 39:49–54.
- Coombe, B.G. 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. *Plant Physiol.* 35:241–250.
- Cortell, J. and J. Kennedy. 2006. Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* 54:8510–8520.
- Dokoozlian, N. K., and W.M. Kliewer. 1995. The light environment with grapevine canopies. I. Description and seasonal changes during fruit development. *Am. J. Enol. Vitic.* 46: 2:209-218.
- Ebadi, A., May, P. and Coombe, B. 1996. Effect of short-term temperature and shading on fruit-set, seed and berry development in model vines of *Vitis vinifera*, cvs Chardonnay and Shiraz. *Aust. J. Grape Wine Res.* 2:1-8.
- Gubler, W.L. Bettiga, and D. Heil. 1991. Comparisons of hand and machine leaf removal for *Botrytis* bunch rot. *Am. J. Enol. Vitic.* 42: 233-236.
- Hardie, W.J., and S.J. Aggenbach. 1996. Effects of site, season and viticultural practices on grape seed development. *Aust. J. Grape Wine Res.* 2:21-24.
- Hunter, J.J. 2000. Implications of seasonal canopy management and growth compensation in grapevine. *S. Afr. J. Enol. Vitic.* 21:2:81-91.
- Hunter, J. and H. Visser. 1990. The effect of defoliation on growth characteristics of *Vitis vinifera* L cv. Cabernet Sauvignon II. Reproductive growth. *S. Afr. J. Enol. Vitic.* 11:26-32.
- Hunter, J., Volscheck, C, G., J. Marais, J, J. and G.W. Fouche. 2004. Composition of Sauvignon blanc grapes as affected by pre-veraison canopy manipulation and ripeness level. *S. Afr. J. Vitic.* 25:13–17.
- Intrieri, C., Filippetti, I., Allegro, G.M. Centinari, and S. Poni. 2008. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 14:25-32.

- Johnson, L.F, and L.L. Pierce. 2004. Indirect measurement of leaf area index in California North Coast vineyards. *HortSci*. 39:2:236-238.
- Keller, M. 2009. Managing grapevines to optimize fruit development in a challenging environment: a climate change primer for viticulturists. *Aust. J. Grape Wine Res.* 16:56- 69.
- Koblet, W., Candolfi-Vasconcelos, M.C. Zweifel, W, and G.S. Howell. 1994. Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot noir grapevines. *Am. J. Enol. Vitic.* 45:181-187.
- Kliewer, W.M, and N.K. Dokoozlian. 2005. Leaf area/crop weight ratios of grapevines: Influence of fruit composition and wine quality. *Am. J. Enol. Vitic.* 56:2:170–180.
- Lopes, C, and P.A. Pinto. 2005. Easy and accurate estimation of grapevines leaf area with simple mathematical models. *Vitis*. 44:2:55-61.
- Morrison, J, and A. Noble. 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes on fruit and wine sensory properties. *Am. J. Enol. Vitic.* 41:193-200.
- Ollat, N, and J. Gaudillere. 1998. The effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* 49:251-258.
- Percival, D.C. Fisher, K.H, and J.A. Sullivan. 1994. Use of fruit zone leaf removal with *Vitis vinifera* L. cv. Riesling grapevines. II. Effect on fruit composition, yield, and occurrence of bunch rot (*Botrytis cinerea* Pers: Fr.). *Am. J. Enol. Vitic.* 45:2:133-140.
- Pereira, G., Gaudillere, J-P., Pieri, P., Hilbert, G., Maucourt, M., Deborde, C. A. Moing, and Rolin. D. 2006. Microclimate influence on mineral and metabolic profiles of grapeberries. *J. Agric. Food Chem.* 54:6765-6775.
- Petrie, P.R. Trought, M.C.T. and G.S. Howell. 2000a. Fruit composition and ripening of Pinot noir (*Vitis vinifera* L.) in relation to leaf area. *Aust. J. Grape Wine Res.* 6:46-51.
- Petrie, P.R. Trought, M.C.T. and G.S. Howell. 2000b. Growth and dry matter partitioning of Pinot noir (*Vitis vinifera* L.) in relation to leaf area and crop load. *Aust. J. Grape Wine Res.* 6:40-45.
- Poni, S., Casalini, L., Bernizzoni, F.S. Civardi, and C, Intrieri. 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am. J. Enol. Vitic.* 57:397-407.
- Poni, S., Bernizzoni, F.S. Civardi, and N, Libelli. 2009. Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. *Aust. J. Grape Wine Res.* 15:185–193.
- Price, S., Breen, P. Valladao, M, and B, Watson. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* 46:2:187-194.

Ristic, R., Downey, M., Iland, P., Bindon, K., Francis, L., Herderich, M., and Robinson, S. 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Aust. J. Grape Wine Res.* 13:53-65.

Ristic, R., and P, Iland. 2005. Relationships between seed and berry development of *Vitis vinifera* L. cv Shiraz: developmental changes in seed morphology and phenolic composition. *Aust. J. Grape Wine Res.* 11:43–58.

Spayd, S.E. Tarara, J.M. Mee, D.L. and J.C, Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53:171-182.

Smart, R., and M, Robinson, 1991. *Sunlight into Wine*. Winetitles Ltd, Ashford. South Australia.

Smart, R. 1985. Principles of grapevine canopy microclimate manipulation with implications for yield and quality. *Am. J. Enol. Vitic.* 36:3:230-239.

Smith, S, and C, Codrington. 1988. Viticultural and oenological implications of leaf removal for New Zealand vineyards. *In: Proc. Sec. Intl. Symp. Cool Climate Vitic. Oenol*, Auckland, New Zealand. R. Smart, R. Thornton, S. Rodriguez, J. Young (Eds). Pp 127-133.

Strong, D, and Azarenko, A. 2000. Relationship between trunk cross-sectional area, harvest index, total tree dry weight and yield components of "Starkspur Supreme Delicious" apple trees. *J. Amer. Pomol. Soc.* 51:1:22-27.

Vasconcelos, S.C. and S, Castagnoli. 2000. Leaf canopy structure and vine performance. *Am. J. Enol. Vitic.* 51:4:390–396.

Table 1. Canopy characteristics using data from Point Quadrat analysis obtained one week post veraison in the 2007-2008 and 2008-2009 seasons. Statistical significance ($P < 0.05$) between leaf removal treatments (i.e. within each column) using Tukey's procedure are indicated by different letters.

Treatment	LLN		Exterior clusters (%)		Interior clusters (%)		Gaps (%)	
	2008	2009	2008	2009	2008	2009	2008	2009
NLR	3.4 b	1.9 b	31 b	58 b	69 b	42 b	0.05 a	4 a
LR7	1.0 a	1.3 a	63 a	87 a	37 a	13 a	0.1 b	18 b
LR30	1.0 a	1.3 a	61 a	82 a	39 a	18 a	0.4 c	34 c
LRV	1.0 a	1.3 a	71 a	83 a	29 a	17 a	0.2 b	17 b
Sig	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 2. Average leaf area per meter row, leaf size, leaf number and leaf area to fruit weight ratio in 2007-2008 and 2008-2009. Statistical significance between treatments (i.e. within each column) using Tukey's procedure are indicated by different letters.

Treatment	Leaf area (cm ² m/row)		Average leaf size (cm ²)		Leaf Number (m/row)		Leaf:fruit ratio (cm ² /g)	
	2008	2009	2008	2009	2008	2009	2008	2009
NLR	5302 b	3388	98 b	45	54	78	16	16
LR7	4455 ab	3130	86 ab	40	53	79	14	18
LR30	3542 a	3204	70 a	38	47	85	8	15
LRV	5302 b	3408	80 ab	38	50	91	8	16

Table 3. Mean seed number, mean seed weight and the percent of seeded berries in all treatments in 2009. (ns = not significant)

Treatment	Mean seed no. /berry	Mean seed wt (g)	Seeded berries (%)
NLR	1.6	0.1154	98
LR7	1.2	0.0842	87
LR30	1.5	0.0787	73
LRV	1.5	0.1383	88
Sig	ns	ns	ns

Table 4. Clusters per vine, berries per cluster and cluster weight, for all treatments.
(ns = not significant)

Treatment	Clusters/vine	Berries/cluster	Cluster wt (g)	Cluster wt (g)
	2009	2009	2008	2009
NLR	29 a	81 a	105 a	89 a
LR7	29 a	81 a	93 a	84 a
LR30	26 a	80 a	98 a	77 a
LRV	26 a	73 a	103 a	93 a
Sig	ns	ns	ns	ns

Table 5. The effect of leaf removal treatment on berry ripeness parameters in 2008 and 2009. A statistically significant difference was observed for Brix between years ($P < 0.01$) but not pH or TA g/L.

Treatment	Brix		TA (g/L)		pH	
	2008	2009	2008	2009	2008	2009
NLR	22.4	24.2	9.8	8.3	3.11	3.11
LR7	22.7	24.5	10.7	8.3	3.14	3.06
LR30	22.8	24.7	10.0	8.8	3.20	3.12
LRV	23.0	24.5	9.6	8.3	3.19	3.11
Sig	ns	ns	ns	ns	ns	ns

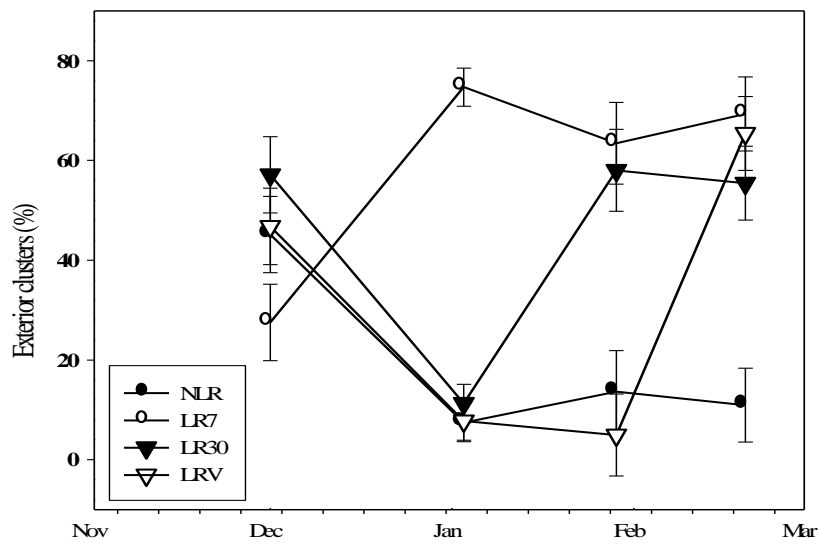


Figure 1. Percentage of exposed clusters per treatment measured on four occasions during the 2007-2008 growing season with bars representing standard error of the means.

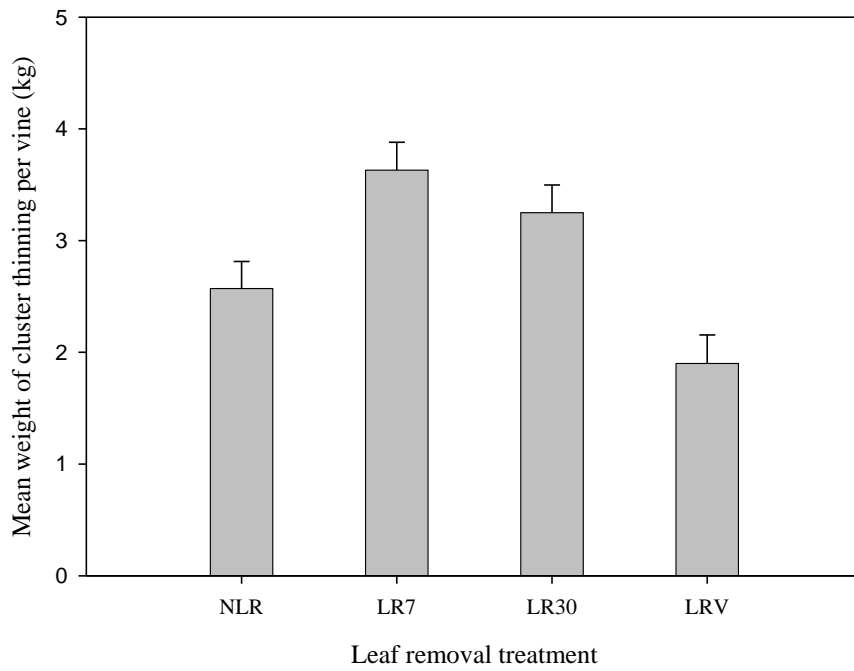


Figure 2. Fruit weight per vine removed at cluster thinning in 2007-2008. Bars represent standard error of the means.

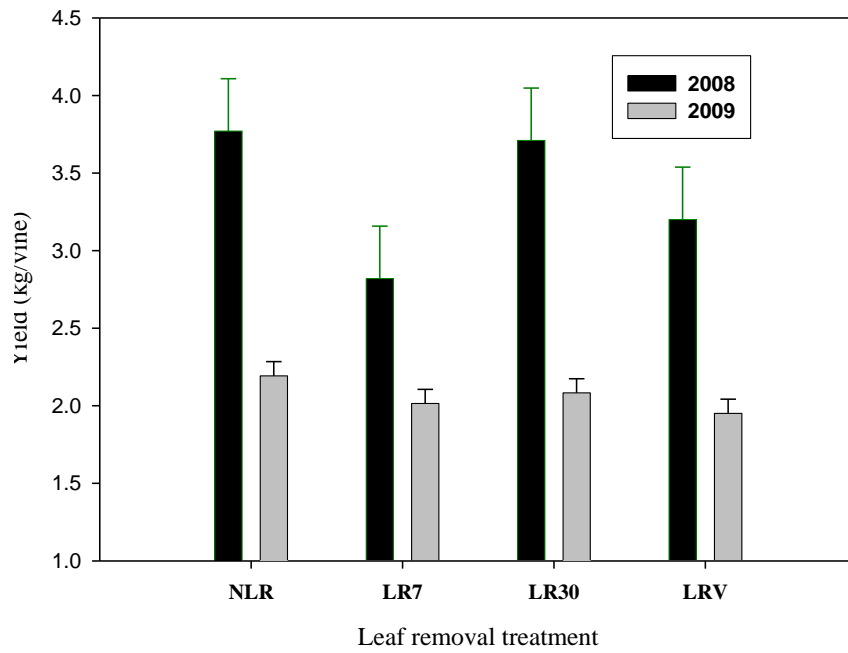


Figure 3. The affect of timing of leaf removal on yield per vine for 2007-2008 and 2008-2009. Bars represent the standard error of the means. There is a statistically significant difference between years ($P < 0.05$).

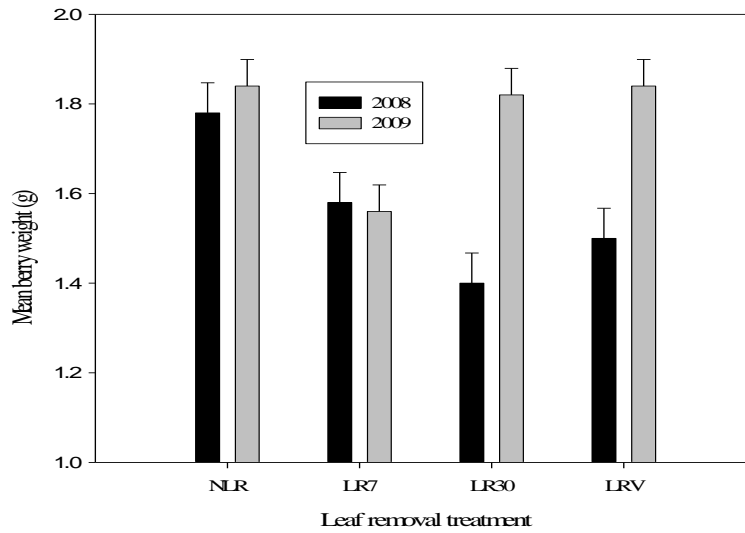


Figure 4. Mean berry weight at harvest. Bars represent standard error of the means. Significant differences were observed within the 2007-2008 ($P < 0.01$) and 2008-2009 ($P < 0.005$) seasons, but not between years.

Chapter 4

(A preparation for submission to *Journal International des Science de la Vigne et du Vin*)

Effect of protein-tannin ratio and tannin concentration on the bovine serum albumin (BSA) -based precipitation method.

B.S. Kemp^a, R. Harrison^{*a} and R. N. Hider^a

^a Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.

* Corresponding author: fax: + 64 3 325 3850. Mail address: Centre for Viticulture and Oenology, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand. E-mail address: Roland.Harrison@lincoln.ac.nz (R. Harrison)

Short running title: Protein-tannin ratio and concentration

Abstract

Aims: The main objective of the study was to investigate the reason for the lack of precipitate occurring in red wine tannin analysis using the existing bovine albumin serum (BSA) method that measures total tannin concentrations in grapes and wine. This was achieved by altering the wine sample volume which resulted in a change in wine tannin to protein ratio.

Methods and results: Seven New Zealand red wines were assayed according to the BSA method using a range of protein (BSA) and wine concentrations achieved by varying wine dilutions and the volume of the model wine solution. Maximum precipitation was observed at lower wine/protein ratio in diluted wines and tannin precipitation increased as protein concentration increased. It was observed that the estimation of tannin concentration in red wine is a product of tannin/protein ratio and BSA concentration. Consequently, the methylcellulose (MCP) assay was performed to independently determine tannin concentration in red wines. Results indicate that tannin/protein ratio, BSA concentration and possibly tannin composition affect BSA tannin precipitation.

Conclusion: For the BSA assay there appears to be a region of low tannin/protein ratio within which lower wine tannin concentrations can be determined. Overall it is suggested that tannin precipitation is linearly related to tannin concentration.

Significance and impact of study: Results showed the limits of the BSA method for low tannin wines and the difficulty in using the method for wines with unknown tannin concentrations.

Keywords: Bovine serum albumin (BSA), methylcellulose precipitation (MCP), tannin, wine.

Introduction

According to their structure, wine tannins are divided into two classes, hydrolysable tannins which originate in oak and condensed tannins/proanthocyanidins which originate in grape skins, seeds and stalks. Proanthocyanidins, the most abundant tannins found in red wine, are formed from the polymerisation of flavan-3-ol subunits such as catechin, epicatechin, epicatechin gallate and/or epigallocatechin (Obradovic, 2005, Herderich *et al.*, 2005). Hydrolysable tannins further subdivide into two classes according to their configuration: gallotannins, which are comprised of polygalloyl esters of glucose and ellagitannins, comprised of esters of ellagic acid (Herderich *et al.*, 2005).

Methods previously utilised to analyse tannin concentration include colorimetric derivatisation, gravimetric, chromatographic, and protein and polysaccharide precipitation assays (Herderich and Smith, 2005, Smith *et al.*, 2005, Schofield *et al.*, 2001). Polymeric tannin compounds in wine are identified by their ability to complex with, and precipitate proteins. This property has been used by scientists as a method to selectively remove tannins from solution and consequently determine their concentration (Makkar, 1989). The classic method of Hagerman and Butler (1978) involves the use of bovine serum albumin (BSA) and precipitation of a protein-tannin complex, followed by its separation and dissolution. The phenolics present in the dissolved complex are determined spectrophotometrically at 510 nm by the addition of ferric chloride (Harbertson and Spayd, 2006). Grape berry skins, seeds and wine tannins have been investigated using this method (Seddon and Downey, 2008, Harbertson *et al.*, 2002, Harbertson *et al.*, 2008, Kennedy *et al.*, 2006). A modified version has been used to estimate long and short chain polymeric pigments in grapes and wines (Harbertson *et al.*, 2003).

Tannin-protein associations are thought to entail cross-linking of separate protein molecules by tannin that acts as a polydentate ligand on the protein surface involving hydrophobic effects and hydrogen bonds (Carvalho *et al.*, 2004). The interaction with globular proteins such as bovine serum albumin involves only the surface exposed amino acid residues, whereas proline rich linear proteins (which represent 70-80% of human salivary protein) involve face-to-face stacking with amino acid residues (Carvalho *et al.*, 2004, Deaville *et al.*, 2007). Additionally, precipitation by the two tannin classes differ as it is thought that hydrolysable tannins form a hydrophobic coating on the surface of the protein while condensed tannins form hydrogen-bonded cross-links between protein molecules (Harbertson

and Spayd, 2006, Hagerman *et al.*, 1998). Furthermore, Deaville *et al.*, (2007) reported that gallotannins bind more strongly to BSA than ellagitannins due to the aromatic rings in the hydroxydiphenyl groups of ellagitannins that are inhibited by intramolecular biphenyl linkages. Binding of polyphenols (proanthocyanidins) to protein depends on the number and location of hydroxyl groups on the aromatic ring (monophenols << meta-diphenols << ortho-diphenols < vicinal triphenols) and molecular size (Siebert, 1999).

A recent investigation of the influence of sample dilution on the reliability of tannin analysis by protein precipitation concluded that tannin concentrations of both diluted and concentrated samples were systematically underestimated (Jensen *et al.*, 2008) explained by a precipitation threshold and insufficient protein for precipitation, respectively. More recently, Brooks *et al.*, (2008) presented data which indicated that one implementation of the BSA tannin precipitation assay (Harbertson *et al.*, 2002) does not meet criteria for acceptable precision and recovery. Other studies have indicated that all protein precipitation assays are potentially compromised by their inability to measure the tannin precipitated directly, requiring a subsequent colorimetric (or other) assay (Sarneckis *et al.*, 2006). Consequently, an alternative assay based on precipitation of tannins with methylcellulose has been used in a number of studies (Seddon and Downey, 2008, Sarneckis *et al.*, 2006, Mercurio and Smith, 2008).

The purpose of this study was to investigate BSA-tannin precipitation from wines. We were prompted to undertake this research prior to analysis of wines from viticultural field trials of which, it was predicted, several would have low to medium tannin concentrations. Furthermore, observations in our laboratory noted that variable quantities of precipitate (including no precipitate) could be produced from some undiluted wines.

The study was purely an investigation into the use of an existing and widely-used protein precipitation assay to assess the effect of dilution on low to medium tannin wines, and also to ascertain whether the previously reported threshold effect might be overcome by the simple expedient of increasing the quantity of wine tannin relative to BSA. However, a number of factors have been found to influence protein-polyphenol interaction including: type of protein, protein concentration, tannin concentration, tannin size, degree of galloylation, pH, alcohol concentration and the ionic strength of the medium (Siebert *et al.*, 1996). Because a simple adaptation of an existing analytical method was sought, kinetic parameters such as reaction temperature and reaction time were not studied, and other factors, specifically final

pH and ethanol concentration, were not held constant. Nevertheless, Prigent *et al.*, (2009) found no effect of ionic strength ($I = 0.023$ to 0.087) on the solubility α -lactalbumin (a globular protein) in the presence of proanthocyanidins even after an extended incubation period (3 days). Additionally, temperature in the same study only had an effect at $40\text{ }^{\circ}\text{C}$ so our analysis was carried out at room temperature according to Harbertson *et al.*, (2002) although Jensen *et al.*, (2008) extended the incubation time from 10 to 30 minutes for dilution experiments. With regard to alcohol concentration, Serafini *et al.*, (1997) found that ethanol in the range 0-22 % proportionally reduced tannin precipitation from red wine after BSA addition, although significant differences versus alcohol-free wine were only observed at higher concentrations ($\geq 11\%$). Similarly, Siebert *et al.*, (1996) found that the effect of alcohol concentration on haze induced in apple juice with tannin acid (hydrolysable tannin) was relatively small. Hagerman and Butler (1978) found that BSA was significantly precipitated by condensed tannins when the pH of the mixture was between 3.0 and 5.0, although they recommended a pH range of 4 to 5 for maximum precipitation. Similarly, De Freitas and Mateus (2001) found that procyanidin oligomers bind extensively to BSA at around pH 4.5.

Materials and Methods

1. Wine samples

Seven New Zealand red wines were selected to obtain a broad range of possible tannin concentrations. These were (alcohol concentration in parentheses): Corbans Private Bin Syrah 2004 (13.8 %), Crossroads Pinot noir 2004 (13.0 %), Muddy Water Pinot noir 2004 (14.1 %), Red Rock Merlot Malbec 2006 (14.0 %), Saint Clair Pinot noir 2006 (13.5 %), Te Mata Gamay noir 2007 (13.0 %) and Vidals Cabernet Sauvignon 2002 (14.0 %).

2. Chemicals

Bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), triethanolamine (TEA), ferric chloride, methyl cellulose solution, (+)-catechin and (-)-epicatechin and ammonium sulphate were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). All chemicals were analytical grade.

3. BSA precipitation assay

Wines were assayed for tannins according to Harbertson *et al.*, (2002) except that a range of protein (BSA) and wine concentrations were used (Table 1). Samples were centrifuged using

a Heraeus Sepatech Biofuge15 at 17,300 g for 5 minutes. Absorbance was measured at 510 nm using a Unicam Helios Alpha Spectrometer. Distilled water was used as the blank. By varying the amounts and concentration of BSA and the amount and dilution of wine samples, it was possible to prepare mixtures ranging in wine/protein ratio (0.031, 0.063, 0.125, 0.250 and 0.500 mL mg⁻¹ BSA) for different final BSA concentrations (0.40, 0.67, 1.00, 1.33, 1.60 and 1.80 mg BSA mL⁻¹). The amounts and concentrations in each mixture (1.5 mL total volume) are given in Table 1. BSA stock solutions were prepared in a buffer consisting of 0.20 M acetic acid and 0.17 M NaCl, adjusted to pH 4.9. Wine samples were diluted as appropriate in a model wine consisting of 5 g L⁻¹ potassium bitartrate in 12 % v/v ethanol and adjusted to pH 3.3. Thus, the row labelled 0.67 mg mL⁻¹ BSA (Table 1) corresponds to a wine dilution series utilising standard conditions from Harbertson *et al.*, (2002). Results from such a dilution series have recently been reported by Jensen *et al.*, (2008). In our study, results for dilution series at other BSA concentrations were achieved although this entailed varying the volume of the pH 4.9 buffer solution which was combined with the (appropriately diluted) wine; i.e. from 1.20 to 0.15 mL (in a total volume of 1.5 mL) for BSA concentrations of 0.4 and 1.8 mg mL⁻¹, respectively. The pH levels of the solutions (Table 1) were found to be in the recommended range (4.2 to 4.9) for protein precipitation. Ethanol concentrations in the final mixtures varied from *c.* 2.4 % (depending on the wine) for the row labelled 0.40 mg mL⁻¹ BSA (Table 1) to *c.* 10.8 % for the row labelled 1.80 mg mL⁻¹ BSA.

4. Methylcellulose precipitation (MCP) assay

The MCP assay was performed according to Mercurio *et al.*, (2007) on five of the seven wines (the Crossroads Pinot noir 2004 and Muddy Water Pinot noir 2004 were not analysed). MCP analyses were carried out twice in triplicate.

5. Statistical analyses

A variable number of replicates (up to 6) were used in the determination of tannin by the BSA precipitation assay. Consequently, average standard errors were calculated as the harmonic mean of the pooled variance from replicate analyses using Excel 2003, Windows XP, Microsoft, USA. Graphs were produced using SigmaPlot version 2002, Windows version 8.02 (SPSS Inc, Cranes Software International Pty Ltd, Melbourne, Australia).

Results and Discussion

Data showing the effect of wine/protein ratio and the apparent effect of BSA concentration on assayed tannin concentration for one of the wines (Corbans Private Bin Syrah 2004) are presented in Figure 1. Results varied from 0 to 380 mg catechin equivalents (CE) L⁻¹ wine across the full range of solution conditions. With a BSA concentration of 0.67 mg mL⁻¹, the standard condition of the Harbertson *et al.*, (2002) assay, all results ranged from 0 to 230 mg CE L⁻¹ wine. The other wines showed similar patterns: Red Rock Merlot Malbec 2006, a wine with a high tannin concentration, gave results of 0 to 860 and 250 to 680 mg CE L⁻¹, respectively; and Te Mata Gamay noir 2007, the wine with the lowest tannin concentration, gave results of 0 to 100 and 0 mg CE L⁻¹, respectively (data not shown).

The ranges in the results obtained in this study appear to be much greater than those of Jensen *et al.*, (2008) who reported underestimation of up to 27 % for their dilution series. Nevertheless, we believe that results from the two studies are compatible. It is apparent from the data of Jensen *et al.*, (2008) that estimated tannin concentrations declined most markedly at high dilution factors and that this effect was more apparent and occurred at lower dilutions for lower tannin concentration wines. The same pattern was observed in our study but wines had generally lower tannin concentrations and the maximum dilution was greater (16-fold compared to 10-fold); Figure 2 shows data for our range of wines at a BSA concentration of 0.67 mg mL⁻¹ and can be compared with Figure 2 of Jensen *et al.*, (2008). In our study, tannin concentrations determined with the standard conditions of the Harbertson *et al.*, (2002) assay were in the range 0-600 mg CE L⁻¹, similar to results reported by Mercurio and Smith (2008) for a range of Australia red wine varieties. It is interesting to note that BSA-precipitable tannin for the three wines with clearly the lowest tannin concentrations (Muddy Water Pinot noir 2004, Crossroads Pinot noir 2004 and Te Mata Gamay noir 2007) increased continually with increasing wine/protein ratio (up to 0.5, undiluted wine) in contrast to the other wines for which maximum or plateau values were obtained at lower wine/protein ratios (diluted wines). These results indicate that low tannin wines such as Pinot noir may not require dilution if the BSA method is used to analyse total tannin concentration. When the protein precipitation assay was carried out using a range of BSA concentrations at a defined wine/protein ratio, it appeared that tannin precipitation by protein increased as the concentration of protein increased although it should be noted that pH decreased and ethanol concentration increased across this series. Figure 3 shows the results obtained with the

undiluted wines (column labelled 0.5 mL wine mg⁻¹ BSA in Table 1), but equivalent data (not shown) were obtained for the range of wine dilutions investigated.

It is clear that the wine/protein ratio, along with other factors, markedly affect the precipitation of tannin from solution. Other studies, albeit involving hydrolysable tannins, have suggested that there exists a critical point for precipitation of the tannin-BSA complex from solution which is strongly dependent on the tannin/BSA ratio in the reaction solution (Silber *et al.*, 1998). Studies with condensed tannins suggest that the quantity of insoluble precipitate from the formation of complexes with protein increases rapidly up to a maximum and remains relatively constant thereafter (De Freitas and Mateus, 2002; Hagerman and Butler, 1978). Since wines contain different concentrations of tannin, these would occur at different wine/BSA ratios. In order to investigate whether our data conformed to such a model, we have plotted the quantity of tannin precipitated by BSA as a function of MCP tannin and BSA concentrations in the solution mixture (Fig. 4) for five of the wines (i.e. those assayed by both BSA and methylcellulose precipitation (MCP) methods). This allowed all the data from these wines to be plotted on a tannin concentration as opposed to wine dilution basis. Because the estimated concentration of tannin in each wine appears to be a function of a number of factors, we used MCP tannin as an independent measure of the tannin concentration.

Figure 4 indicates that the relationship between tannin precipitation and tannin/protein ratio is similar for all the wines, although there is some variation in the amount of tannin precipitated by BSA at seemingly equal MCP tannin concentrations and for different wines (Fig. 4, inset) which could be interpreted to indicate some variability in tannin/protein stoichiometry and might be related to tannin composition. A recent report regarding the validity or otherwise of the Harbertson *et al.*, (2002) assay ascribes significant inter-laboratory variation to non-stoichiometric protein-tannin precipitation (Brooks *et al.*, 2008). Recently, Mercurio and Smith (2008) found that methylcellulose complexes and precipitates all tannins and pigmented polymers observable by HPLC whereas BSA does not. Harbertson *et al.*, (2003) previously reported that dimeric and trimeric procyanidins do not complex with BSA, therefore two classes of pigments are labelled as large polymeric pigments (LLP: precipitate with BSA), and small polymeric pigments (SPP: do not precipitate with BSA) (Mercurio and Smith, 2008). This observation would suggest that the MCP assay could be better suited to low tannin wines that might have a higher proportion of SPP than other wines. Although,

Harbertson and Spayd (2006) suggested that if protein is available in extreme excess, then it can remove all phenolics rather than selectively removing polymers (tannins). It may also be that methylcellulose has a higher binding affinity to hydrolysable tannins than the globular protein, BSA, which could marginally affect the tannin concentration results in both assays. There is also some evidence (from the small but consistent increase in slope) that at higher BSA concentrations a greater proportion of the tannin in solution is precipitated (in line with Figure 3), but the increase is quite minor and certainly does not indicate dissolution of the tannin-BSA complex at high BSA concentration. In contrast to the results of Jensen *et al.*, (2008), Figure 4 shows no evidence, over the range of tannin concentrations investigated, for a situation in which tannin concentration exceeds the capacity of BSA for precipitation of that tannin (i.e. there is no maximum in precipitated tannin with increasing tannin concentration in the solution mixture). Overall, it seems that tannin precipitation from these mixtures is linearly related to tannin concentration since protein is in excess (for the range 0.40-1.80 mg BSA mL⁻¹) and the effect of BSA concentration on precipitate formation is relatively small.

It is clear from our data that both wine/protein (i.e. tannin/protein) ratio and apparent BSA concentration affect estimated wine tannin concentration. In particular, there appears to be a region at low tannin/protein ratio within which lower wine tannin concentrations are determined (Figures 1 and 2) although overall Figure 4 suggested that tannin precipitation from these mixtures is linearly related to tannin concentration. Nevertheless, it seems that when the quantity of tannin in the solution mixture is < 0.1 mg MCP tannin mL⁻¹, either no precipitate with BSA is formed or it is not possible to recover it adequately with the techniques used in this study (Fig. 4). This was exemplified by the linear regression between tannin measured by BSA and that measured by MCP (BSA tannin concentration mg catechin equivalents L⁻¹ = 0.31 × MCP tannin concentration mg epicatechin equivalents L⁻¹ - 82; R² = 0.832, P < 0.05), which is also very similar to that determined by Mercurio and Smith (2008) for a range of Australian dry red wines except for a difference in the intercepts. (That on the x-axis correspond to c. 800 mg epicatechin equivalents L⁻¹ in the Australian study and 260 mg L⁻¹ for the New Zealand wines, although should be noted that a total of forty one wines were analysed in the Mercurio and Smith (2008) study compared to only five here). These observations support the suggestion made by Jensen *et al.*, (2008) of a threshold tannin level for precipitation with BSA to occur (260 mg epicatechin equivalents L⁻¹ MCP tannin from our regression between BSA and MCP assays, or approximately 100 mg L⁻¹ from Fig. 4,

insert). Our results have shown that tannin/protein ratio (Fig. 2), BSA concentration (Fig. 3) and possibly tannin composition affect tannin precipitated by BSA (Fig. 4, insert) although overall there is a strong relationship between tannin concentration in the solution mixture and tannin precipitated by BSA.

Acknowledgements

The authors gratefully acknowledge Marie de Beauchesne (Agrocampus, Rennes, France) for analytical assistance, Richard Sedcole (Lincoln University) for statistical advice and the financial support of the Lincoln University Research Fund and the New Zealand Society of Viticulture and Oenology.

References

- BROOKS. L. MCCLOSKEY. L. MCKESSON. D. SYLVAN. M. 2008. Adams-Harbertson protein precipitation-based wine tannin method found invalid. *Journal of AOAC International*. 91, 1090-1094.
- CARVALHO. E. MATEUS. N. DE FREITAS. V. 2004. Flow nephelometric analysis of protein-tannin interactions. *Analytica Chimica Acta*. 513, 97-101.
- DEAVILLE. E. GREEN. R. MUELLER-HARVEY. I. WILLOUGHBY. I. FRAZIER. R. 2007. Hydrolysable tannin structures influence relative globular coil protein binding strengths. *Journal of Agricultural and Food Chemistry*. 55, 4554-4561.
- DE FREITAS. V. MATEUS. N. 2002. Nephelometric study of salivary protein-tannin aggregates. *Journal of the Science of Food and Agriculture*. 82, 113-119.
- HAGERMAN. A. E. BUTLER. L. G. 1978. Protein precipitation method for quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*. 26, 809-812.
- HARBERTSON. J. F. DOWNEY. M. O. 2009. Investigating differences in tannin levels determined by methylcellulose and protein precipitation. *American Journal of Enology and Viticulture*. 60, 246-249.
- HARBERTSON. J. F. HODGINS. R. E. THURSTON. L. N. SCHAFFER. L. J. REID. M. S. LANDON. J. L. ROSS. C. F. ADAMS. D. O. 2008. Variability of tannin concentration in red wines. *American Journal of Enology and Viticulture*. 59, 210-214.
- HARBERTSON. J. F. KENNEDY. J. A. ADAMS. D. O. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah and Pinot noir berries during ripening. *American Journal of Enology and Viticulture*. 53, 54-59.
- HARBERTSON. J. F. PICCIOTTO. E. A. ADAMS. D. O. 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture*. 54, 301-306.
- HARBERTSON, J. F. SPAYD. S. E. 2006. Measuring phenolics in the winery. *American Journal of Enology and Viticulture*. 57, 280-288.
- HERDERICH. M. BIRSE. M. DAMBERGS. R. HOLT. H. ILAND. P. LATTEY. K. SMITH. P. 2005. Grape and wine tannins – an overview on current research, emerging applications, and future challenges. ASVO Seminar Proceedings. Advances in Tannin and Tannin Management. Adelaide, Australia.
- JENSEN. J. S. WERGE. H. H. M. EGEBO. M. MEYER. A. S. 2008. Effect of wine dilution on the reliability of tannin analysis by protein precipitation. *American Journal of Enology and Viticulture*. 59, 103-105.

KENNEDY. J. A. FERRIER. J. HARBERTSON. J. F. PEYROT DES GACHONS. C. 2006. Analysis of tannins in red wines using multiple methods: correlation with perceived astringency. *American Journal of Enology and Viticulture*. 57, 481-485.

MAKKAR. H. P. S. 1989. Protein precipitation methods for quantification of tannins: a review. *Journal of Agricultural and Food Chemistry*. 37, 1197-1202.

MERCURIO. M. D. DAMBERGS. R. G. HERDERICH. M. J. SMITH. P. A. 2007. High throughput analysis of red wine and grape phenolics - adaptation and validation of methylcellulose precipitable tannin assay and modified Somers colour assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry*. 55, 4651-4657.

MERCURIO. M. D. SMITH. P. A. 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. *Journal of Agricultural and Food Chemistry*. 56, 5528-5537.

OBRADOVIC. D. 2005. Grape-derived tannins and their application. ASVO Seminar Proceedings. Advances in Tannin and Tannin Management. Adelaide, Australia.

PRIGENT. S. VORAGEN. A. VAN KONINGSVELD. G. BARON. A. RENARD. C. GRUPPEN. H. 2009. Interactions between globular proteins and procyanidins of different degrees of polymerization. *Journal of Dairy Science*. 92, 5843-5853

SARNECKIS. C. J. DAMBERGS. R. G. JONES. P. MERCURIO. M. HERDERICH. M. J. SMITH. P. A. 2006. Quantification of condensed tannins by precipitation with methylcellulose: development and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*. 12, 39-49.

SCHOFIELD. P. MBUGUA. D. M. PELL. A. N. 2001. Analysis of condensed tannins: A review. *Animal Feed Science and Technology*. 91, 21-40.

SEDDON. T. J. DOWNEY. M. O. 2008. Comparison of analytical methods for the determination of condensed tannins in grape skin. *Australian Journal of Grape and Wine Research*. 14, 54-61.

SERAFINI, M., MAINIA, G. and FERRO-LUZZI, A. 1997. Effect of ethanol on red wine tannin-protein (BSA) interactions. *Journal of Agricultural and Food Chemistry*. 45, 3148-3151.

SILBER. M. L. DAVITT. B. B. KHAIRUTDINOV. R. F. HURST. J. K. 1998. A mathematical model describing tannin-protein association. *Analytical Biochemistry*. 263, 46-50.

SIEBERT. K. J. 1999. Protein-polyphenol hazes in beverages. *Food Technology*. 53, 54-57.

SIEBERT. K. J. 2006. Haze formation in beverages. *Lebensmittel Wissenschaft und Technologie*. 39, 987-994.

SIEBERT. K. J. CARRASCO. A. LYNN. P. Y. 1996. Formation of protein-polyphenol haze in beverages. *Journal of Agricultural and Food Chemistry*. 44, 1997-2005.

SMITH. P. SARNECKIS. C. JONES. P. DAMBERGS. R. HERDERICH. M. 2005. Development and application of a simple and robust assay for quantitation of tannins in grape and wine samples. . ASVO Seminar Proceedings. Advances in Tannin and Tannin Management. Adelaide, Australia.

Table 1. Volumes used to prepare mixtures ranging in wine/protein at different final BSA concentrations.

Wine/protein (mL mg ⁻¹)		0.03			0.06			0.13			0.25			0.50			Final solution pH
Final BSA (mg mL ⁻¹)	Stock BSA (mg mL ⁻¹)	Wine (μL)	Model wine (μL)	Stock BSA (μL)	Wine (μL)	Model wine (μL)	Stock BSA (μL)	Wine (μL)	Model wine (μL)	Stock BSA (μL)	Wine (μL)	Model wine (μL)	Stock BSA (μL)	Wine (μL)	Model wine (μL)	Stock BSA (μL)	
0.40	0.50	19	281	1200	38	262	1200	75	225	1200	150	150	1200	300	0	1200	4.9
0.67	1.0	31	469	1000	63	437	1000	125	375	1000	250	250	1000	500	0	1000	4.9
1.00	2.0	47	703	750	94	656	750	188	562	750	375	375	750	750	0	750	4.8
1.33	4.0	63	937	500	125	875	500	250	750	500	500	500	500	1000	0	500	4.7
1.60	8.0	75	1125	300	150	1050	300	300	900	300	600	600	300	1200	0	300	4.5
1.80	18	84	1266	150	169	1181	150	338	1012	150	675	675	150	1350	0	150	4.2

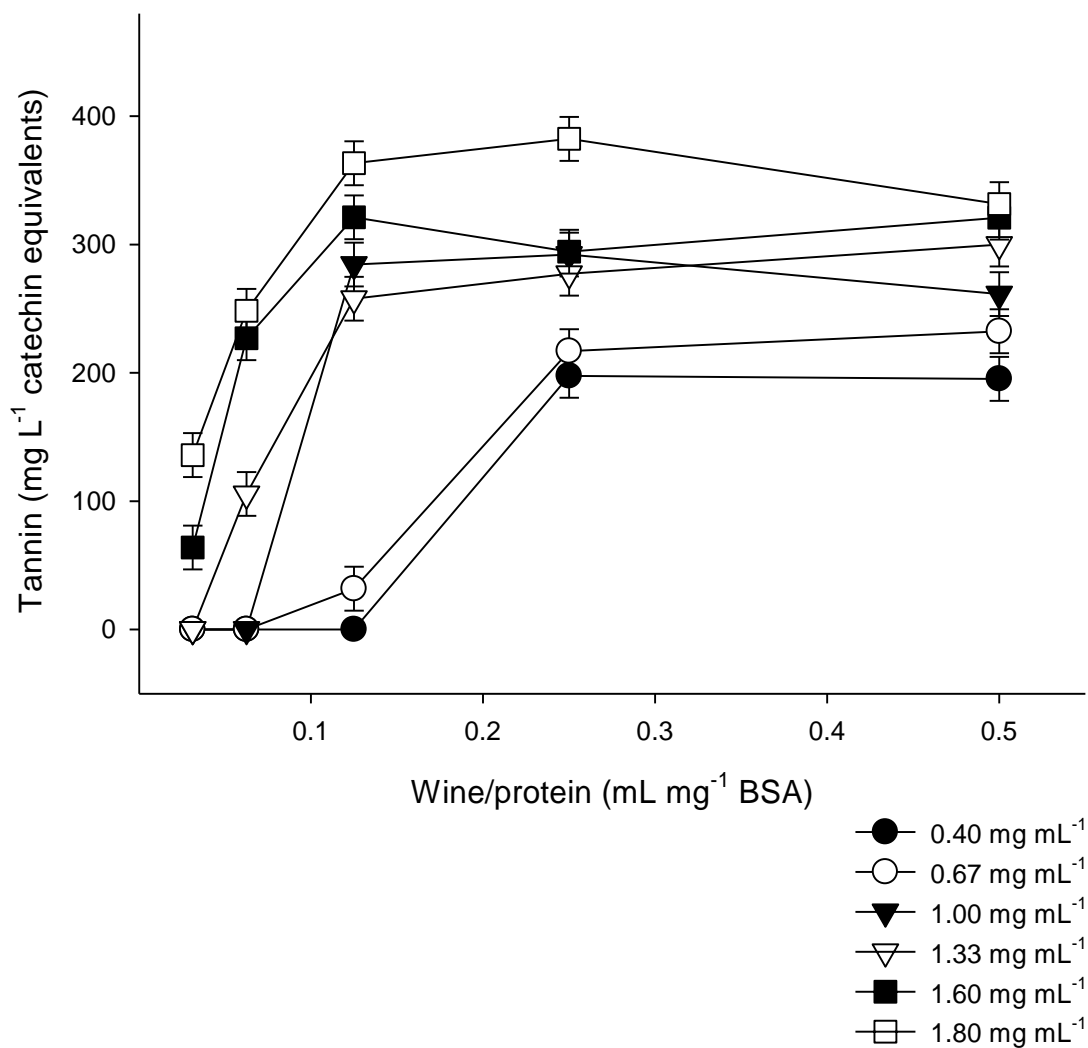


Figure 1. The effect of wine/protein ratio on tannin concentration determined with the BSA precipitation assay using a range of BSA concentrations for a 2004 New Zealand Syrah wine. Error bars show the average standard error, calculated as the harmonic mean of the pooled variance from replicate analyses.

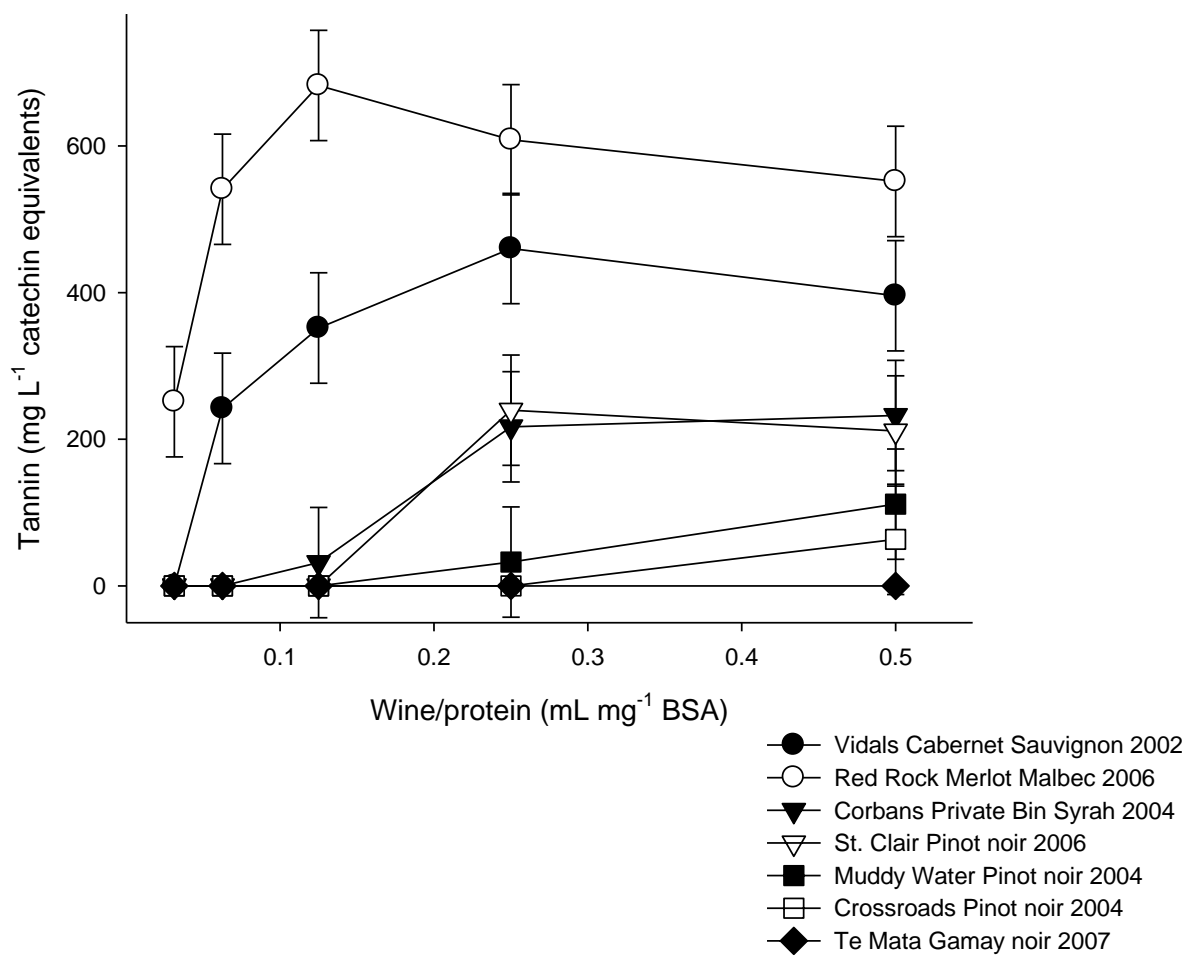


Figure 2. The effect of wine/protein ratio on tannin concentration determined using the BSA precipitation assay for a range of New Zealand red wines. [BSA] = 0.67 mg mL⁻¹. Error bars show the average standard error, calculated as the harmonic mean of the pooled variance from replicate analyses.

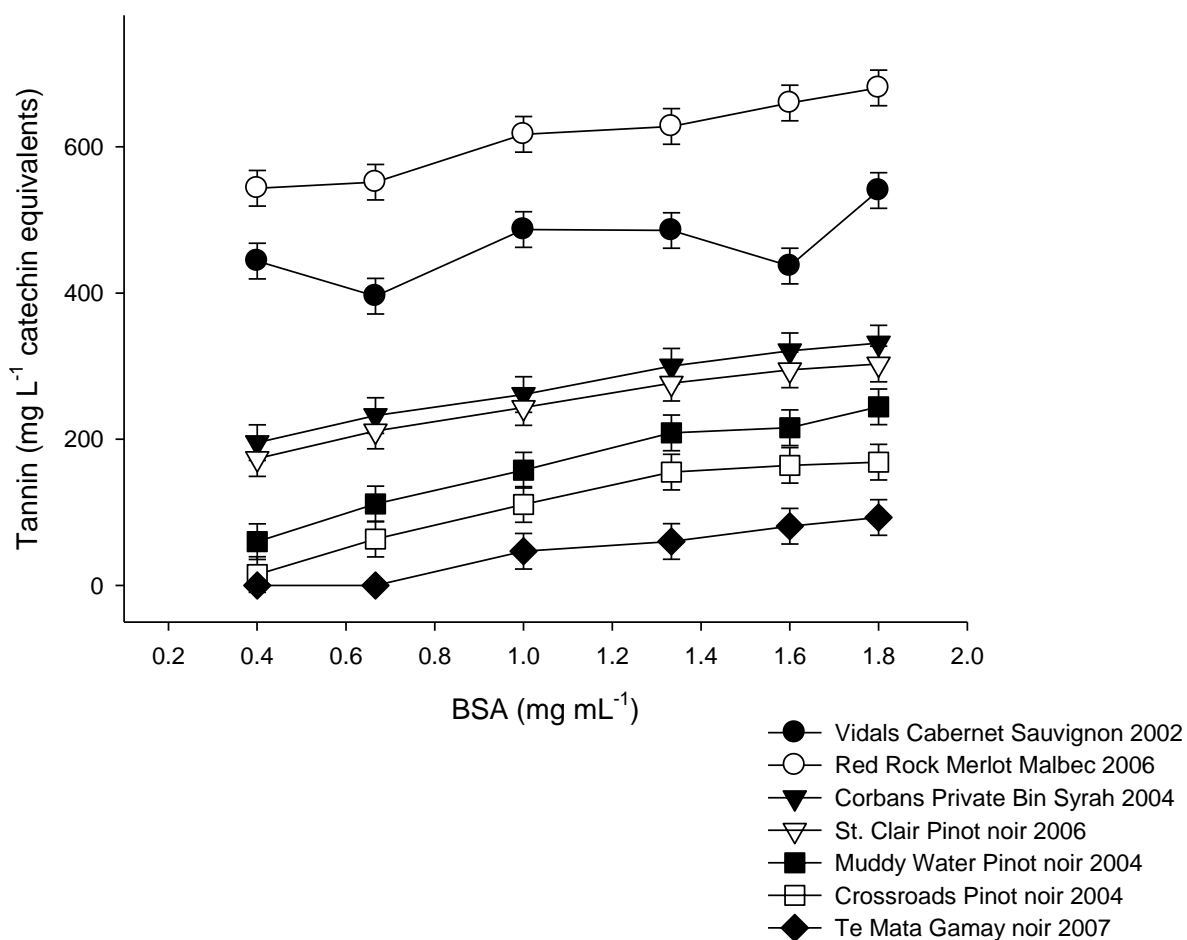


Figure 3. The effect of protein concentration on tannin concentration determined using the BSA precipitation assay for a range of New Zealand red wines. Wine/BSA = 0.5 mL mg⁻¹. Error bars show the average standard error, calculated as the harmonic mean of the pooled variance from replicate analyses.

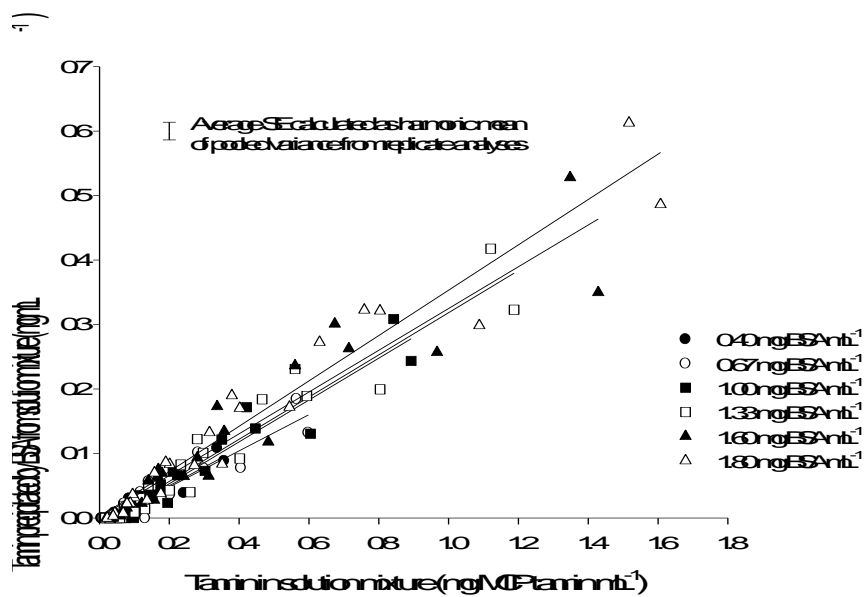


Figure 4. Plot of tannin precipitated using a range of BSA concentrations for five New Zealand red wines. Lines are regressions at each BSA concentration.

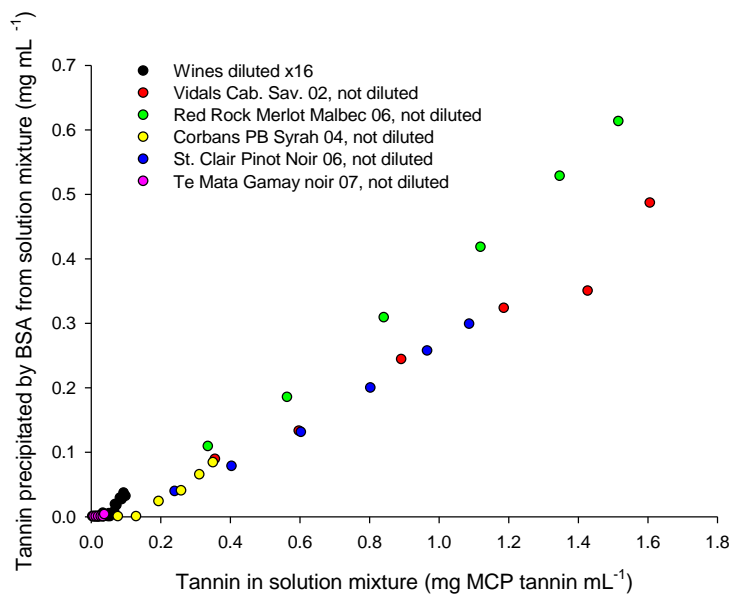


Figure 4. Insert: Subset of data for two wine dilutions.

Chapter 5

(A preparation for submission to Australian Journal of Grape and Wine Research)

Effect of mechanical leaf removal and timing on the concentrations and composition of phenolics in *Vitis vinifera* L. Pinot noir wines.

B. S. Kemp^a, R. Harrison^{a*}, G. L. Creasy^a

^a Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.

* Corresponding author. Fax: +64 3 3253850. Mail address: Centre for Viticulture and Oenology, Faculty of Agriculture & Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, Canterbury, New Zealand. Email: roland.harrison@lincoln.ac.nz (R. Harrison)

Short running title: Leaf removal effects on wine phenolics

Abstract

Mechanical leaf removal was applied to Pinot noir vines in a commercial vineyard in Waipara, New Zealand in the 2007-2008 and 2008-2009 growing seasons. Leaf removal treatments were designed to coincide with tannin synthesis at flower development and tannin polymerisation at veraison. Fruit zone treatments consisted of **NLR**: no leaf removal (control), **LR7**: leaf removal from the fruiting zone 7 days after flowering, **LR30**: leaf removal from the fruiting zone 30 days after flowering, **LRV**: leaf removal at 5% berry colour change (by visual assessment). No statistical difference was observed in wine pH, titratable acidity, alcohol, residual sugar, volatile acidity or free and total SO₂ amongst treatments. Tannin concentration in berries at harvest showed no statistical difference between treatments, but a statistically significant difference was observed during winemaking and in the bottled wine in both years. LR7 had the highest tannin concentration in the bottled wine in 2007-2008, but LR30 had the highest concentration in 2008-2009 and NLR had the lowest in both years. The mean degree of polymerisation (mDP) showed no statistical difference between treatment wines. The 2009 wines had increased tannin concentrations compared to 2008. Flavan-3-ol concentrations were highest in LR7 wines and an increase in the ratio of 2,3-*trans* to 2,3-*cis* was observed with earlier leaf removal. These results suggest that the timing of mechanical leaf removal increases tannin concentration, but has no influence on the mDP. Increased severity of defoliation and/or higher alcohol levels in the 2008-2009 growing season appeared to be responsible for the statistically significant differences in total tannin concentration compared to the previous year.

Keywords: Leaf removal, mechanical, Pinot noir, tannin, mDP

Introduction

The cultural practice of leaf removal is carried out in cool climate vineyards worldwide to increase air movement in the cluster zone, in order to prevent disease and to increase berry ripening, colour and flavour. Exposure of clusters to sunlight and higher temperatures during the growing season can be achieved either by manual or mechanical means. Previous studies of leaf removal effects on grape and wine composition include research by Arnold and Bledsoe (1990), Crippen and Morrison (1996), Haselgrove et al. (2000), Ollat and Gaudillere (1998) and Zoecklein et al. (1992). Viticultural practices that increased Pinot noir cluster exposure resulted in wines with higher phenolic concentrations and improved wine colour (Cortell et al. 2007b, Price et al. 1995).

Phenolics in the skins and seeds of grape berries include flavan-3-ols which belong to the flavonoid group (e.g. catechin and epicatechin) (Cheynier 2005, Downey et al. 2003). These monomeric compounds are also encountered as oligomers and polymers and are referred to as condensed tannins or proanthocyanidins because they release anthocyanidins when heated under acidic oxidative conditions (Cheynier 2005). Among proanthocyanidins two subclasses exist. First, procyanidins found in skins and seeds consisting of (epi) catechin units which possess dihydroxylated extension units and release cyanidin upon oxidative heating in concentrated acidic media (Cheynier 2005). Second, prodelfinidins found only in grape skins and are derived from (epi) gallo catechin and possess trihydroxylated extension units that release delphinidin (Cheynier 2005). However, proanthocyanidins exist that have both di- and trihydroxylated units (Herderich et al. 2005, Broussaud et al. 2001, Pascual-Teresa et al. 2000, Dixon et al. 2005). Seed proanthocyanidins are partly galloylated procyanidins with a mean degree of polymerisation (mDP) from 1 to 20 (Cheynier 2006). Skin proanthocyanidins that contain both procyanidin and prodelfinidin units are much larger than seed tannins and can have mean degree of polymerization (mDP) of ~ 30 (Souquet et al. 1996 as cited by Cheynier 2006). Proanthocyanidins are primarily responsible for astringency, the tactile sensation described as mouth drying or puckering when tasting red wine (Cortell et al. 2008, Gawel et al. 1998). The monomers contribute significantly to bitterness. Consequently both monomers and tannins are important contributors to red wine taste and mouthfeel (Cortell et al. 2008).

The main period of tannin synthesis in seeds occurs immediately after fruitset with the maximum rate observed around veraison (Downey et al. 2006). The level of tannin in skins is

high at flowering and accumulation continues from fruitset, until one to two weeks post-veraison (Downey et al. 2006, Kennedy et al. 2001, Bogs et al. 2005). In conducting trials concerning vineyard cultural effects on grape and wine tannin it is important to consider these tannin synthesis and accumulation periods. The influence of environmental factors and cultural practices on tannin accumulation, polymerisation and extractability remain largely unknown (Downey et al. 2006, Keller 2009). However, studies of proanthocyanidins in berry skins and seeds of Shiraz and Cabernet Sauvignon using shade boxes to artificially exclude light from clusters and minimise differences in temperature and humidity have occurred (Downey et al. 2004, Cortell et al. 2006). Also a study regarding leaf removal timing and severity on Sauvignon blanc berries was undertaken by Bledsoe et al. (1988), but the impact on wine composition was not investigated. Colour differences in red wines have been observed from artificial cluster shading trials of Shiraz and Pinot noir trials (Joscelyne et al. 2007, Cortell et al. 2007b). Both studies reported elevated wine colour with increased cluster exposure suggesting that the timing of cluster exposure by leaf removal affects wine colour. Therefore, this research was conducted to investigate the effects of mechanical leaf removal and its timing on Pinot noir phenolic accumulation and concentration in the resultant wines.

Materials and Methods

Vineyard experimental design

Complete details of the vineyard design, replication and viticultural measurements have been reported previously (Chapter 3). In summary, leaf removal treatments were: NLR, no leaf removal (control); LR7, leaf removal from the fruiting zone 7 days after flowering; LR30, leaf removal from the fruiting zone 30 days after flowering; and LRV, leaf removal at 5% colour change at veraison (by visual assessment). LR7 was applied on 20th December 2008 and 12th December 2009, LR30 was performed on 15th January 2008 and 5th January 2009 the LRV was applied 11th February 2008 and 13th February 2009. Early flowering in the 2008-2009 season resulted in earlier treatment application in December and January compared to the previous season.

Winemaking

An addition of SO₂ at a rate of 50 ppm was added to the fruit at harvest to protect the grapes from microbial spoilage on the journey from the vineyard to the winery at Lincoln University (approximately 74 km). For wine production in 2008 and 2009, grapes from the replicates of

each treatment were pooled and subsequently divided into three equal lots and placed in picking bins for 12 hours at 4 °C. Fruit was then de-stemmed, crushed and the must, skins and seeds were placed into three replicate 30 L fermentation vessels in 2008 and 10 L vessels in 2009. The must was placed in a refrigerator and underwent a 24 hour cold-soak at 4° C. The wines were then placed in a 28 °C room and inoculation with Elegance yeast (1 g/L), from the Institute of Burgundy, (AB Mauri, Sydney, Australia) occurred in two stages: half in the morning and the rest in the afternoon. All twelve fermentations were completed within 5 days in each year and were monitored twice daily for temperature and °Brix by hydrometer. Clinitest (Bayer New Zealand, Auckland) tablets were used to confirm the end of fermentation. Microvinification temperatures did not exceed 30 °C and five punch downs were conducted daily. Wines were moved to 18 °C room post fermentation and punch downs continued until the caps remained submerged. Wines were pressed in a 40 kg capacity vertical hydraulic bladder press to 1.2 bars into demijohns and samples of the pressed wines, skins and seeds post-fermentation were removed and frozen at -20 °C for later analysis. Wines were inoculated with malolactic starter culture, placed in the 18 °C room and progress monitored by thin layer chromatography. Following malolactic fermentation, SO₂ was added at a rate of 40 ppm with subsequent further additions made periodically to maintain 20 ppm free-SO₂ according to the method of Iland et al. (2000). Filtration and bottling occurred on 22nd September 2008 and 22nd June 2009, respectively, for the two harvests.

Standard must and wine analyses

Must analysis including °Brix, pH, and titratable acidity were carried out using standard protocols (Iland et al. 2000). Analyses of alcohol, residual sugar by Rebelein method (g/L), pH, volatile acidity (g/L), free and total SO₂ were performed by Pacific Rim Oenology Services, Blenheim Marlborough, New Zealand. Spectral colour analysis was performed according to Iland et al. (2000) using a UV-VIS Spectrophotometer (Unicam Helios, Spectronic *Unicam*, Cambridge, U.K). Acetaldehyde, sodium metabisulfite and hydrochloric acid for colour spectral analysis were purchased from Sigma-Aldrich, Auckland, New Zealand.

Sample preparation of whole berries, skins and seeds

Whole berries and post fermentation skins and seeds were prepared using a modified method from Sarneckis et al. (2007). Whole berries ($n = 100$) from monitor vines in the three replicate treatment rows were collected, de-stemmed and frozen at -20 °C at harvest on 20th

April 2008 and 5th April 2009. Skins and seeds post fermentation samples were removed from the fermentation vessels post pressing and frozen at -20 °C until analysis. Defrosted fruit was homogenised using a Polytron PT 3100 homogeniser for 5 minutes at 22,360 g, although whole berries required extensive grinding with pestle and mortar prior to machine homogenisation to break down the seeds. Aqueous ethanol (50% v/v) was added to approximately 1 g of grape homogenate (10 mL) or 2 g of post fermentation sample (20 mL) and mixed on a Shafter Orbital shaker for 60 minutes at 30 rpm followed by centrifugation at 1,960 g for 5 minutes.

Methylcellulose assay for tannin concentration

Total tannin was determined using the 1 mL assay by Sarneckis et al. (2007) as modified by and Mercurio et al. (2007) using epicatechin (Sigma-Aldrich New Zealand, Auckland, New Zealand) as the standard. Methylcellulose solution (0.04%, Sigma-Aldrich, M-0387, Sydney, Australia, 1500 cP viscosity at 2%) and saturated ammonium sulphate solution (Sigma-Aldrich A4915, Auckland, New Zealand) were prepared according to Sarneckis et al. (2007). All samples were passed through a 0.45 µm filter prior to analysis. Sample volumes were 100 µL for grape homogenate and post fermentation skins, 250 µL for must and 25 µL for wine and post-fermentation seeds. Measurements were carried out at 280 nm (Unicam Helios UV-VIS Spectrophotometer).

Mean degree of polymerisation (mDP) by acid catalysis in the presence of excess phloroglucinol

A modified analytical method based on that by Kennedy and Jones (2001) was used. Each wine sample (4 mL) was reduced to 0.2-0.3 mL by rotary evaporation at 40 °C and 3 mL of deionised water was added. A 0.36 g C₁₈ SEP-PAK cartridge (WAT051910, Global Science, Auckland, New Zealand) was activated using 5 mL methanol, 7.5 mL ethyl acetate (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand), then 7.5 mL deionised water. The sample was applied to the C₁₈ cartridge and washed with deionised water (7.5 mL) and allowed to dry with nitrogen gas at a flow rate of 1 L/min for 60 minutes. Ethyl acetate (5 mL) was added to the C₁₈ cartridge to ensure all monomeric material was completely discarded. To remove proanthocyanidins, the cartridge was eluted with 5 mL methanol (HPLC grade, Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand). The extracted solution then reduced by rotary evaporation using a bath temperature at 30 °C to less than 1 mL before being reconstituted with methanol to a final volume of 1 mL. The solution was

transferred to a 1.5 mL Eppendorf tube and stored at -20°C until acid hydrolysis was performed.

For the blank reagent, 0.06 g ascorbic acid (Sigma-Aldrich New Zealand, Auckland, New Zealand) was added to 800 μ L of HPLC grade methanol (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand) and 49.8 μ L of concentrated hydrochloric acid (Sigma-Aldrich New Zealand Ltd, Auckland, New Zealand). The solution was sonicated for 20 minutes and once cooled HPLC grade methanol (2150 μ L) was added. For the phloroglucinol reagent, the method was the same as the blank but instead of methanol addition after cooling, 0.301 g of phloroglucinol dissolved in 600 μ L of HPLC grade methanol was added to the solution and HPLC methanol (1550 μ L) was added. All reagents were prepared on the day of analysis to prevent reagent degradation.

The blank or phloroglucinol reagent (0.5 mL) was added to each sample (0.5 mL) and heated at 50 °C for 20 minutes. To stop the reaction 5 mL of 40 mM sodium acetate was added and left for 60 minutes at room temperature. The solution was mixed using a syringe and passed through a 13 mm 0.45 μ m PTFE filter (Grace, 2165 catalogue, Taiwan) into an HPLC vial with the first few drops discarded.

Liquid chromatography was performed on a Shimadzu 2010 equipped with two binary LC-20AD pumps, SIL-20AC auto-sampler, SPD-M20A PDA detector and 2010EV mass spectrophotometer with ElectroSpray ionization (ESI) probe operated in negative ion mode. The mass spectrometer heat block temperature was 200 °C and the curved desolvation line (CDL) temperature was 250 °C. Both nebulizing and drying gas were nitrogen, the nebulizing gas flow at 1.5 L/min and drying gas pressure at 0.14 MPa. Detector voltage was 1.5 kV, interface voltage was 4.5 kV and CDL voltage was -45 V. The PDA detector wavelength collected data from 250 nm to 700 nm and quantification was performed at 280 nm. The mobile phase solvent A was 2% acetic acid in water and solvent B was 2% acetic acid in methanol (MeOH). The flow rate was 0.8 mL/min and a flow splitter (Upchurch, UK) was installed to direct 0.2 mL/min flow to the mass spectrophotometer. The linear solvent gradient was 5% B to start, raised to 10% B in 5 minutes and to 40% in 25 minutes and quickly to 100% in 0.5 minutes, maintained at 100% B for 5.5 minutes and reduced to 5% B in 0.5 minutes. The column was re-equilibrated at 5% solvent B for 5.5 minutes before the

next injection. The column used was LUNA C-18, 250 x 4.6 mm, particle size 5 μ m (Phenomenex, USA).

Catechin, epicatechin, epicatechin gallate and epigallocatechin were determined from their respective standard curves although the latter two were found to be not present in the samples analysed. The phloroglucinol adducts (-PG) were estimated as follows: concentrations of catechin-PG, epicatechin gallate-PG and epigallocatechin-PG were calculated using the standard curve of the flavan-3-ol monomer and corrected to allow for the difference in molar absorptivity according to Kennedy and Jones (2001). Catechin was not present as a phloroglucinol adduct. Concentrations were converted to catechin equivalents using the appropriate molecular weights. The mDP information was calculated by subtracting the phloroglucinol adducts from the corresponding blank.

Analysis of flavan-3-ol monomers and quercetin by HPLC

The method was modified from Meagher et al. (2004) and Ibern-Gomez et al. (2002). Wine samples were analysed by injection of 5.0 μ L on to a HPLC system (Agilent 1100 series, Germany) equipped with a quaternary pump, a thermostat, an autosampler and a DAD detector. The column was a LUNA C-18, 250 x 4.6 mm, particle size 5 μ m held at 25 °C (Phenomenex, USA). The elution solvents were: 2 % acetic acid in deionised water (A) and 2 % acetic acid in methanol (B). The linear pump gradient was 5% solvent B, increase to 10% B by 5 minutes, 40% B by 30 minutes, 100% B by 40 min and held until 45 min, then reduced to 5% B at 45.5 minutes and maintained until 51 minutes. The flow rate was constant at 1.0 mL/min. All wine samples were filtered using a 0.45 μ m PTFE, 13 mm syringe filter from Grace Davision (Auckland, New Zealand). Catechin, epicatechin, epigallocatechin, gallic acid, epicatechin gallate and quercetin standards were purchased from Sigma Aldrich, Auckland, New Zealand, and prepared at concentrations of 0, 12.5, 25, 50 and 100 ppm. The detector scanned between 250 and 650 nm and quantification was carried out at 280 nm. ChemStation software (Agilent Technologies, Inc., California USA) was used to identify and quantify compounds.

Statistical analyses

Two-way analysis of variance (ANOVA) with contrasts was carried out using GenStat 11 (VSNi, Hemel Hempstead, UK). Graphs were produced using SigmaPlot version 2002, Windows version 8.02 (SPSS Inc, Cranes Software International Pty Ltd, Melbourne, Australia). Spearman rank correlation was carried out to determine correlations between spectral analysis and tannin concentrations using XLSTAT version 2010.2, (Paris, France).

Results

Wine analysis

On the basis of standard chemical parameters, wines from each treatment and from both years were remarkably similar (Table 1). However, the 2009 wines had higher alcohol concentration, slightly greater residual sugar concentration, moderately higher pH values and lower volatile acidity and total SO₂ concentrations. The berries in 2009 were harvested at 25 °Brix compared to 23 °Brix in 2008 which would be expected to correspond with greater alcohol concentration and higher pH.

Spectral analysis

Significant differences between leaf removal treatments were found for SO₂ resistant pigments, total phenols and chemical age ($P < 0.05$, Table 2). Chemical age is described as the ratio of SO₂ resistant pigments to total red pigments and relates the proportion of monomeric compounds to polymeric compounds to aging (Somers and Evans 1977). Lowest SO₂ resistant pigments were found in NLR wines and the highest chemical age was in LR30 wines in 2008 and 2009. NLR wines had the lowest total phenols and lowest colour density in both years. There were statistically significant differences between vintages as the 2009 wines had increased colour density, total red pigments, SO₂ resistant pigments, anthocyanins, total phenols and chemical age. However, there was no significant interaction between leaf removal treatments and year. Significant differences were found between NLR and those wines made from vines that had leaf removal, especially regarding wine colour density, SO₂ resistant pigments, chemical age and total phenols.

Total tannin concentration by methylcellulose precipitation (MCP) assay

No difference ($P > 0.05$) was observed between treatments with regards to extractable tannin concentration in whole berries at harvest in either year (Table 3). However, a difference was observed between years as concentrations were lower in 2008 in NLR, LR7, LR30 but not LRV. Must tannin concentrations were variable with no significant differences determined. However, there were significant differences in the concentration of wines after pressing and at bottling due to leaf removal treatment ($P < 0.05$) and year ($P < 0.001$). Tannin concentration in the 2009 wines was greater and showed smaller relative differences between treatments, although no ($P > 0.05$) treatment \times year interaction was determined. In 2008, a two-fold increase in tannin levels was observed in LR7 compared to NLR in the bottled wine. The highest total tannin concentrations in the 2009 wines at the post-pressing stage were in LR7 and LR30 respectively while NLR and had a somewhat lower value. In both years, a statistically significant difference ($P < 0.01$) between no leaf removal and leaf removal treatments was determined. Amongst the leaf removal treatments, tannin concentrations in bottled wines were lowest for LRV wines in both years although the difference between this timing and the earliest LR7 treatment was only significant at $P < 0.10$.

There was a significant difference ($P < 0.001$) between years regarding tannin extraction from post-fermentation grape seeds. Total extractable tannins remaining in the seeds in 2009 were much lower than the previous year (Table 4). A significant difference between treatments for skins was also observed. In 2008, lowest values were obtained for NLR whereas low values were obtained for all treatments in 2009. Similarly, values for LR7 were greater than LRV in both years. Tannin concentration was positively correlated with alcohol (% v/v) ($P < 0.05$), wine colour density ($P < 0.01$), wine colour hue ($P < 0.01$), total phenolics ($P < 0.01$) and the estimate of SO₂ resistant pigments ($P < 0.001$).

Proanthocyanidin composition and concentration by acid catalysis in the presence of phloroglucinol

Total proanthocyanidin concentrations in the treatment wines by acid catalysis in the presence of phloroglucinol showed no significant difference between NLR and leaf removal treatments ($P > 0.05$) (Figure 1). However, analysis of contrasts suggested that the concentration was greater in the LR7 treatment compared to the LRV treatment ($P < 0.05$). There was a significant difference between years ($P < 0.05$) in agreement with the methylcellulose wine tannin analysis ($P < 0.001$). It should be noted that the methylcellulose

assay was performed on wines two weeks after bottling in 2008, whereas the acid catalysis was performed one year later on the 2008 wines. Whilst it is difficult to compare the results of these analytical tannin methods, a similar pattern was observed with regards to treatment effects in tannin concentration in bottled wines (Table 3).

Analysis of the terminal and extension subunit concentrations of wine proanthocyanidins revealed a significant difference between treatments (Table 5). The lowest concentration of terminal units in 2008 and 2009 was in NLR wines. However, LRV wines had the lowest concentration of extension units in 2008 and 2009. Vintage variation was significant ($P < 0.01$) as the 2009 wines all had higher concentrations of terminal and extension units compared to 2008.

The majority of terminal subunits consisted of catechin with epicatechin being present at a lower percentage (Table 6). Extension units consisted mostly of epicatechin with a lower proportion of epigallocatechin and epicatechin gallate. The only significant difference in terminal and extension units was in the concentration of epicatechin gallate. All treatment wines in 2009 had slightly higher concentrations than the 2008 wines. However, there was no significant difference in the mean degree of polymerisation (mDP) between treatments or between years (Table 6).

Flavan-3-ol monomers

Significant differences in the concentrations of total flavan-3-ol monomers in wines were determined (Figure 2). Concentrations were lower in 2008 compared to 2009. In 2008, NLR, LR7 and LR30 had similar concentrations which were greater than LRV. In 2009, NLR had the lowest concentration of flavan-3-ol monomers and the leaf removal treatments had similar concentrations.

The predominate flavan-3-ol monomer present in the Pinot noir wines in both years was catechin, with greater concentrations determined in 2009 compared to 2008 in all wines. NLR had the lowest catechin concentration in 2009 ($P < 0.01$) whereas LR7, LR30 and LRV had similar concentrations; in 2008, catechin concentrations were greater in LR7 and LR30 compared to NLR and LRV. Epicatechin was the next most abundant flavan-3-ol monomer and concentrations were lower in all wines in 2009 compared to 2008 ($P < 0.05$). The highest concentration was in NLR wines in 2008 with leaf removal treatments having lower values,

whereas the opposite trend was observed in 2009 with the lowest concentration in NLR compared to higher concentrations in the leaf removal treatments.

Gallocatechin and epigallocatechin were found in lower quantities in wine than catechin and epicatechin. This was also true for epicatechin gallate. Significant interactions between treatment and year were determined for all three species (Table 7) and consistent trends were difficult to identify.

Wines were also analysed for quercetin because of its role in response to increased UV-B exposure (Price et al. 1995). Significant effects of treatment and year were determined as well as a statistically significant interaction (Table 8). However, a relatively consistent pattern was observed between treatments, with lowest concentrations for NLR and highest for early leaf removal (LR7 or LR30). Concentrations in 2009 were higher than those in 2008 ($P < 0.001$).

Discussion

Wine analysis

No significant differences in standard wine parameters (alcohol concentration, pH, titratable acidity, volatile acidity, residual sugar concentration and free and total SO₂ concentrations) due to leaf removal treatments (Table 1) are in agreement with Tardaguila et al. (2008). Similarly, Percival et al. (1994) found that leaf removal had no influence on total soluble solids accumulation in Riesling berries although other studies have found leaf removal at fruitset reduced total soluble solids concentration (Chorti et al. 2010, Joscelyne et al. 2007, Poni et al. 2006). Higher °Brix levels in berries at harvest in 2009 accounts for the higher alcohol and residual sugar levels in the wines although differences in °Brix between years might ultimately be attributed to the increased fruit exposure in 2009 compared to 2008 (Chapter 3). The significant difference in pH between years and the lack of any significant difference in titratable acidity suggests vintage variation across all treatments. One explanation could be that malic acid and potassium concentrations varied (Bledsoe et al. 1988) in berries at harvest in 2008 compared to 2009. Higher volatile acidity in 2008 may be due to lower cluster exposure. Observed differences in total SO₂ between years could be due to the later bottling of the 2008 wines as no difference was observed between years for free SO₂. These results indicate that the timing of mechanical leaf removal had no impact on common berry ripening parameters, as discussed above (Chapter 3), or standard wine analyses, but a vintage variation was observed across all treatments.

Spectral analysis

Red wine colour is due to a number of factors that include grape variety, type and concentration of anthocyanins, pH, free SO₂ concentration and the extent of polymerisation and copigmentation (Versari et al. 2008). In this study, significant differences due to vintage were observed in wine colour density, total red pigments, SO₂ resistant pigments, concentration of anthocyanins and chemical age. The higher alcohol concentration in the second year of the study would have assisted with increased colour extraction (Canals et al. 2005). NLR had the lowest colour in agreement with Tardaguila et al. (2008) who found a significant difference in colour intensity measured by UV-VIS spectrophotometer between Grenache wines from varying leaf removal treatments. In their study, early leaf removal (corresponding to LR30 in this study) had higher colour intensity compared to non-defoliated and other defoliated treatment wines.

Cortell et al. (2007a, b) studied the influence of vine vigour on Pinot noir anthocyanins and pigmented polymers in wines. Low vigour vineyards were characterised by greater light exposure in the fruiting zone (Smart 1985, Cortell et al. 2007a, Jackson and Lombard 1993). Low vigour vines produced wines with higher concentrations of anthocyanins and bisulfite-resistant pigments, greater diversity of anthocyanins, greater colour density, and higher percentage of red pigments and reduced hue (Cortell et al. 2007a).

The estimation of SO₂ resistant pigments showed that levels were significantly less in NLR compared to leaf removal treatments suggesting a lower level of polymers present in wines in both years (Versari et al. 2007). These results are in agreement with Ristic et al. (2007) who reported that wines produced from Shiraz grapes which had light exclusion boxes applied prior to flowering, had lower SO₂ resistant pigments than light exposed fruit, although their spectral analyses did not prove statistically significant until three years post bottle aging. It is well documented that polymeric pigments are more stable to SO₂ bleaching than monomeric pigments although Versari et al. (2008) provided evidence that polymeric pigments can be partly bleached by SO₂. However, no relationships between SO₂ bleaching and total tannin concentrations in wines determined by the BSA assay results were found (Versari et al. 2008, Adams and Harbertson 1999, Harbertson et al. 2002).

Total tannin concentration during winemaking

Whole berries

Whole berry tannin concentration difference between years could be due to a higher concentration of non-extractable proanthocyanidins in 2008 caused by increased interaction with cell wall components (Hanlin et al. 2009). Tannins bind to proteins and polysaccharides in berry cell walls both covalently and non-covalently and this is an important factor for extraction of tannins from fruit during winemaking. Increased cluster exposure from greater defoliation in 2009 due to partial cane removal, hail and leaf removal may have resulted in increased extraction, although Eglington et al. (2004) using HPLC analysis of Cabernet Sauvignon berry homogenate samples, confirmed that increased grape maturity was associated with reduced grape tannin concentration. Therefore in 2009, increased cluster exposure combined with increased alcohol (25 °Brix as opposed to 23 °Brix in 2008) may have facilitated tannin extraction. In studies by Kallithraka et al. (1995) and Harbertson and Downey (2009), it was concluded that 70% acetone extracted the highest concentration of total phenols compared to methanol and ethanol. Increased efficiency of berry tannin extraction may be better able to reveal differences in tannin concentration between treatments. In both years the total tannin concentration in our Pinot noir berries was within the range (3.35-5.96 mg/g) reported by Mercurio et al. (2007) for grape homogenate extracts with using 50% ethanol. Regardless of the analytical method, no relationship has been established between total tannin per berry (grape homogenate or seed and skin analysis) and the amount of tannin in the resulting wine (Harbertson et al. 2002, Mercurio et al. 2007, Cortell et al. 2005, Pastor del Rio and Kennedy 2006).

Must

Must at harvest had low total tannin concentrations in both years which was undoubtedly due to low tannin extraction at this early stage of winemaking. The somewhat lower total tannin concentration in the 2009 must samples compared to 2008 may be due to the greater maturity of the fruit in the second year. Grapes harvested at greater maturity have been found to have less extractable total tannins at this stage of winemaking (De Frietas et al. 2000).

Wine

All completed wines post malolactic fermentation, SO₂ adjustment and two weeks bottle aging exhibited a decrease in total tannins in all treatments compared to concentrations post pressing. This decrease is unlikely to be due to malolactic fermentation as there are no

reports concerning the ability of lactic acid bacteria to degrade tannin (Vaquero et al. 2004). The most likely reason for tannin concentration decreasing in both years is probably the wine oxygenation or absorption by yeast lees (Mazauric and Salmon 2006). Condensation reactions involving monomers, proanthocyanidins and anthocyanins result in the formation of relatively stable polymeric pigments of high molecular weight (Remy et al. 2000, Santas-Buelga and Scalbert 2000). These compounds may have partially precipitated during wine storage or been affected by SO₂ addition (Tao et al. 2007). It is well documented that SO₂ bleaches monomeric anthocyanin derived pigments to a greater extent than polymeric pigments, but further studies are required regarding SO₂ degradation of proanthocyanidins (Versari et al. 2007).

The statistically significant effect of treatment on tannin concentration (Table 3) may be due to a combination of berry size, tannin concentration and tannin extractability. Another plausible explanation for this variation is that skin tannin composition was diverted towards shorter polymer lengths that were more readily extracted into wine (Hanlin et al. 2009). The wines produced from NLR had the lowest total tannin concentrations in both years and were similar to LRV. These results suggest that earlier leaf removal treatments (LR7 and LR30) compared to later leaf removal (LRV) or no leaf removal (NLR) resulted in wines with higher total tannin concentrations. The bottled wine total tannin concentration in both years was lower than reported by Mercurio et al. (2007) for Shiraz and Merlot which is probably due to varietal differences.

Skins post fermentation

Due to the difference in total tannin concentration observed throughout the winemaking procedure an investigation into tannins remaining in skins and seeds post fermentation was carried out using the MCP assay (Table 4). As previously mentioned, skin and seed tannins are not fully extracted during the winemaking process partly due to absorption by grape and yeast cell walls combined with interaction with polysaccharides and proteins (Cerpa-Calderon and Kennedy 2008). However, some polymerised proanthocyanidins remain intact and are difficult to extract under wine conditions (Cerpa-Calderon and Kennedy 2008). The skins with the lowest total tannin concentration remaining post fermentation were NLR in 2008 and LRV in 2009. NLR also had the lowest low tannin concentration in the bottled wines in both years. In 2009 less tannin remained in the skins than in 2008, most likely due to the higher alcohol facilitating extraction in the second year (Canals et al. 2005). Additionally,

changes in the berry from early cluster exposure such as skin thickness, cell size and cell wall properties may have aided tannin extraction (Ristic et al. 2007). Further studies regarding the effect of the timing of leaf removal on berry skin cell wall composition pre-and post fermentation are required.

Seeds post fermentation

Skin tannin is extracted early in fermentation whereas seed tannins are extracted at the end of fermentation, so higher levels remained in all seeds post fermentation than in skins (Des Gachons and Kennedy 2003). In 2008 and 2009, a statistically significant pattern similar to skins emerged with a reduction in tannin concentration post fermentation. Explanations for this phenomenon include increased alcohol content facilitated seed tannin extraction (there was a positive correlation between alcohol and tannin concentration in wine), variation between years in tannin extractability, alterations to cell wall composition, severity of leaf removal or a combination of all of these.

Proanthocyanidin composition and concentration in Pinot noir wines

Cortell et al. (2005) found no difference between terminal unit composition and concentration in wines produced from sites differing in vigour and catechin terminal units accounted for between 75 and 88 %. Catechin terminal units in the present study accounted for 64%-74% of the total terminal units in 2008 and 2009. Epicatechin made up the remainder of terminal units. Mattivi et al. (2009) found that Pinot noir skins had the highest percentage of catechin terminal units and the highest amount of catechin in the seeds compared to Cabernet Sauvignon, Carmenere, Marzemino, Merlot, Syrah and Teroldego.

Extension unit composition was dominated by epicatechin (Table 6). However, there were small but statistically significant differences in the proportion of epicatechin gallate, with higher values in 2009 compared to 2008. The study by Aron and Kennedy (2007) regarding Pinot noir phenolic polymers states that epicatechin gallate extension units are derived solely from the seeds of grapes.

An important observation from this study is the lack of a significant difference in mDP of treatment wines (Table 6). Pastor Del Rio and Kennedy (2006) reported lower mDP values in Pinot noir wine, but their calculations may have included wine flavan-3-ol monomers whereas in this study monomeric compounds were eliminated using ethyl acetate (Sun et al.

1998). Hayasaka and Kennedy (2003) reported a value for mDP of 8.3 in three year old Pinot noir wine. Aron and Kennedy (2007) found Pinot noir wines at the end of winemaking had mDP between 5.04 and 5.99, which are slightly lower than the values reported in this study but analysis occurred several months later. Whilst significant progress has been made regarding flavan-3-ol synthesis pathways, the biochemistry of proanthocyanidin polymerization, specifically in late berry development has not been elucidated (Gagne et al. 2009). A possible explanation for a lack of significant differences in this study is that tannin polymerisation is not reliant upon cluster sunlight exposure and increased cluster temperatures unlike flavan-3-ol synthesis at flowering. Indeed, these results indicate that another mechanism within the berry at veraison, such as enzymatic activity by anthocyanidin reductase (ANR) or leucoanthocyanidin reductase (LAR) could be involved in proanthocyanidin polymerisation (Yu Xie and Dixon 2005, He et al. 2008, Gagne et al. 2009).

The increase in total proanthocyanidins in the wines in 2009 could be explained by the increase in seed tannins and/or decreased vigour combined with increased cluster exposure that may have altered berry skin composition (Cortell et al. 2005). The result may have been more extractable short chain polymers since cell wall material has a preferential binding affinity to larger sized proanthocyanidins (Hanlin et al. 2009, Bindon et al. 2010). Alternatively, as a result of tannin-tannin condensation reactions the production of new proanthocyanidin molecules can lead to an increase or decrease in mDP depending upon the amount of monomeric flavanols present (Tao et al. 2007, Monagas et al. 2005). The difference in total tannin and proanthocyanidin concentration between years could furthermore be attributed to 2008 wines aging in bottle for one year. Jorgensen et al. (2004) showed a decline in the apparent mDP of proanthocyanidins in Pinot noir when wines were exposed to oxygen to imitate bottle aging.

Flavan-3-ol monomers

In 2008, the highest total flavan-3-ol monomers concentrations were found in the early defoliated treatment wines (LR7 and LR30). In 2009 the lowest concentration was found in the NLR wines. These results in 2009 suggest that leaf removal and cluster exposure increased flavan-3-ol concentrations in wines. Results concerning catechin in wine are in agreement with studies regarding Pinot noir berries (Goldberg et al. 1995, Mattivi et al. 2009). Pinot noir berries contain predominantly catechin flavan-3-ol monomers and catechin

terminal units with a lower occurrence of epicatechin, epigallocatechin and galocatechin compared to other varieties (Goldberg et al. 1995, Mattivi et al. 2009). Separate biosynthetic pathways of flavan-3-ols have been identified and leucoanthocyanidin reductase (LAR) catalyses the reduction of 2*R*,3*S*,4*S*-flavan-3,4-diols to the corresponding 2*R*,3*S*-flavan-3-ols i.e. catechin (Maugé et al. 2010). A second enzyme, anthocyanidin reductase (ANR), has been shown to exhibit an epimerase activity in grapes that results in the production of 2*S*,3*S*- and 2*S*,3*R*-flavan-3-ols i.e. epicatechin (Maugé et al. 2010). The lower catechin concentrations in NLR and the latest leaf removal timing (LRV) supports the idea that leaf removal and cluster exposure in LR7 and LR30 may have increased LAR activity (Maugé et al. 2010). Similarly, enhanced cluster exposure in 2009 compared to 2008 resulted in higher catechin concentrations in wines. These results support the view that cluster exposure favours catechin synthesis by increasing LAR activity. A similar pattern was found in epicatechin concentrations which increased in wines in 2009, except for NLR, but results were not statistically significant between treatments.

Galocatechin, epigallocatechin, and epicatechin gallate are found in smaller amounts in the wines. Previous studies report that they are present as dimers, trimers and polymers including galocatechin oligomers (Fulcrand et al. 1999, Pascual-Teresa et al. 2000). This suggests that trihydroxylated units, which represented 20% of total tannin units in the wine extract investigated by Fulcrand et al. (1999), are randomly distributed in wine and grape proanthocyanidin structures. The contribution of these compounds to wine mouthfeel and taste is currently unknown. The increase in galocatechin and small increase in epigallocatechin in 2009 indicate a similar response to leaf removal and cluster exposure as that found for catechin, namely a preference for 2,3-*trans* rather than 2,3-*cis* flavan-3-ols. Higher proportions of trihydroxylated anthocyanins were reported in exposed Shiraz berries compared to artificially shaded ones, which suggest cluster exposure could increase synthesis of the B-ring trihydroxylated flavanoids (Downey et al. 2004). Alternatively epicatechin and epigallocatechin may have been integrated into dimers, trimers and proanthocyanidins in preference to catechin and galocatechin (Cortell et al. 2005). This phenomenon, reported for the first time by Cortell et al. (2005) in wine tannin research, requires further investigation and the possible impact on sensory analysis evaluated.

Results for quercetin concentrations in wine are in agreement with Price et al. (1995) who found that quercetin concentrations increased with increased sun exposure. Quercetin

derivatives (Jaakola and Hohtola 2010) appear to be the flavonoids that are most often reported to increase with increasing light irradiation (Jaakola and Hohtola 2010). Quercetin is of importance for copigmentation, colour stability and aging (Boulton 2001). Wines made from grapes low in copigmentation cofactors such as quercetin will have low colour density as observed in NLR wines.

Conclusion

The naturally shaded non-defoliated fruit produced wines with lower colour, flavan-3-ols, proanthocyanidins and total tannin concentrations compared to the defoliated wines. The early leaf removal treatments, LR7 and LR30, had the highest total tannin and proanthocyanidin concentration in 2008 and 2009, respectively. Vintage variation occurred as 2009 wines were higher in proanthocyanidins, flavan-3-ols, total tannin concentrations and colour. This variation could be attributed to increased cluster exposure and defoliation as well as greater alcohol level in the second year. Wine flavan-3-ol composition was affected by defoliation timings, but no differences between proanthocyanidin composition and mDP between treatments were observed. Monitoring tannin concentration during winemaking enabled identification of leaf removal treatment effects at specific stages of winemaking. This approach could be adapted for other cultural and environmental studies which investigate viticultural impacts on wine tannin concentration and might enable appropriate extraction techniques to be employed to increase concentrations without exogenous enzyme applications and improve the final mouthfeel and aging ability of the wine.

Acknowledgements

The authors wish to acknowledge the generous support of Pegasus Bay Winery for material contributions and staff support in carrying out the trial. The financial assistance of Lincoln University and New Zealand Winegrowers is also acknowledged. Grateful appreciation is extended to Richard Hider for his assistance with the proanthocyanidin isolation and Jenny Zhao for her assistance with the LC-MS and RP-HPLC analyses.

References

- Adams, D. and Harbertson, J. (1999) Use of alkaline phosphatase for the analysis of tannins in grape and red wines. *American Journal of Enology and Viticulture*. **50**, 247-252.
- Arnold, R. and Bledsoe, A. (1990) The effect of various leaf removal treatments on the aroma and flavor of Sauvignon blanc wines. *American Journal of Enology and Viticulture*. **41**, 74-76.
- Aron, P. and Kennedy, J. (2007) Compositional investigation of phenolic polymers isolated from *Vitis vinifera* L. Cv. Pinot noir during fermentation. *Journal of Agricultural and Food Chemistry*. **55**, 5670-5680.
- Bindon, K., Smith, P. and Kennedy, J. (2010) Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *Journal of Agricultural and Food Chemistry*. **58**, 2520-2528.
- Bledsoe, A., Kliewer, W. and Marois, J. (1988) Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon Blanc grapevines. *American Journal of Enology and Viticulture*. **39**, 49-54.
- Bogs, J., M.O. Downey, J.S. Harvey, A.R. Ashton, G.J. Tanner, and Robinson. S.P. (2005) Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevines leaves. *Plant Physiology*. **139**, 652-663.
- Boulton, R. (2001) The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*. **52**, 67-87.
- Broussaud, F., Cheynier, V., Asselin, C. and Moutounet, M. (1999) Flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *American Journal of Enology and Viticulture*. **50**, 277-284.
- Canals, R., Llaudy, M., Valls, J., Canals, J. and Zamaora, F. (2005) Influence of ethanol concentration on the extraction of colour and phenolic compounds from skins and seeds of Tempanillo grapes at different stages of ripening. *Journal of Agriculture and Food Chemistry*. **53**, 4019-4025.
- Cerpa-Calderon, F. and Kennedy, J. (2008) Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *Journal of Agricultural and Food Chemistry*. **56**, 9006-9014.
- Cheynier, V. (2005) Polyphenols in foods are more complex than often thought. *American Journal of Clinical Nutrition*. **81**, 223-2239.
- Cheynier, V. (2006) Structure and Properties of Wine Pigments and Tannins. *American Journal of Enology and Viticulture*. **57**, 298-305.

- Chorti, E., Guidoni, S., Ferrandino, A. and Novello, V. (2010) Effect of different cluster sunlight exposure levels to ripening and anthocyanin accumulation in Nebbiolo grapes. *American Journal of Enology and Viticulture*. **61**, 23-30.
- Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2005) Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry*. **53**, 5798-5808.
- Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2007a) Influence of vine vigour on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *Journal of Agricultural and Food Chemistry*. **55**, 6575-6584.
- Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2007b) Influence of Vine vigour on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins. 2. Anthocyanins and Pigmented polymers in wine. *Journal of Agricultural and Food Chemistry*. **55**, 6585-6595.
- Cortell, J. and Kennedy, J. (2006) Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. *Journal of Agricultural and Food Chemistry*. **54**, 8510–8520.
- Cortell, J., Silvertsen, H., Kennedy, J. and Heymann, H. (2008) Influence of vine vigour on Pinot noir fruit composition, wine chemical analysis, and wine sensory analysis. *American Journal of Enology and Viticulture*. **59**, 1:1-10.
- Crippen, D. and Morrison, J. (1996) The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. *American Journal of Enology and Viticulture*. **37**, 243-247.
- Dixon., R., Xie, De-Yu. and Sharma, S. (2005) Tansley Review: Proanthocyanidins-a final frontier in flavonoid research? *New Phytologist* **165**, 9-28.
- Downey, M., Dokoozlian, N. and Kristic, P. (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture*. **57**, 257-268.
- Downey, M., Harvey, J. and Robinson, S. (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*. **9**, 15-27.
- Downey, M., Harvey, J. and Robinson, S. (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research*. **10**, 55-73.
- Eglinton, J., Griesser, M., Henschke, P., Kwiatowski, M., Parker, M. and Herderich, M. (2004) Yeast- mediated formation of pigmented polymers in red wine. *Red wine color: Revealing the mysteries*. ACS Symposium series 886, Washington DC, USA. Pp 7-12.

- Frietas, V., Glories, Y. and Monique, A. (2000) Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *American Journal of Enology and Viticulture*. **51**:4:397-403.
- Fulcrand, H., Remy, S., Souquet, J., Cheynier, V. and Moutounet, M. (1999) Study of wine tannin oligomers by on-line liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*. **47**, 1023-1028.
- Gagne, S., Lacampagne, L., Claisse, O. and Geny, L. (2009) Leucoanthocyanidin reductase and anthocyanidin reductase gene expression and activity in flowers, young berries and skins of *Vitis vinifera* L. cv. Cabernet Sauvignon during berry development. *Plant Physiology and Biochemistry*. **47**, 282-290.
- Gawel, R. (1998) Red wine astringency: a review. *Australian Journal of Grape and Wine Research*. **4**, 74-95.
- Goldberg, D., Karumanchiri, A., Tsan, E. and Soleas, G. (1995) Catechin and epicatechin concentrations in wines: Regional and cultivar-related differences. *American Journal of Enology and Viticulture*. **49**, 23-34.
- Hanlin, R., Hrmova, M., Harbertson, J. and Downey, M. (2009) Review: Condensed tannin and grape cell wall interaction and their impact on tannin extractability into wine. *Australian Journal of Grape and Wine Research*. **16**, 173 – 188.
- Harbertson, J. and Downey, M. (2009) Investigating differences in tannin levels determined by methylcellulose and protein precipitation. *American Journal of Enology and Viticulture*. **60**, 246-249.
- Harbertson, J., Kennedy, J. and Adams, D. (2002) Tannins in skins and seeds of Cabernet Sauvignon, Syrah and Pinot noir berries during ripening. *American Journal of Enology and Viticulture*. **53**, 54-59.
- Harbertson, J. and Spayd, S. (2006) Measuring phenolics in the winery. *American Journal of Enology and Viticulture*. **57**, 280-288.
- Hardie, W.J. and Aggenbach, S.J. (1996) Effects of site, season and viticultural practices on grape seed development. *Australian Journal of Grape and Wine Research*. **2**, 21-24.
- Haselgrove, L., Botting, D., Van Heeswijck, R., Hoj. P., Dry, P., Ford, C. and Iland, P. (2000) Canopy microclimate and berry composition: The effect of cluster exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research*. **6**, 136-140.
- Hayasaka, Y. and Kennedy, J. (2003) Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Australian Journal of Grape and Wine Research*. **9**, 210-220.
- He, F., Pan, Q., Shi, Y. and Duan, C. (2008) Review: Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules*. **13**, 2674-2703.

Herderich, M. and Smith, P. (2005) Analysis of Grape and Wine Tannins: Methods, applications and challenges. *Australian Journal of Grape and Wine Research*. **11**, 205-214.

Herderich, M., Bell, S., Holt, H., Ristic, R., Birchmore, W., Thompso, K., Iland, P. (2004) Grape maturity and tannins: the impact of viticultural treatments on grape and wine tannins. Proc. 12th Australian Wine Industry Technical Conference, Melbourne, Victoria, 24th-29th July 2004. Pp 79-84.

Ibern-Gómez, M., Andrés-Lacueva, C., Lamuela-Raventós, R. and Waterhouse. A. (2002) Rapid HPLC analysis of phenolic compounds in red wines. *American Journal of Enology and Viticulture*. **53**, 218-221.

Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000) Techniques for Chemical Analysis and Quality Monitoring During Winemaking. Patrick Iland Wine Promotions, South Australia.

Intrieri, C., Filippetti, I., Allegro, G., Centinari, M. and Poni, S. (2008) Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.) *Australian Journal of Grape and Wine Research*. **14**, 25-32.

Jaakola, L. and Hohtola, A. (2010) Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell and Environment*. doi: 10.1111/j.1365-3040.2010.02154.x

Jackson, D. and Lombard, P. (1993) Environmental and management practices affecting grape composition and wine quality – a review. *American Journal of Enology and Viticulture*. **44**, 409-430.

Jorgensen, E., Marin, A. and Kennedy, J. (2004) Analysis of the oxidative degradation of proanthocyanidins under basic conditions. *Journal of Agricultural and Food Chemistry*. **52**, 2292-2296.

Joscelyne, V., Downey, M., Mazza, M. and Bastian, S. (2007) Partial shading of Cabernet Sauvignon and Shiraz vines altered wine colour and mouthfeel attributes, but increased exposure had little impact. *Journal of Agricultural and Food Chemistry*. **55**, 10888-10896.

Kallithraka, S., Garcia-Viguera, C., Bridle, P. and Bakker, J. (1995) Survey of solvents for the extraction of grape seed phenolics. *Phytochemical Analysis*. **6**, 265-267

Kennedy, J. and Jones, G. (2001) Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry*. **49**, 1740-1746.

Kennedy, J.A., Y. Hayasaka, S. Vidal, E.J. Waters, and Jones. G.P. (2001) Composition of grape skin proanthocyanidins at different stages of berry development. *Journal of Agricultural and Food Chemistry*. **49**, 5348-5355.

Keller, M. (2009) Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research*. **16**, 56–69.

- Mattivi, F., Vrhovsek, U., Masuero, D. and Trainotti, D. (2009) Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Australian Journal of Grape and Wine Research*. **15**, 27-35.
- Maugé, C., Granier, T., Langlois d'Estaintot, B., Gargouri, B., Manigand, C., Schmitter, J., Chaudière, J. and Gallois, B. (2010) Crystal structure and catalytic mechanism of leucoanthocyanidin reductase from *Vitis vinifera*. *Journal of Molecular Biology*. **397**, 1079-1091.
- Mazauric, J. and Salmon, J. (2006) Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees. *Journal of Agricultural and Food Chemistry*. **54**, 3876–3881.
- Meagher, L., Lane, G., Sivakumaran, S., Tavendale, M. and Fraser, K. (2004) Characterization of condensed tannins from *Lotus* species by thiolytic degradation and electro spray mass spectrometry. *Animal Feed Science and Technology*. **117**, 151–163.
- Mercurio, M., Damberg, R., Herderich, M. and Smith, P. (2007) High throughput analysis of red wine and grape phenolics – adaption and validation of methylcellulose precipitable tannin assay and modified Somers colour assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry*. **55**, 4651-4657.
- Monagas, M., Begona, B. and Gomez-Cordoves, C. (2005) Updated knowledge about the presence of phenolic compounds in wine. *Critical Reviews in Food Science and Nutrition*. **45**, 85-118.
- Obradovic, D. (2004) Grape-derived tannins and their application. 12th Australian Wine Industry Technical conference, Melbourne, Victoria, 24th-29th July 2004.
- Ollat, N. and Gaudillere, J. (1998) The effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *American Journal of Enology and Viticulture*. **49**, 251-258.
- Pascual-Teresa, S., Rivas-Gonzal, J. and Santos-Buelga, C. (2000) Prodelphinidins and related flavanols in wine. *International Journal of Food Science and Technology*. **35**, 33-40.
- Pastor del Rio, J. and Kennedy, J. (2006) Development of Proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *American Journal of Enology and Viticulture*. **57**, 125-132.
- Peleg, H., Gacon, K., Schlich, P. and Noble, A. (1999) Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture*. **79**, 1123-1128.
- Percival, D.C., Fisher, K.H. and Sullivan, J.A. (1994) Use of fruit zone leaf removal with *Vitis vinifera* L. cv. Riesling grapevines. II. Effect on fruit composition, yield, and occurrence of bunch rot (*Botrytis cinerea*). *American Journal of Enology and Viticulture*. **45**, 133-140.

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S. and Intrieri, C. (2006) Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *American Journal of Enology and Viticulture*. **57**, 397-407.

Price, S., Breen, P., Valladao, M. and Watson., B. (1995) Cluster sun exposure and quercetin in Pinot noir grapes and wine. *American Journal of Enology and Viticulture*. **46**, 187-194.

Remy, S., Fulcrand, H., Babarbe, B., Cheynier, V. and Moutounet, M., (2000) First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *Journal of the Science of Food and Agriculture*. **80**, 745-752.

Richards, A. (2001) *Alien vision: exploring the electromagnetic spectrum with imaging technology*. Published by the International Society for Optical Engineering, Washington, USA. **1**, 1-6.

Ristic, R., Downey, M., Iland, P., Bindon, K., Francis, L., Herderich, M. and Robinson, S. (2007) Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research*. **13**, 53-65.

Roby, G., Harbetson, J., Adams, D. and Matthews, M. (2004) Berry size and vine water deficits as factors in wine grape composition: anthocyanins and tannins. *Australian Journal of Grape and Wine Research*. **10**, 100-107.

Santos-Buelga, C. and Scalbert, A. (2000) Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *Journal of Agricultural and Food Chemistry*. **80**, 1094-1117.

Sarneckis, C. J., Dambergs, R.G., Jones, P., Mercurio, M., Herderich, M. and Smith, P., (2006) Quantification of condensed tannins by precipitation with methylcellulose: development of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*. **12**, 39-49

Seddon, T. and Downey, M. (2008) Comparison of analytical methods for the determination of condensed tannins in grape skin. *Australian Journal of Grape and Wine Research*. **14**, 54–61.

Somers, C. and Evans, M. (1977) Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO₂, “chemical age”. *Journal of the Science of Food and Agriculture*. **28**, 279-287.

Smart, R. (1985) Principles of grapevine microclimate manipulation with implications for yield and quality. *American Journal of Enology and Viticulture*. **36**, 230-239.

Sun, B., Leandro, C., Ricardo da Silva, J. and Spranger, I. (1998) Separation of grape and wine proanthocyanidins according to their degree of polymerisation. *Journal of Agricultural and Food Chemistry*. **46**, 1390-1396.

Tao, J., Dykes, S. and Kilmartin, P. (2007) Effect of SO₂ concentration on polyphenol development during red wine micro-oxygenation. *Journal of Agricultural and Food Chemistry*. **55**, 6104-6109.

Tarara, J., Lee, J., Spayd, S. and Scagel, C. (2008) Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanins in Merlot grapes. *American Journal of Enology and Viticulture*. **59**, 235-247.

Tardaguila, J., Diago, M., Martinez-de Toda, F., Poni, S. and Vilanova, M. (2008) Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of CV. Grenache grown under non-irrigated conditions. *Journal International de la Vigne et du Vin*. **42**, 221-229.

Vaquero, I., Marcobal, A. and Muñoz, R. (2004) Tannase activity by lactic acid bacteria isolated from grape must and wine. *International Journal of Food Microbiology*. **96**, 199-204

Versari, A., Boulton, R. and Parpinello, G. (2007) Analysis of SO₂-resistant polymeric pigments in red wines by high-performance liquid chromatography. *American Journal of Enology and Viticulture*. **58**, 523-525.

Versari, A., Boulton, R. and Parpinello, G. (2008) A comparison of analytical methods for measuring the colour components of red wines. *Food Chemistry*. **106**, 397-402.

Yu Xie, D. and Dixon, R. (2005) Tannins and Related Polyphenols (Part 2) Review: Proanthocyanidin biosynthesis – still more questions than answers? *Phytochemistry*. **66**, 2127-2144.

Zoecklein, B., Wolf, T., Duncan, N. and Judge, J. (1992) Effects of fruit zone leaf removal on yield, fruit composition and fruit rot incidence in Chardonnay and Riesling (*Vitis vinifera* L.) grapes. *American Journal of Enology and Viticulture*. **43**, 139-148.

Table 1. Wine analyses post-bottling.

Treatment	Year	pH	Titrateable acidity (g/L)	Volatile acidity (g/L)	Alcohol (% v/v)	Free SO₂ (ppm)	Molecular SO₂ (ppm)	Total SO₂ (ppm)	Residual sugar (g/L)
NLR	2008	3.66	4.8	0.53	12.8	22	0.30	69	1.2
	2009	3.87	4.8	0.26	14.1	19	0.16	33	1.5
LR7	2008	3.65	4.9	0.45	13.1	9	0.12	47	1.2
	2009	3.94	4.7	0.28	14.1	24	0.18	44	1.5
LR30	2008	3.62	4.9	0.49	12.8	9	0.14	47	1.2
	2009	3.90	4.7	0.28	14.0	15	0.13	31	1.6
LRV	2008	3.58	5.1	0.47	12.9	11	0.19	43	1.2
	2009	3.91	4.6	0.26	14.1	12	0.09	24	1.6
Significance									
	Treatment	NS	NS	NS	NS	NS	NS	NS	NS
	Year	***	NS	***	***	NS	NS	**	***
	Tr × Year	NS	NS	NS	NS	NS	NS	NS	NS

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 2. Spectral analysis of the treatment wines by the method from Iland et al. (2000).

Treatment	Year	Colour density (a.u.)	Colour hue	Total red pigments (a.u.)	SO ₂ -resistant pigments (a.u.)	Anthocyanins (mg/L)	Chemical age	Total phenols (a.u.)
NLR	2008	1.67	1.00	5.43	0.49	92	0.094	19.9
	2009	4.44	0.86	12.59	0.89	222	0.071	31.3
LR7	2008	3.09	0.87	5.82	0.75	92	0.128	26.1
	2009	4.55	1.00	12.23	1.02	211	0.084	36.6
LR30	2008	2.84	0.92	5.93	0.66	97	0.112	27.6
	2009	5.31	0.92	13.88	1.08	242	0.078	39.5
LRV	2008	3.00	0.82	5.44	0.59	89	0.109	21.4
	2009	5.03	0.91	14.26	1.04	251	0.073	37.7
Significance								
Treatment		NS	NS	NS	*	NS	*	**
Year		***	NS	***	***	***	***	***
Tr × Year		NS	NS	NS	NS	NS	NS	NS
<i>Contrasts</i>								
NLR vs LR		*	NS	NS	**	NS	*	***
LR7 vs LRV		NS	NS	NS	NS	*	*	NS

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 3. Total tannin concentrations (epicatechin equivalents) during the winemaking process by the methylcellulose precipitation assay.

Treatment	Year	Whole berries (mg/g fresh weight)	Must (mg/L)	Pressed wine (mg/L)	Bottled wine (mg/L)
NLR	2008	3.34	137	315	247
	2009	4.03	55	692	675
LR7	2008	3.51	55	653	615
	2009	4.00	51	788	741
LR30	2008	3.51	94	485	419
	2009	3.87	47	816	807
LRV	2008	3.82	122	517	394
	2009	3.61	39	780	729
<i>Significance</i>					
	Treatment	NS	NS	*	*
	Year	*	NS	***	***
	Tr × Year	NS	NS	NS	NS
<i>Contrasts</i>					
	NLR vs LR	NS	NS	**	**
	LR7 vs LRV	NS	NS	NS	NS

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 4. Tannin extracted (mg/g epicatechin equivalents) from Pinot noir post fermentation skins and seeds.

Treatment	Year	Seeds post fermentation DW (mg/g)	Skins post fermentation DW (mg/g)
NLR	2008	139.7	6.1
	2009	36.5	5.8
LR7	2008	142.8	10.3
	2009	22.6	5.3
LR30	2008	164.4	9.8
	2009	20.1	5.1
LRV	2008	138.2	7.2
	2009	11.0	4.8
Significance			
	Treatment	NS	***
	Year	***	NS
	Tr × Year	NS	NS
<i>Contrasts</i>			
	NLR vs LR	NS	*
	LR7 vs LRV	NS	**

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 5. Concentrations of total terminal and extension units in treatment wines.

Treatment	Year	Terminal units (mg/L)	Extension units (mg/L)
NLR	2008	4.58	32.7
	2009	7.94	59.7
LR7	2008	6.89	52.5
	2009	9.90	57.3
LR30	2008	5.99	40.3
	2009	9.63	63.6
LRV	2008	4.74	29.9
	2009	8.05	51.6
Significance			
Treatment		*	*
Year		***	**
Tr × Year		NS	NS
Contrasts			
NLR vs LR		NS	NS
LR7 vs LRV		*	**

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 6. Proanthocyanidin mean degree of polymerisation (mDP) and composition in all treatment wines.

Treatment	Year	Terminal Units			Extension Units		
		mDP	Catechin (%)	Epicatechin (%)	Epicatechin (%)	Epicatechin gallate (%)	Epigallocatechin (%)
NLR	2008	8.30	66	34	73	1.6	25
	2009	8.52	72	28	77	1.8	21
LR7	2008	8.63	68	33	79	1.6	20
	2009	6.77	67	33	78	1.7	20
LR30	2008	7.82	64	36	88	1.9	11
	2009	7.62	73	27	79	2.0	20
LRV	2008	7.31	68	32	73	1.2	26
	2009	7.49	74	26	77	1.8	21
Significance							
	Treatment	NS	NS	NS	NS	NS	NS
	Year	NS	NS	NS	NS	*	NS
	Tr × Year	NS	NS	NS	NS	NS	NS
Contrasts							
	NLR vs LR	NS	NS	NS	NS	NS	NS
	LR7 vs LRV	NS	NS	NS	NS	NS	NS

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 7. Flavan-3-ol monomer composition of Pinot noir treatment wines.

Treatment	Year	Catechin (mg/L)	Epicatechin (mg/L)	Epicatechin gallate (mg/L)	Gallocatechin (mg/L)	Epigallocatechin (mg/L)
NLR	2008	84.6	42.9	1.06	0.28	4.48
	2009	104.7	36.1	1.40	3.42	2.94
LR7	2008	93.2	39.3	1.20	2.54	4.36
	2009	123.7	44.3	1.79	3.02	2.33
LR30	2008	88.4	39.6	1.11	1.21	4.33
	2009	121.4	41.3	1.74	3.81	2.06
LRV	2008	70.6	32.7	1.10	0.22	2.45
	2009	122.9	45.5	1.65	3.10	2.34
<i>Significance</i>						
	Treatment	**	NS	***	*	**
	Year	***	*	***	***	***
	Tr × Year	**	***	*	*	**
<i>Contrasts</i>						
	NLR vs LR	**	NS	***	NS	**
	LR7 vs LRV	**	NS	*	*	**

Significance levels are: NS is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

Table 8. Quercetin concentrations in treatment wines.

Treatment	Year	Quercetin (mg/L)
NLR	2008	0.63
	2009	0.26
LR7	2008	1.30
	2009	5.35
LR30	2008	2.46
	2009	4.40
LRV	2008	1.94
	2009	0.75
<i>Significance</i>		
	Treatment	***
	Year	***
	Tr ×Year	***
<i>Contrasts</i>		
	NLR vs LR	***
	LR7 vs LRV	***

Significance levels are: * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

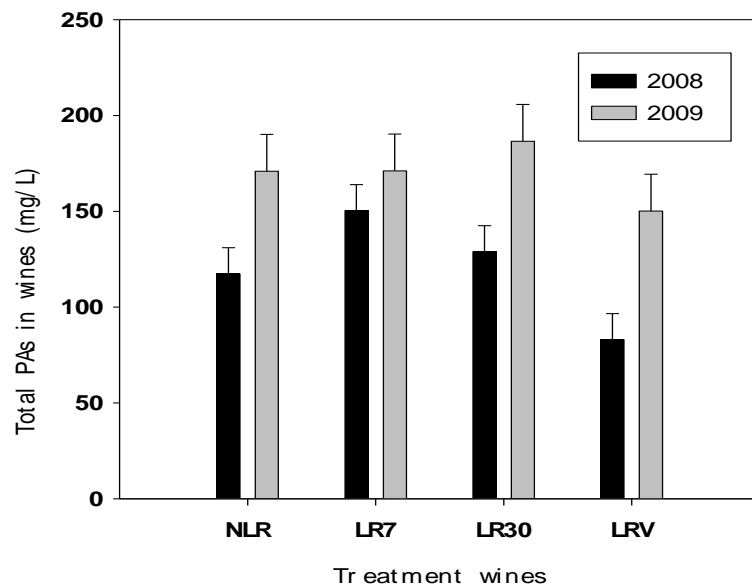


Figure 1. Total proanthocyanidins in wines in 2008 and 2009 by acid catalysis in the presence of phloroglucinol. Bars represent the standard error of the means.

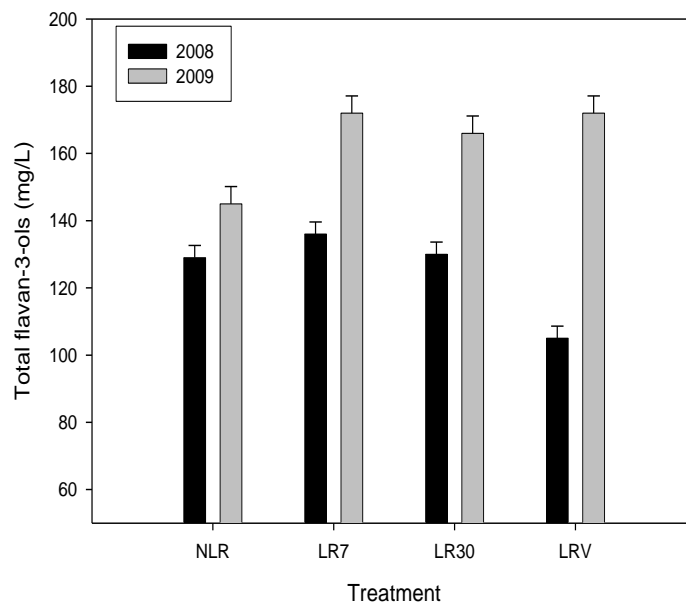


Figure 2. Total flavan-3-ol monomers in Pinot noir wines in 2008 and 2009. ANOVA revealed significant differences between treatments and between years ($P < 0.001$). Bars represent the standard error of the means.

Chapter 6

(A preparation for submission to Australian Journal of Grape and Wine Research)

Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry stable isotope dilution analysis technique to identify “green” and “fruity” volatile aroma compounds in Pinot noir wine.

B. S. Kemp^a, J. Breitmeyer^b, R. Harrison^{a*}, R. Sherlock^b, G. L. Creasy^a

^a *Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.*

^b*Department of Soils and Physical Sciences, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.*

* Corresponding author. Fax: +64 3 3253850. Mail address: Centre for Viticulture and Oenology, Faculty of Agriculture & Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, Canterbury, New Zealand. Email: roland.harrison@lincoln.ac.nz (R. Harrison)

Short running title: HS-SPME-GC-MS-SIDA for “fruity” and “green” compounds.

Abstract

A method was developed to quantify fourteen volatile organic compounds (VOCs) which contribute to the “green” and “fruity” aromas in Pinot noir wine. The method employs stable isotope dilution combined with headspace solid phase microextraction and gas chromatography-mass spectrometry (HS-SPME-GC-MS-SIDA). For each Pinot noir wine sample, three C₆ alcohols, one C₆ aldehyde, eight esters and two C₁₃-norisoprenoids were quantified separately on the mass spectrometer in both scan and selective ion mode by reference to one of six deuterated analogues added as a mixed internal standard to each sample. The separate standard curves prepared for each targeted VOC showed satisfactory repeatability and linearity ($r^2 > 0.9982$). The method requires minimal sample preparation and is suitable for quantifying a wide range of VOCs across a range of chemical classes and molecular weights.

Keywords: Pinot noir, C₆ alcohols, C₆ aldehydes, ethyl esters, C₁₃ norisoprenoids, aroma.

Introduction

Wine aroma derives from the presence in the wine of more than 800 volatile organic compounds (VOCs) (Canuti et al. 2009) including alcohols, esters, acids, terpenes and terpenoids, norisoprenoids, thiols, aldehydes and ketones. However, most of these VOCs are not odour active suggesting that their contribution to wine aroma is minimal (Canuti et al. 2009). Studies on Pinot noir wine have shown that the most odour active VOCs present include alcohols, short chain fatty acids, and ethyl and acetate esters. Important individual odour active VOCs include: acetovanillone, benzaldehyde, benzyl alcohol, β -damascenone, ethyl and methyl vanillate, 3-ethylthio-1-propanol, eugenol, linalool, methionol, methional, γ -nonalactone, γ -octalactone, 2-phenylethanol, *trans*-linalool oxide, vanillin and whisky lactone (Fang and Qian et al. 2005 and 2006, Brander et al. 1980, Miranda-Lopez et al. 1992, Schreier. 1980).

Purported “fruity” ethyl esters are produced during fermentation and their formation can be affected by yeast strain, nitrogen level, temperature and oxygen availability (Fang et al. 2006). Ethyl esters in wines are principally generated as yeast metabolism products formed during the fermentation process and are rarely present to any extent in the grape berry itself. The C₁₃-norisoprenoids also tend to have “fruity” aromas and are formed from elevated levels of carotenoids pre-veraison and by post-veraison degradation by ultraviolet (UV) radiation (Keller 2009, Polaskova et al. 2008). In addition, Escudero et al. (2007) observed that “fruity” aromas in red wines can be enhanced by C₁₃-norisoprenoids. The “green” C₆ alcohols and aldehydes (1-hexanol, *cis*-3-hexen-1-ol, *trans*-3-hexen-1-ol and hexanal respectively) are derived from microbial mediated cleavage of the C-C double bonds in linoleic and linolenic acids, by lipoxygenase and alcohol dehydrogenate enzymes (Salinas et al. 2004). They are present in the grapes and are mainly formed pre-fermentation during transportation, crushing, pressing, must heating and grape maceration (Oliveira et al. 2006).

Headspace Solid Phase Micro Extraction Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) is a technique that is being increasingly utilised to quantify wine aroma compounds. HS-SPME is a rapid and solvent-free sampling technique based on the sorption characteristics (adsorption and absorption) of fibre coating materials (Jung and Ebeler, 2003). When applied to wine analysis, the VOC analytes in a wine sample are encouraged to volatilise into the headspace above the sample in a sealed vial. Then they are sorbed onto a fibre coated with polymer which is introduced into the headspace. Following sorption, the

VOC analytes are thermally desorbed in the heated injection port of a gas chromatograph for separation, identification and quantification. The GC detector most widely employed for this type of analysis is the mass spectrometer (MS), also called a mass selective detector (Jung and Ebeler 2003).

For any HS-SPME analysis preliminary investigations are needed to optimise the analytical conditions. For example, consideration has to be given to the amount and nature of the salt that is typically added to the sample to encourage migration of the VOCs into the sealed vial headspace. SPME fibre extraction temperature, desorption temperature and the choice of SPME fibre for the selectivity of the target compounds are also important considerations (Siebert et al. 2005). The influence of extraction temperature is particularly relevant since it affects both the thermodynamics and kinetics of VOC extraction from the headspace (Noguerol-Pato et al. 2009). It is important also to optimise extraction time for the target analytes to ensure steady-state equilibrium between the fibre and the vapour phase especially if a range of aroma compounds of differing volatilities are being investigated (Noguerol-Pato et al. 2009).

Ethanol in the headspace above a wine sample competes with other VOCs for sorption sites on the SPME fibre and exerts a considerable negative influence on their sorption and quantification. This influence of ethanol, together with other possible matrix effects, all need to be taken into account if accurate quantification is to be achieved (Kalua and Boss, 2008). The negative influence of ethanol is reduced markedly by the simple expedient of diluting the wine sample with water. All these considerations are discussed in detail by Siebert et al. (2005) in their description of the development of a HS-SPME-GC-MS procedure to quantify 31 fermentation-derived aroma compounds present in both red and white wines.

To achieve accurate quantification of any particular VOC in wine requires the addition to the wine sample of a close chemical analogue to act as an internal standard. The best analogue is an isotopically labelled version of the naturally occurring VOC. In particular, deuterated variants are favoured since they will not be present in the original sample itself and they have almost identical sorption and retention properties to their natural counterparts. Siebert et al. (2005) employed synthetic poly-deuterated chemical analogues as internal standards in what is known as the 'Stable Isotope Dilution Analysis' (SIDA) procedure. Ideally, a separate deuterated analogue should be added to the wine sample for each VOC that requires

quantification. However, in practice a single deuterated compound can serve as the internal standard for a number of chemically related VOCs. In this current study six deuterated internal standards were used to quantify effectively fourteen important aroma volatiles.

The aim of this current work was to develop an analytical method using HS-SPME-GC-MS-SIDA to quantify specific aroma compounds in Pinot noir wine associated with “fruity” and “green” aromas. This work was undertaken to support a parallel study of the effects of viticultural treatments on aroma compounds in the Pinot noir wines produced from a field trial and those results are reported elsewhere (Chapter 7). The method described in this current work defines the optimum extraction and analysis conditions for a range of compounds (Table 1) which vary in their volatility and retention time.

Materials and methods

The concentrations of fourteen important aroma compounds (8 esters, 3 C₆-alcohols, 1 C₆ aldehyde and 2 C₁₃-noisoprenoids) detailed in Table 1, were determined using a modification of the HS-SPME-GC-MS-SIDA method described previously by Parr et al. (2007) to measure methoxypyrazine concentrations in Sauvignon blanc wine. Whereas those researchers used just one poly-deuterated internal standard, this current study used six poly-deuterated internal standards (Table 2). Four bottles of unoaked Pinot noir wines (two from 2008 vintage and two from 2009) produced at the Lincoln University Winery from grapes grown in the Waipara region of North Canterbury, South Island, New Zealand were used for method development. The wines were analysed in duplicate and the final analyte concentrations represent the mean of the two subsamples analysed.

Of the fourteen aroma compounds three (hexanal, β -ionone, β -damascenone) were analysed on a separate run due to the unavailability of internal standards d₁₂-hexanal and d₃- β -ionone and calibration standards hexanal and β -damascenone at the outset of the experimentation.

Aroma standards and chemicals

Of the six deuterated internal standards used, two (d₁₃-hexan-1-ol, and 1-phenyl-d₅-ethanol) were purchased from Sigma–Aldrich New Zealand (Table 2). The d₁₂-hexanal used was purchased from SiVac Pty Ltd (New South Wales, Australia). The d₅-ethyl esters (d₅-ethyl butanoate and d₅-ethyl octanoate) and d₃- β -ionone were all synthesised by Lincoln

University, Christchurch, New Zealand. The d₃-β-ionone was synthesised using the protocol developed by Kotseridis et al. (1998). The purity of this standard was determined by using GC-MS analysis of a 5 mg/L standard solution.

Method for production of d₅-ethyl butanoate and d₅-ethyl octanoate

The d₅-ethyl esters were prepared by reacting equi-molar amounts of their acid chlorides with the required amounts of d₆-ethanol (Isotec, Sigma-Aldrich New Zealand Ltd, certified 99.66 atom% deuterated). Synthesis was carried out in 10 mL flasks at 60 °C under reflux conditions for several hours. After cooling, the ethyl ester products were transferred to a separatory funnel, washed with saturated NaHCO₃, then rinsed with saturated NaCl and finally dried using anhydrous Na₂SO₄. The purity of these deuterated standards was verified (Table 2) by injection of a 1.5 µL diluted sample (500 µg/L in hexane) into a Shimadzu GC-MS-QP2010. The GC-MS was fitted with a Restek-5MS fused silica capillary column (30.0 m x 0.25 mm i.d. x 0.25 µm, Shimadzu, Japan). The purity was calculated by comparing the deuterated standard peak area with the total peak area for the chromatogram (data not included here).

The purity of all solvents and analytical standards were tested and validated using GC-MS prior to their use. All of the fourteen (non-deuterated) standards used to generate standard curves for quantitative analysis (Table 3), were obtained from commercial suppliers Sigma–Aldrich, New Zealand Ltd and Merck, New Zealand Ltd. The purity of those compounds was stated by the manufacturer and is reported in Table 3.

Standard and working solutions

Standard solutions were prepared for each of the fourteen aroma compounds in 100% ethanol (Scharlau Chemie S.A, Spain) HPLC Grade ACS ISO UV-VIS. The concentration of each standard solution was individually selected so that when combined with the other standards into a composite standard and then diluted to working strength, a range of concentrations was achieved which fully spanned the anticipated concentrations of the aroma compounds in the wine. The concentrated composite working standard was made by adding appropriate amounts of the standards for the individual compounds to a solution of 10% ethanol in deionised water. This composite standard was then split into small amber vials and stored at -20 °C until it was used. Standards for GC-MS analysis were prepared on the day by serially

diluting this concentrated composite working standard. Each standard vial was prepared in duplicate and standardised to an ethanol concentration of 1.4% (10-fold dilution of a 14% ethanol solution). Standard solutions were also prepared in 100% ethanol for each of the deuterated internal standards. Composite standards were made for the deuterated internal standards; this was performed as described for the non-deuterated standards above.

SPME fibre and conditioning

A 2 cm long Stable flex DVB/CAR/PDMS combination SPME fiber (p/n 57348-U, 50/30 μm thickness, 24 gauge) was selected for this work (Supelco Bellefonte, PA, USA, through Sigma- Aldrich, Australia). Prior to use the SPME fibre was conditioned at 270 $^{\circ}\text{C}$ in the injection port for 1 hour. Prior to sample analysis the SPME fibre was conditioned in helium for 10 minutes at 270 $^{\circ}\text{C}$ in a fibre conditioning station attached to the Combi-Pal auto-sampler used with the Shimadzu GC-MS instrument.

Sample preparation

All wine samples were diluted immediately prior to analysis. This sample dilution involved pipetting 0.9 mL of wine and 8.06 mL of deionised water into 20 mL SPME sample vials followed by 40 μL of the composite deuterated internal standard solution (a 10 fold dilution of the wine) (Table 2). A 5-fold dilution was used initially but some peak shapes showed signs of column overloading, so a 10-fold dilution was used in accordance with Siebert et al. (2005). Crystalline sodium chloride (3.0 g) was added to the SPME vial just prior to capping. Samples were incubated initially for 10 minutes at 60 $^{\circ}\text{C}$ during which time the vial was agitated at 500 rpm. After 10 minutes the SPME fibre was exposed to the headspace of the vial for a period of 60 minutes at 60 $^{\circ}\text{C}$, during which time the headspace volatiles were adsorbed onto the fibre. No agitation was used during the 60 minute extraction period. This was because SPME needles often broke when using agitation with the CTC Combi-Pal autosampler.

GC-MS instrumentation

Automated GC-MS analysis was carried out on a Shimadzu GC-MS-QP2010 gas chromatograph–mass spectrometer (quadrapole) equipped with a CTC Combi-Pal autosampler using Version 5.0 of Shimadzu’s GC-MS solutions data acquisition software. The chromatography was performed using two GC columns in series: a Rtx-Wax 30.0 mm x

0.25 mm ID x 0.5 μ m film thickness (Polyethylene Glycol - Restek, Bellefonte, PA, USA) and a Rxi-1MS 15 m x 0.25 mm ID x 0.5 μ m (100% dimethyl polysiloxane - Restek, Bellefonte, PA, USA). The helium carrier gas was set to a constant linear velocity of 33.5 cm s⁻¹ and the injector was operated in splitless mode for 3 minutes then switched to a 20:1 split ratio. The column oven was held at 35 °C for 3 minutes (during desorption of the SPME fiber), then increased to 250 °C at 4 °C min⁻¹ and held at this temperature for 10 minutes. Total run time was 67 minutes with each aroma compound eluting at the times shown (Tables 2 and 3). The interface and MS source temperatures were set at 250 °C and 200 °C respectively and the MS was operated in electron impact mode (EI) at ionization energy of 70V. Full scan mode was used for 10 of the 14 analytes with selected ions (Tables 2 and 3) being used for the quantification of each aroma compound during post-run data analysis. Hexanal, β -ionone, β -damascenone, and ethyl cinnamate were analysed using single ion monitoring (SIM) mode. The selected ions used for each are listed in Table 3.

Statistical analysis

Limits of detection (LODs) and limits of quantification (LOQs), standard deviations, mean values and graphs were calculated and produced using Excel 2003, Windows XP, Microsoft, USA.

Results and discussion

Method optimisation and extraction conditions

SPME fibre

Previous work by Howard et al. (2005), Martí et al. (2003), Tat et al. (2005) and Noguero-Pato et al. (2009) has shown that DVB/CAR/PDMS SPME fibres are the most suitable for wine aroma profiling due to their sensitivity for different classes of analytes specifically ethyl esters, C₆ alcohols and fatty acids. Silva-Ferreira and Guedes De Pinho (2003) and Noguero-Pato et al. (2009) used a DVB/CAR/PDMS SPME fibre to analyse alcohols, and C₁₃ norisoprenoids in white wine. Rodrigues et al. (2008) used the same type of SPME fibre for the analysis of the same compounds as well as ethyl esters in white wines, beer and whiskys. Fan et al. (2010) found that the DVB/CAR/PDMS SPME fibre extracted more polar and middle polar compounds compared to carboxen/PDMS or the PDMS fibre and was the most suitable SPME fibre to extract volatile compounds from grapes. The volatile compounds extracted in their study included hexanal, ethyl esters, β -damascenone, β -ionone and C₆

alcohols. Canuti et al. (2009) found that the DVB/CAR/PDMS fibre was more suited to C₁₃ norisoprenoids and alcohols, as well as the more polar aldehydes. Therefore, the DVB/CAR/PDMS SPME fibre was utilised in our method due to its demonstrated suitability for the analysis of different molecular weight aroma compounds in the Pinot noir wines.

Adsorption temperature and time

Adsorption temperature is an important part of the SPME process as higher temperatures will increase the partial vapour pressure of analytes in the headspace but simultaneously sorption time onto the fibre will decrease, especially for volatile components (Antalick et al. 2010). In the current study four extraction temperatures (40, 50, 60 and 70°C) and three extraction times (40, 50 and 60 minutes) yielded twelve temperature/time combinations which were investigated in triplicate. A summary of results is shown in Figures 1 and 2. The same mixed diluted standard was put into each of 36 vials, 3 g of NaCl was added and the vials were capped. The vials were then allowed to incubate with agitation for 10 minutes at the selected temperature (40, 50, 60 or 70 °C) before headspace extraction for the selected time (40, 50 or 60 minutes). The concentrations of the twelve aroma compounds in the mixed diluted standard were selected to represent the approximate concentrations in diluted wine as follows: ethyl acetate 36443 µg/L, ethyl isobutyrate 100 µg/L, ethyl butanoate 48.2 µg/L, ethyl pentanoate 1.30 µg/L, ethyl hexanoate 78.1 µg/L, hexanol 651 µg/L, trans-3-hexen-1-ol 39.0 µg/L, cis-3-hexen-1-ol 39.0 µg/L, ethyl octanoate 65.1 µg/L, ethyl decanoate 26.0 µg/L, β-ionone 0.117 µg/L, ethyl cinnamate 1.30 µg/L.

Results showed that adsorption of the ethyl esters and C₆ alcohols onto the SPME fibre decreased with increasing extraction temperature. Conversely adsorption of β-ionone and ethyl cinnamate increased with increasing extraction temperature. Carrillo et al. (2006) found that the best response for β-ionone was obtained at 70 °C with an extraction time of 60-75 mins without agitation. Antalick et al (2010) showed that aromatic esters such as ethyl cinnamate increased with increasing temperature whilst straight chain esters such as ethyl butanoate decreased with increasing temperature. Noguero-Pato et al. (2009) found the optimal extraction temperature for esters ethyl hexanoate and ethyl decanoate was 42.5 °C. This is consistent with the trend seen in Figure 1. Their work also showed that ethyl butanoate and C₆ alcohols were best extracted at 25 °C indicating that adsorption by the SPME fibre of these aroma compounds decreases with increasing temperature.

In this current work the optimisation process focused on β -ionone and ethyl cinnamate as they were expected to show the lowest concentration of the fourteen analytes. Improving the sensitivity for these two compounds was a priority. It was decided that 60 °C was the best extraction temperature to accommodate all twelve analytes.

It was found that increasing extraction time resulted in an increase in peak areas for all twelve aroma compounds tested. Antalick et al. (2010) found that for aromatic esters, the sorption increases markedly until 30 minutes, then more gradually from 30-60 minutes. Noguero-Pato et al. (2009) reported that for low volatility compounds such as β -ionone, 45 minutes is not enough to reach equilibrium. Additionally sorption of the volatile C₆ alcohols and ethyl hexanoate (with boiling points lower than 200° C) decrease from 45-90 minutes. Antalick et al. (2010) showed that for less volatile esters sorption is rapid until 5 minutes and continues to increase gradually until 45 minutes then declines thereafter. Fan et al. (2005) reported that the sorption of short chain fatty acid esters decreases whilst the long chain fatty acid esters increase with increasing extraction time.

An adsorption time of 60 minutes was used in our study as all twelve aroma compounds tested showed a maximum peak area within the GC-MS at the selected extraction temperature of 60 °C. Initial optimisation tests did not include hexanal or β -damascenone due to their unavailability at the time of testing. Nevertheless, when these compounds became available the final selected temperature/time combination (60 °C for 60 minutes) proved very suitable for quantifying those compounds too.

Desorption time and temperature

Desorption of the volatile compounds occurred when the fiber was inserted into the GC injection port for 10 minutes at 270 °C. An initial desorption time of 5 minutes resulted in a carryover effect on the fibre so desorption time was increased to 10 minutes to allow for the cleaning of the remaining adsorbed compounds without damaging the fibre (Antalick et al. 2010). Carrillo et al. (2006) used a desorption temperature of 260 °C for 7 minutes, while Fan et al. (2010) desorbed the SPME fibre at 250 °C for 5 minutes. The recommended conditioning temperature range is 250–270 °C for a DVB/CAR/PDMS 50/30 μ m SPME fibre (Supelco Bellefonte, PA, USA). Therefore a desorption temperature of 270 °C was used in

our study to maximise the removal of the most adsorbed aroma compounds, namely the least polar esters.

Matrix effect and sensitivity

Ionic strength of the wine samples

The distribution constants between the liquid phase and the coating are strongly dependent on the wine matrix (Zhang et al. 1994). In wine besides ethanol content, which improves the solubility of organic compounds compared to their solubility in aqueous solution, there are hundreds of compounds, e.g. proteins, amino acids, sugars, polyphenols and tartrates that contribute to the matrix and can interfere in the HS-SPME analysis (De la Calle Garcia et al. 1998). In our study crystalline sodium chloride (3.0 g, 33.3% w/v) was added to the SPME sample vial (9 mL total volume) prior to sealing with aluminum crimp-tops. Noguero-Pato et al. (2009) studied the addition of salt (sodium chloride) and found that 30% w/v enhanced extraction for most of the compounds tested. Carrillo et al (2006) reported that the best responses were obtained with 30% sodium chloride (saturated solution). Howard et al. (2005) explains that virtually all analysis of aroma volatiles in wine use salting out to increase the levels of analytes in the headspace before extraction. The dissociated ions disrupt the sample matrix by decreasing the solubility of the aroma molecules, which then partition more readily into the headspace. Additionally the addition of salting out agents improves extraction efficiency due to a decrease in the solubility of the analytes, thus increasing the amount of analyte sorbed onto the fibre (Canuti et al. 2009). Fan et al. (2010) highlighted that the effect of adding salt also alters the phase boundary, encouraging the volatilization of analytes dissolved in the liquid phase into the headspace.

Sample dilution

Dilution of the wine sample was necessary to prevent column overloading when using splitless injection mode. This served to aid both the chromatography and the SPME fibre extraction procedure as it reduced the competition pressure for sites on the SPME fibre, resulting in a more linear response for most compounds. In the study carried out by Kalua et al. (2008) wine volatile analysis did not show an optimum for dilution suggesting that wine dilution does not affect HS-SPME results. However, Robinson et al. (2009) found that with increasing ethanol content a significant decrease in compound extraction occurs, whereas dilution with deionised water results in a significant increase in analyte sorption on the SPME

Dilution of the wine samples reduced the matrix effect induced by high ethanol concentrations, the ethanol concentration having been effectively diluted by a factor of 10-fold to approximately 1.4 %. The mean values ($\mu\text{g/L}$) of the analyte concentrations were multiplied by 10 to allow for the dilution factor and are presented in Table 5.

Validation and calibration

Linearity

To quantify wine VOCs using HS-SPME-GC-MS-SIDA requires a standard curve to be generated for each VOC. Each standard curve is a graph of the peak area ratio of selected ions (characteristic of the VOC of interest) and of the respective deuterated internal standard plotted against the concentration of the VOC of interest. Seven diluted standards were used to generate the calibration curve for each aroma compound (an example of which is shown in Figure 3). Quadratic curves were fitted for all analytes, due to the curvilinear relationship for some analytes such as ethyl acetate and ethyl decanoate. Figure 3 shows the *cis*-3-hexen-1-ol calibration curve and this near linear relationship was typical for 10 out of the 14 calibration curves. Correlation coefficients (R^2) were greater than 0.9982 for all analytes (Table 3).

Initial ethyl decanoate values in actual wine samples fell beyond the upper limit of the corresponding calibration curve. Subsequent spiked recovery tests showed that both ethyl octanoate and ethyl decanoate were being overestimated by their calibration curves. Further investigation found that the peak area of the internal standard d_5 -ethyl octanoate had declined over a 43 hour run as illustrated in Figure 4. This figure shows a comparison with the d_5 -ethyl butanoate peak areas where only a small decline was observed. The decreasing peak area for d_5 ethyl octanoate had the effect of altering the peak area ratio with the non-deuterated, naturally occurring ethyl octanoate in the samples resulting in an apparent increase in the reported concentration. Antalick et al. (2010) reported that internal standard d_5 -ethyl decanoate decreased significantly after 8 hours in the wine matrix but d_5 -ethyl octanoate did not show signs of instability although it did in this current study. The use of d_5 -ethyl octanoate needs to be tested further to determine if this internal standard can be stabilised or whether an alternative standard should be investigated.

As previously mentioned, β -ionone, β -damascenone and hexanal were analysed on a separate GC-MS analysis run to the esters. Like d_5 -ethyl octanoate it was found that d_3 - β -ionone also degraded during an analysis run on the GC-MS causing a similar over estimation of the

concentration for both β -ionone and β -damascenone. However this degradation did not occur to the same extent as for d₅ethyl octanoate. Check standards were placed throughout the 67.5 hour run to monitor the overestimation. A check standard is defined as a calibration standard run as a sample in contrast to a calibration standard which is a standard used to construct a calibration curve. A linear drift calculation was applied to correct the sample results using the comparison of the check standards to the calibration standards. Sample results were divided by the ratio of the concentration of the check standard to the calibration standard. This ratio was applied incrementally with the first sample measurement assigned a value of 1 and then increasing to the value of the check standard/calibration standard concentration ratio. The applied correction factor was method validated by assessing the spiked recoveries for the two analytes, namely 97.9% and 97.4% for β -damascenone and β -ionone respectively.

Limits of detection and quantification.

The limits of detection (LODs) were estimated as the concentration of the analyte that produces a signal-to-noise ratio of three times the standard deviation (concentration for signal/noise = 3) (Antalick et al. 2010). The limit of quantification (LOQs) is the concentration of the signal-to-noise ratio of ten times the standard deviation (concentration for signal/noise = 10). LOD values ranged from 0-0.085 $\mu\text{g/L}$ and LOQ values ranged from 0.000-0.283 $\mu\text{g/L}$ as shown in Table 4. No analytes were found to be below these concentration limits for the wine samples reported in Table 5.

Repeatability and Reproducibility

Spiked recovery tests

A spiked recovery test was used to assess the matrix effect on each analyte. Selected wine samples were spiked with an aliquot of the concentrated composite working standard to the concentration level of standard 3 (seven standards were used in the calibration curve). The spiked wine samples were then diluted 10 times (0.9 mL wine, 8.06 mL deionised water, 40 μL internal standards) in the SPME vial. Spiked samples were analysed in duplicate and compared to their unspiked partners. The concentration difference was then compared with the expected analyte concentration, as determined by standard 3 of the calibration curve, and reported as a percentage (Table 4). Four samples of a Pinot noir wine were used for the spiked recovery test with the mean results reported in Table 4. Two of the fourteen analytes exhibited high spiked recovery results, two of which can be explained by the instability of d₅-

ethyl octanoate (see *Linearity* above). Ethyl acetate had a recovery result of 119% which indicates that the use of the internal standard d₅ ethyl butanoate may not be suitable for this compound. Siebert et al (2005) utilised d₈-ethyl acetate as the internal standard for ethyl acetate and reported that for spiked wine samples the accuracy and repeatability of the analyses was <5% RSD (relative standard deviation) for all concentrations investigated within the calibration range. Siebert et al (2005) also highlighted the need for a 1/100 dilution for ethyl acetate to improve the accuracy of quantification. This was able to be addressed in our current work by reducing the MS detector gain during the period where ethyl acetate elutes.

Repeatability and reproducibility was also checked by calculating the standard deviation (SD) of the wine aroma concentrations (sample repeats) and confirming that the coefficients of variations did not exceed 20% as shown in Table 5.

Concentrations of aroma compounds in Pinot noir wines

The concentrations of the target analytes in each of the four Pinot noir wines from 2008 and 2009 vintages are listed in Table 5. It is difficult to compare results for these specific aroma compounds with previous research due to the limited number of published papers regarding Pinot noir aromas. However, the concentrations of the C₆ alcohols and the aldehyde hexanal are lower in our study than in the work by Oliveira et al. (2006) who identified hexanol, trans-3-hexen-1-ol and cis-3-hexen-1-ol in red wines from Portugal using GC-MS. These differences could be due to varietal concentration variations as well as differences in the methods employed. In the study by Fang and Qian (2004), two “green” aromas showed potential importance in Pinot noir, namely trans-3-hexen-1-ol and cis-3-hexen-1-ol. It was reported that trans-3-hexen-1-ol had higher aroma extract dilution analysis (AEDA) values than cis-3-hexen-1-ol. Similarly, the concentration of trans-3-hexen-1-ol was far greater than cis-3-hexen-1-ol in our study.

Fang and Qian (2006) employed a stir bar sorptive extraction-gas chromatography – mass spectrometry (SBSE-GC-MS) technique to investigate thirty three Pinot noir wine aroma compounds. The extraction theory for SBSE is the same as for SPME but with a higher phase ratio coating (Salinas et al. 2004). The extraction polymer is placed in the liquid phase for SBSE rather than the headspace and requires modification to the GC inlet for automated sampling (Canuti et al. 2009). The range of concentrations reported by Fang and Qian (2006)

for ethyl hexanoate and ethyl octanoate using an SBSE method was lower than in our study but this could be due to the different analytical methods employed as SIDA was not used, time and temperature conditions used, climate, and winemaking techniques. However, ethyl cinnamate concentrations in our study range from 1.04-2.79 µg/L and show an agreement with the concentrations observed by Fang and Qian (2006) which were 1.92-6.36 µg/L.

β-Ionone concentrations ranged from 0.38-0.63 µg/L in our study in agreement with results by Fang and Qian (2006) who identified the compound using SBSE. Additionally, Noguero-Pato et al. (2009) used HS-SPME-GC-MS to detect β-ionone in red wine from Mençía, Spain and reported a concentration of 0.45 µg/L. β-damascenone concentrations ranged from 3.02-4.17 µg/L in our study whereas Kotseridis et al. (1999) used a GC-MS-SIDA method and reported levels of 2.63-2.89 µg/L in Burgundian Pinot noir.

Conclusion

A HS-SPME-GC-MS-SIDA method was developed for the simultaneous identification and quantification of fourteen important aroma compounds in Pinot noir wine. Three C₆ alcohols, one C₆ aldehyde, eight esters and two C₁₃-norisoprenoids were quantified separately on the mass spectrometer in both scan and selective ion mode by reference to one of six deuterated analogues added as a mixed internal standard to each sample. Sample preparation is minimal and simply requires a 10-fold dilution with deionised water and the addition of sodium chloride. Deuterated internal standards were used to provide improved accuracy in the quantification of the analytes. However, one deuterated internal standard, d₅-ethyl octanoate, appeared to degrade slowly within the diluted wine matrix and this exaggerated the reported analyses for its non-deuterated, naturally occurring, analogue in the wine which appeared not to degrade. The degradation of this particular deuterated standard requires further investigation. The method was validated by the measurement of linearity, repeatability and reproducibility through spiked recovery experiments. With the possible exception of ethyl octanoate, the method is therefore a reliable analytical tool to measure viticultural and oenological treatment affects especially in the examination of fruity and green aromas in Pinot noir wines.

Acknowledgements

The assistance of Len Ibbotson for synthesising the deuterated esters and Elizabeth Tomasino for formulating the primary and secondary standards is gratefully acknowledged. Also we thank Lincoln University and New Zealand Winegrowers for funding the research and Pegasus Bay Winery in kind contributions.

References

- Antalick, G., Perello, M. and de Revel, G. (2010) Development, validation and application of a specific method for the Quantitative determination of wine esters by Headspace-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry. *Food Chemistry*. **121**, 1236-1245.
- Brander, C., Kepner, R. and Webb, A. (1980) Identification of some volatile aroma compounds of wine in *Vitis vinifera* cultivar Pinot noir. *American Journal of Viticulture and Enology*. **31**, 69-74.
- Canuti, V., Conversano, M., Li Calzi, M., Heymann, H., Matthews, M. and Ebeler, S. (2009) Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. *Journal of Chromatography A*. **1216**, 3012-3022.
- Carrillo, J., Garrido-Lopez, A. and Tena, M. (2006) Determination of volatile oak compounds in wine by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Chromatography A*. **1102**, 25-36
- De la Calle Garcia, D., Reichenbacher, M., Danzer, K., Hurlbeck, C., Bartsch, C. and Feller, K. (1998) Analysis of wine bouquet components using headspace solid-phase microextraction-capillary gas chromatography. *Journal of High Resolution Chromatography*. **21**, 373–377.
- Escudero, A., Campo, E., Farina, L., Cacho, J. and Ferreira, V. (2007) Analytical characterisation of the aroma of five premium red wines. Insights into the role of odour families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*. **11**, 4501-4510.
- Fan, W., Xu, Y., Jiang, W. and Li, J. (2010) Identification and quantification of impact aroma compounds in four non-floral *Vitis vinifera* varieties of grapes. *Journal of Food Science*. **75**, 81-88.
- Fan, W. and Qian, M. (2005) Headspace solid phase microextraction and gas chromatography-olfactory dilution analysis of young and aged Chinese “Yanghe Daqu” liquors. *Journal of Agricultural and Food Chemistry*. **53**, 7931-7938.
- Fang, Y. and Qian, M. (2004) Aroma compounds in Oregon Pinot noir wine determined by aroma extract dilution analysis (AEDA). *Flavour and Fragrance Journal*. **20**, 22-29.
- Fang, Y. and Qian, M. (2006) Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *Journal of Agricultural and Food Chemistry*. **54**, 8567-8573.
- Herderich, M., Bell, S., Holt, H., Ristic, R., Birchmore, W., Thompson, K. and Iland, P. (2004) Grape maturity and tannins: the impact of viticultural treatments on grape and wine tannins. Conference proceedings, Twelfth Australian Wine Industry Technical Conference, Melbourne, Victoria. 24th – 29th July. 2004. Pp 79-84.

Howard, K. L., Mike, J. H. and Riesen, R. (2005) Validation of a solid-phase micro extraction method for headspace analysis of wine aroma components. *American Journal of Enology and Viticulture*. **56**, 37-45.

Jung, D., M. and Ebeler, S., E. (2003) Headspace solid-phase microextraction method for the study of the volatility of selected flavour compounds. *Journal of Agricultural and Food Chemistry*. **51**, 200-205.

Kalua, C. and Boss, P. (2008) Sample preparation optimisation in wine and grapes, dilution and sample/headspace volume equilibrium theory for headspace solid-phase microextraction. *Journal of Chromatography A*. **1192**, 25-35.

Keller, M. (2009) Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research*. **16**, 56–69

Kotseridis, Y., Baumes, R. and Skouroumounis, G., K. (1998) Synthesis of labelled [²H₂]2-methoxy-3-isobutylpyrazine, [²H₃]α-ionone, and [²H₃]β-ionone, for quantification in grapes, juices and wines. *Journal of Chromatography A*. **824**, 71-78.

Kotseridis, Y., Baumes, R. and Skouroumounis, G. (1999) Quantification of free and hydrolytically liberated β-damascenone in red grapes and wines using a stable isotope dilution assay. *Journal of Chromatography A*. **849**, 245-254.

Martí, M. P., Mestres, M., Sala, C., Busto, O. and Guasch, J. (2003) Solid-phase micro-extraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *Journal of Agricultural and Food Chemistry*. **51**, 7861-7865.

Miranda-Lopez, R., Libbey, L. and Watson, B. (1992) Identification of additional odor-active compounds in Pinot noir wines. *American Journal of Enology and Viticulture*. **43**, 90-92.

Noguerol-Pato, R., Gonzalez-Barreiro, C., Cancho-Grande, B. And Simal-Gandra, J. (2009) Quantitative determination and characterisation of the main odourants on Mencia monovarietal red wines. *Food Chemistry*. **117**, 473-484.

Oliveira, J., Sá, F., Barros, F. and Araújo, I. (2006) C₆-alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta*. **563**, 300–309.

Parr, W., Green, J., White, G. and Sherlock, R. (2007) The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. *Food Quality and Preference*. **18**, 849-861.

Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J. and Camara, J. (2006) Analytical characterisation of the aroma of Tinto Negra Mole red wine: Identification of the main odorant compounds. *Analytica Chimica Acta*. **563**, 154-164.

Polaskova, P., Herszage, J. and Ebeler, S. (2008) Wine flavour: chemistry in a glass. *Chemical Society Review*. **37**, 2478-2489.

- Robinson, A., Ebeler, S., Heymann, H., Boss, P., Solomon, P. and Trengrove, R. (2009) Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry*. **57**, 10313-10322.
- Rocha, S., Ramalheira, V., Barros, A., Delgadillo, I. and Coimbra, M. (2001) Headspace solid phase microextraction (SPME) analysis of flavour compounds in wines. Effect of the matrix volatile composition in the relative response factors in a wine model. *Journal of Agricultural and Food Chemistry*. **49**, 5142–5151.
- Rodrigues, F., Caldeira, M. and Camara, J. (2008) Development of a dynamic headspace solid-phase microextraction procedure coupled to GC-q-MSD for evaluation of the chemical profile of alcoholic beverages. *Analytica Chimica Acta*. **609**, 82-104.
- Salinas, R., Zalacain, A., Pardo, F. and Alonso, G. (2004) Stir bar sorptive extraction applied to volatile constituents evolution during *Vitis vinifera* ripening. *Journal of Agricultural and Food Chemistry*. **52**, 4821-4827.
- Schreier, P. (1980) Wine aroma composition: Identification of additional volatile constituents of red wine. *Journal of Agricultural and Food Chemistry*. **28**, 926-928
- Siebert, T., Smyth, H., Capone, D., Neuwohner, C., Pardon, K., Skouroumounis, G., Herderich, M., Sefton, M. and Pollnitz, A. (2005) Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Analytical and Bioanalytical Chemistry*. **381**, 937-947.
- Siebert, T., Wood, C., Elsey, G. and Pollnitz, A. (2008) Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *Journal of Agricultural and Food Chemistry*. **56**, 3745–3748
- Silva Ferreira, A. and Guedes De Pinho, P. (2003) Analytical method for determination of some aroma compounds in white wines by solid phase microextraction and gas chromatography. *Journal of Food Science*. **68**, 2817-2820.
- Tat, L., Comuzzo, P., Stolfo, I. and Battistutta, F. (2005) Optimization of wine head space analysis by solid-phase micro-extraction capillary gas chromatography with mass spectrometric and flame ionisation detection. *Food Chemistry*. **93**, 361-369.
- Zhang, Z., Yang, M. J. and Pawliszyn, J. (1994) Solid-phase microextraction. A solvent-free alternative for sample preparation. *Analytical Chemistry*. **66**, 844-854.

Table 1 Volatile aroma compounds in Pinot noir wines. Descriptor and odour threshold information taken from Escudero et al. (2007), Robinson et al. (2009), and Fang and Qian (2005).

Analyte	Descriptor	Chemical group	Odour Threshold ($\mu\text{g/L}$)
Ethyl acetate	Blackcurrant/sweet fruity/tart	Ester	12270
Ethyl isobutyrate	Apple/fruity	Ester	20
Ethyl butanoate	Peach/fruity/sweet	Ester	0.02
Ethyl pentanoate	Mint/green fruity	Ester	1-1.5
Ethyl hexanoate	Blackberry/strawberry/fruity/anise	Ester	14
Ethyl octanoate	Green fruity floral/cooked	Ester	580
Ethyl cinnamate	Cherry/fruity/cinnamon	Ester	1.1
Ethyl decanoate	Grapey/fruity	Ester	200
Hexanol	Grass/dry grass/toasted	C ₆ alcohol	8000
Trans-3-hexen-1-ol	Grass/bitter	C ₆ alcohol	400
Cis-3-hexen-1-ol	Fresh cut grass/green	C ₆ alcohol	400
Hexanal	Green grass	C ₆ aldehyde	4.5-5
β -ionone	Berry/violet	C ₁₃ -norisprenoid	0.007
β -damascenone	Fruity/	C ₁₃ -norisprenoid	0.002

Table 2. Internal standard information.

Internal Standards	ISTD ID No	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	CAS No	Supplier
d ₅ -Ethyl butanoate	1	11.78	93	34 (94.96), 106 (15.16)	-	<i>Synthesised by Lincoln University</i>
d ₁₂ -Hexanal	2	12.53	80	64 (271.20), 92 (36.47)		Sivac
d ₁₃ -Hexan-1-ol	3	22.37	62	50 (111.97), 78 (79.19)	16416-34-5	Sigma
d ₅ -Ethyl octanoate	4	26.92	106	74 (106.69), 134 (30.84)	-	<i>Synthesised by Lincoln University</i>
1-Phenyl-d ₅ -ethanol	5	36.72	112	84 (89.20), 127 (28.00)	90162-45-1	Sigma
d ₃ -β-Ionone	6	40.92	180	46 (70.54), 130 (10.86)		<i>Synthesised by Lincoln University</i>

Table 3. Quantification parameters for the fourteen analytes.

Analyte	ISTD ^a Used	RT (mins)	Target Ion m/z	Confirming Ions m/z (% to Target Ion)	Calibration Range µg/L (1/10 dilution)	Standard Curve (R ²) ^b	Purity (%)	CAS No	Supplier
Ethyl acetate	1	6.53	61	70 (94.79), 73 (32.89), 88 (32.97)	0 – 36120.3	0.9982	99.5%	141-78-6	Merck
Ethyl isobutyrate	1	9.60	71	88 (35.83), 116 (27.74)	0 - 99.33	0.9999	99%	97-62-1	Sigma
Ethyl butanoate	1	11.90	88	101 (16.10) 60.00 (34.46)	0 – 47.73	1.0000	99%	105-54-4	Sigma
Hexanal	2	12.87	56	57 (70.60), 75 (25.60), 82 (20.51)	0 – 4.88	0.9994	98%	66-25-1	Sigma
Ethyl pentanoate	1	15.66	88	85 (90.01), 101 (26.37)	0 -1.29	0.9999	98%	539-82-2	Sigma
Ethyl hexanoate	1	19.63	88	99 (51.76), 60 (35.08), 101(27.01)	0 – 77.4	0.9996	98%	123-66-0	Sigma
Hexanol	3	22.84	69	55 (155.61), 84 (18.12)	0 - 645	0.9999	99%	111-27-3	Sigma
Trans-3-hexen-1-ol	3	23.07	67	82 (63.76), 100 (4.85)	0 – 38.7	0.9999	98%	928-97-2	Aldrich
Cis-3-hexen-1-ol	3	23.76	41	67 (89.83), 82 (42.93)	0 – 38.7	0.9998	98%	928-96-1	Fluka
Ethyl octanoate	4	27.06	101	70 (79.43), 129 (28.62)	0 – 64.5	0.9993	99%	106-32-1	Sigma
Ethyl decanoate	4	33.72	88	70 (24.54), 60 (15.74)	0 – 25.8	0.9993	99%	110-38-3	Sigma
β-damascenone	6	37.79	69	121 (58.80), 105 (21.85)	0 – 1.46	0.9986	1.2% ^c	23696-85-7	SAFC
β-ionone	6	40.99	177	135 (19.07), 192 (5.20)	0 – 0.1161	0.9990	96%	14901-07-6	Sigma
Ethyl cinnamate	5	45.46	176	77 (168.00), 131 (455.00)	0 – 1.29	0.9905	99%	60-12-8	Sigma

^a ‘ISTD’ = “internal standard” (see Table 2)

^b a quadratic function was fitted to each aroma compound standard curve. Seven standards were used to generate each curve, the exceptions being hexanal, β-damascenone and β-ionone which all used six.

^c 1.2% wt in 190 proof ethanol

Table 4. Limits of detection (LODs) and quantification (LOQs). Recovery of compounds from spiked samples expressed as percentage ($n = 2$).

Analyte	% Recovery	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)
Ethyl acetate	119	0.038	0.128
Ethyl isobutyrate	100	0.052	0.172
Ethyl butanoate	100	0.018	0.059
Ethyl pentanoate	113	0.001	0.005
Ethyl hexanoate	108	0.058	0.193
Ethyl octanoate	146 ^a	0.069	0.230
Ethyl cinnamate	147 ^b	0.009	0.029
Hexanal	88	0.013	0.045
Hexanol	112	0.085	0.283
Trans-3-hexen-1-ol	96	0.011	0.037
Cis-3-hexen-1-ol	99	0.011	0.038
β -damascenone	98	0.016	0.053
β -ionone	97	0.000	0.002

^a High recovery result due to degradation of the internal standard d_5 ethyl octanoate.

^b Overestimated recovery result shows 1- phenyl d_5 -ethanol was not suitable as the internal standard.

Table 5. The mean concentrations of the duplicate samples of aroma compounds ($n=2$) in the four Pinot noir wines analysed by HS-SPME-GC-MS-SIDA. SD = standard deviation. CV% = coefficient of variation.

Compound	Wine 1 2008	SD	%CV	Wine 2 2008	SD	%CV	Wine 1 2009	SD	%CV	Wine 2 2009	SD	%CV
Ethyl acetate (mg/L)	100.4	±1.3	1.3	93.7	±9.7	10.0	94.5	±9.0	1.0	101.0	±3.7	3.7
Ethyl isobutyrate (µg/L)	327.0	±6.0	2.0	286.4	±7.0	2.4	84.6	±1.0	1.4	111.6	±1.0	1.3
Ethyl butanoate (µg/L)	184.2	±3.0	1.8	171.5	±0.9	0.5	206.0	±0.4	0.2	232.2	±1.0	0.5
Ethyl pentanoate (µg/L)	2.1	±0.01	0.4	2.4	±0.01	0.5	2.4	±0.02	0.7	2.8	±0.1	4.7
Ethyl hexanoate (µg/L)	489.1	±27.0	5.6	539.3	±4.0	0.8	594.5	±3	0.7	542.4	±1.0	0.2
Ethyl octanoate (µg/L)	424.3	±13	3.1	424.4	±0.01	0.01	360.2	±19.0	5.3	426.0	±14.0	3.4
Ethyl cinnamate (µg/L)	2.5	±0.6	22.8	2.8	±0.6	20.0	1.0	±0.05	4.7	1.1	±0.04	3.9
Hexanol (µg/L)	5136	±11.0	0.2	4601	±65	1.4	5618.2	±55	1.0	5985	±29.0	0.5
Trans-3-hexen-1-ol (µg/L)	133.3	±0.6	0.4	112.5	±4.0	3.7	152.0	±4.0	2.4	156.4	±3.0	1.9
Cis-3-hexen-1-ol (µg/L)	55.8	±0.5	0.9	35.9	±0.3	0.8	85.2	±1.0	1.4	89.0	±1.0	1.2
Hexanal (µg/L)	4.7	±0.3	6.9	4.2	±0.1	2.8	3.4	±0.2	5.7	4.1	±0.1	3.1
β-damascenone (µg/L)	3.0	±0.04	1.4	3.9	±0.1	3.0	12.4	±0.1	0.9	14.2	±0.3	2.0
β-ionone (µg/L)	0.38	±0.01	3.1	0.58	±0.01	2.2	0.5	±0.01	0.7	0.6	±0.01	1.9

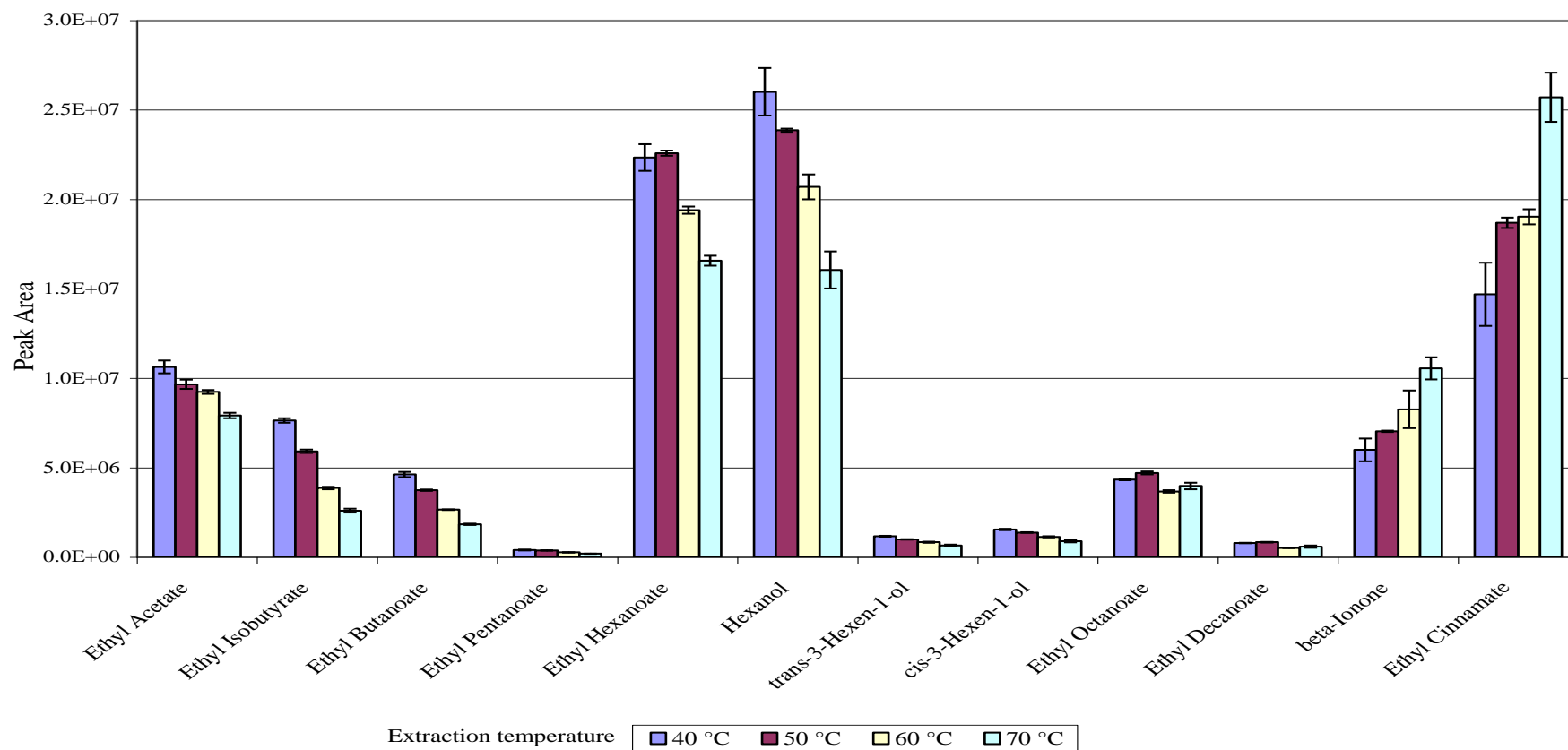


Figure 1. The effect of increasing the adsorption extraction temperature on peak areas with an extraction time of 60 minutes. Different gains were applied to increase the peak areas of some compounds and to decrease others in order to avoid detector overloading. β -Ionone and ethyl cinnamate were analysed in SIM (single ion monitoring) mode with large gains applied. Error bars represent standard error of the means. ($n = 3$)

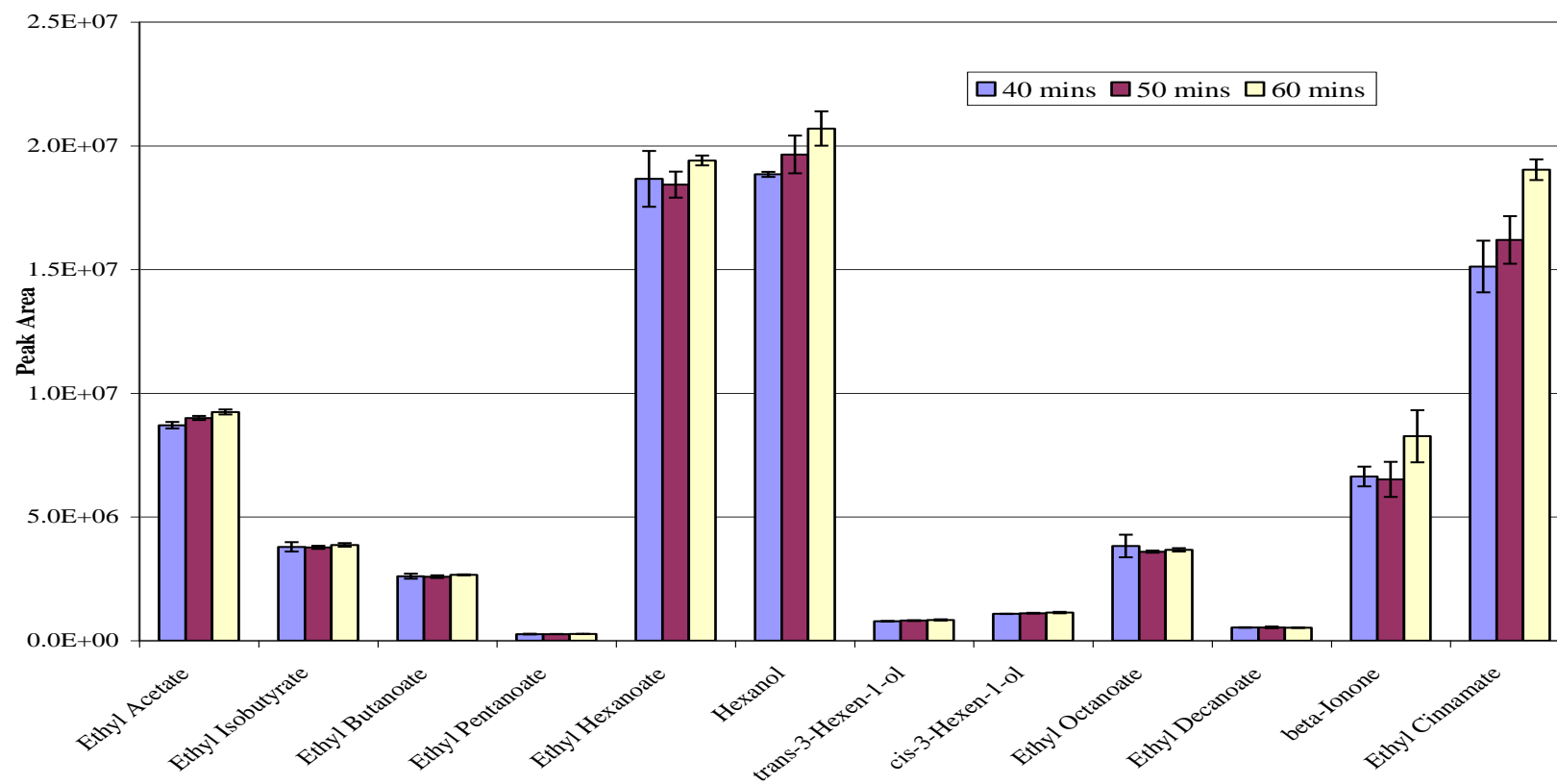


Figure 2. The effect of increasing the adsorption extraction time on peak areas with an extraction temperature of 60 °C. Error bars represent the standard error of the means.

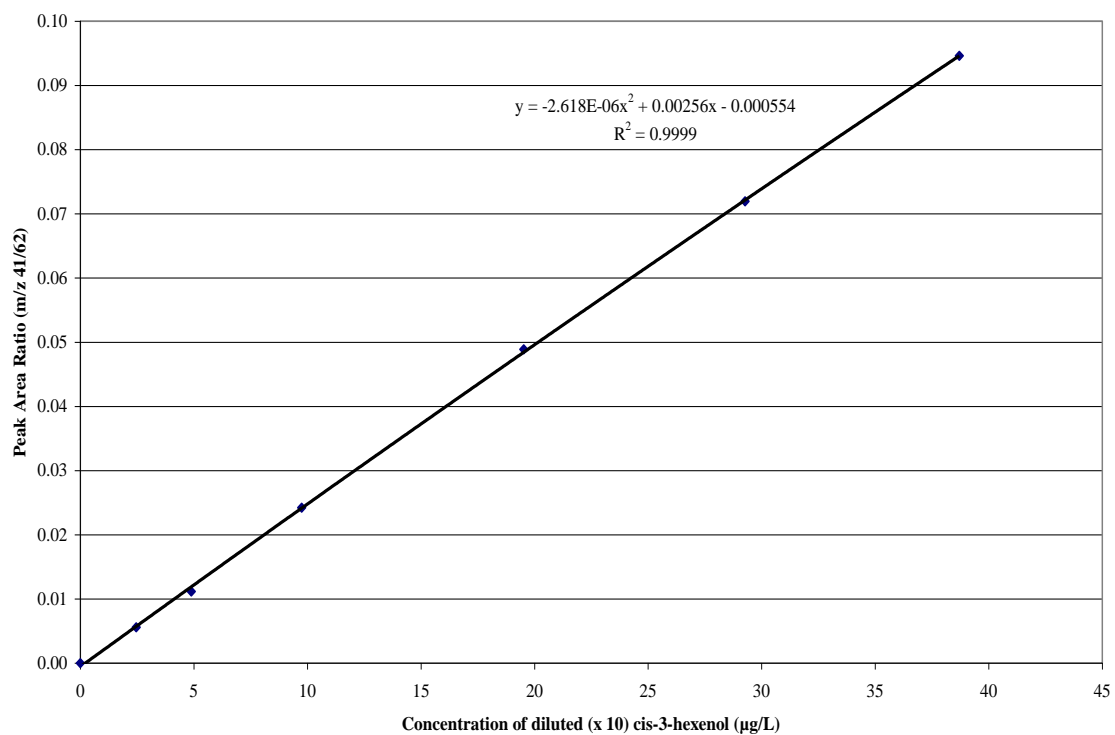


Figure 3. Calibration curve for cis-3-hexen-1-ol using standard diluted concentrations (µg/L) ($n = 2$).

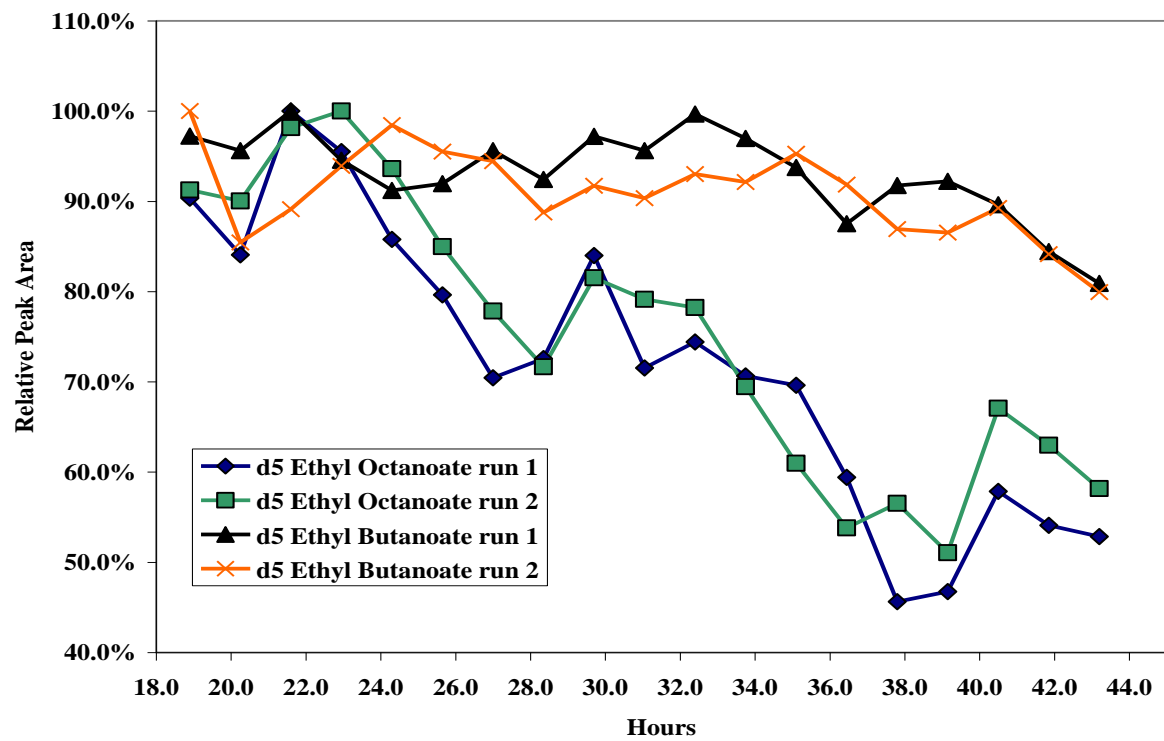


Figure 4. The degradation of two deuterated internal standards during the HS-SPME-GC-MS-SIDA analysis ($n = 2$).

Chapter 7

(A preparation for submission to American Journal of Enology and Viticulture)

The effect of the timing of leaf removal on “green” and “fruity” aroma concentrations and sensory attributes of (*Vitis vinifera* L. cv.) Pinot noir wine by a modified free choice profiling technique.

B. S. Kemp^a, R. Harrison^{a*}, R. Sedcole^b, G. L. Creasy^a.

^a*Department of Wine, Food and Molecular Biosciences,* ^b*Department of Agriculture, Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand.*

* Corresponding author. Fax: +64 3 3253850. Mail address: Centre for Viticulture and Oenology, Faculty of Agriculture & Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, Canterbury, New Zealand. Email: Roland.Harrison@lincoln.ac.nz (R. Harrison)

Short running title: Wine chemical and sensory analysis

Abstract

Mechanical leaf removal was applied to Pinot noir vines in a commercial vineyard in Waipara, New Zealand in the 2007-2008 and 2008-2009 growing seasons. Fruit zone treatments consisted of **NLR**: no leaf removal (control), **LR7**: leaf removal from the fruiting zone 7 days after flowering, **LR30**: leaf removal from the fruiting zone 30 days after flowering, **LRV**: leaf removal at 5% berry color change (by visual assessment). The study investigated the effect of the timing leaf removal on “green” and “fruity” aromas in Pinot noir. Chemical analysis using headspace-solid phase microextraction-gas chromatography-mass spectrometry-stable isotope dilution analysis (HS-SPME-GC-MS-SIDA) was used to analyse fourteen “green” and “fruity” volatile aroma compounds in Pinot noir wines. Ethyl esters showed no discernible pattern between treatments but a difference between years was observed. C₆ compounds and C₁₃ norisoprenoids increased in all wines in 2009. Sensory analysis by winemakers was conducted using a modified-free choice profiling technique. Wines from non-defoliated vines were described as having a rhubarb and lees autolysis aroma and vegetal and candied on the palate. Wines from defoliated vines had increased dark fruit aroma, and dark fruit, tannin and weight on the palate. “Fruity” aroma compounds were found to be above their odor threshold levels in all wines but “green” aroma compounds were found to be below.

Keywords: Pinot noir, leaf removal, HS-SPME-GC-MS-SIDA, modified-free choice profiling.

Introduction

Viticultural factors including light intensity, temperature, crop load, cluster thinning, irrigation and leaf removal have all been reported to affect the final concentrations of volatile compounds in wine (Qian et al. 2009, Chapman et al. 2004, Lee et al. 2007). Zoecklein et al. (2008) reported consistent differences between β -damascenone and *n*-hexanol concentrations in Viognier wines produced from different training systems in Virginia, USA. Wine sensory analysis also indicated an effect of training system on Viognier wine aroma and a difference between years was observed. However, Peterlunger et al. (2002) found little difference between four training systems in Italian Pinot noir wine aroma by sensory analysis but the study lacked chemical aroma analysis. These contradictory results suggest that differences from viticultural effects such as training system could be varietal and/or environment dependent.

With regards to cluster exposure, the highest concentration of flavour and aroma compounds were found in sun-exposed Riesling, Chenin Blanc, and Gewürztraminer fruit compared to shaded fruit (Marais et al. 1992, Reynolds and Wardle et al. 1989, Zoecklein et al. 2008). Elevated levels of Muscat aroma was reported in Golden Muscat wines produced from sun-exposed clusters compared to wine made from shaded clusters (Macaulay and Morris 1993). These results indicate a positive effect of sun exposure on the aroma profile of aromatic white grape varieties. Similarly, Morrison and Noble (1990) suggested that fruity volatile aroma compounds in Cabernet Sauvignon wines may be influenced by light from cluster exposure rather than temperature. Ristic et al. (2007) and Joscelyne et al. (2007) found that Shiraz wines from artificially and naturally shaded grapes were rated lower by sensory analysis for mouthfeel characteristics and fruit flavour than wines from exposed fruit. Gewürztraminer wines produced from grapes grown under three canopy treatments and two crop levels were distinguishable by a sensory panel due to their “vegetative” and “fruity” attributes (Reynolds et al. 1996, Hein et al. 2009). Additionally, severity and timing of leaf removal for canopy management has been shown to alter vegetative aromas and flavour in resultant wines (Arnold and Bledsoe 1990, Hein et al. 2009).

Potent aroma compounds such as leaf aldehydes (hexanal) contribute to the “green” and “herbaceous” aromas and flavours in wines and may consequently contribute to the elusive “green tannin” perception in red wines (Herderich et al. 2004). Additionally, “green” aromas are more readily perceivable in the absence of berry aromas such as “red fruit” and “black

fruit” (Herderich et al. 2004). Chapman et al. (2004) found that grapevine crop reduction increased “vegetal” aromas and reduced “fruity” components in Cabernet Sauvignon wines. Evidence suggests that wines produced from shaded fruit contain more “green” or “vegetal” flavours and sun exposed fruit produces more “fruity” red wines (Hein et al. 2009). The “fruity” aromas in red wine are attributed to ethyl esters, formed enzymatically by yeast during fermentation, and C₁₃ norisoprenoids, formed from light degradation of grape berry skin carotenoids (Sumby et al. 2010, Hein et al. 2009). The “green” and “herbaceous” aromas in wines have been attributed methoxypyrazines (Hein et al. 2009) and to C₆ alcohols and aldehydes which derive from alcohol dehydrogenase and lipoxygenase enzymatic cleavage of linoleic and linolenic fatty acids (Salinas et al. 2004, Ferreira et al. 1995). However, Masino et al. (2008) suggest that *n*-hexanol and trans-3-hexenol are involved in yeast metabolism and could be considered as fermentation by-products. Additionally, alcohols possess a higher propensity to form fruity esters in the presence of carboxylic acids during fermentation than C₆ aldehydes thereby minimizing the “green” and “herbaceous” characters (Kalua and Boss 2009, Salinas et al. 2004).

Researchers use established sensory evaluation techniques to characterise wines from research trials and the central principle is that the method should be matched to the objectives of the test (Perrin et al. 2007, Lawless and Heymann 1998). A sensory evaluation method known as free choice profiling (FCP), developed in the United Kingdom during the 1980s (Williams and Arnold 1985, Perrin et al. 2007), allows individual panellists to choose their own series of attributes with which to assess wines (Arnold et al. 2007). FCP assumes that panellists do not differ in the number and kind of sensory characteristics they perceive, but they do differ in the way they label them (Guy et al. 1989). The distinct advantage of FCP is the avoidance of panel training as panellists only need to be able to use a scale and be familiar with the product (Murray et al. 2001). However, Heymann (1994) suggested that FCP should be used with subjects with prior experience in sensory methods as “sensory naïve” subjects did not produce consistent results. In agreement with this view, Barcenas et al. (2003) found that panellists without sensory experience lacked consensus of attribute scoring even though the attributes were self-generated. Nevertheless, FCP has been used successfully in sensory studies to compare sensory characteristics of orange juices (Aparicio et al. 2007), dairy products (Kirkmeyer and Tepper 2003), inorganic and organic acids (Rubico and McDaniel 1992), Port (Williams and Langron 1984), dry-cured ham (Dolors Guàrdia et al. 2010) and wine (Perrin et al. 2007). Conventional sensory analysis using a

trained panel was compared to FCP analysis carried out by professional wine tasters (Perrin et al. 2007). Results showed that primary characteristics found in wines by conventional sensory analysis were also found by FCP (Perrin et al. 2007). FCP is complemented by the statistical technique generalised procrustes analysis (GPA) (Gower 1975, Murray et al. 2001). GPA can be used on FCP data reduces the information from studies to two or three dimensions, which means FCP can reveal large differences between wines (Murray et al. 2001). Additionally, by allowing attributes to be generated by panellists wine characteristics were identified that may not have been considered using a traditional approach (Perrin et al. 2007). GPA enables a consensus configuration of the wine samples analysed after optimal translation, rotation and shrinking (De Jong et al. 1998). Although often GPA is often associated with variable vocabulary it can also be used on fixed vocabulary data (Meyners et al. 2000).

This research was carried out to investigate whether the timing of leaf removal affects the perception of “green” and “fruity” compounds in Pinot noir wine by sensory and chemical analysis. A modified-free choice profiling method was chosen to enable an expert panel to identify wine differences using their own vocabulary and experience. No studies have been undertaken that used FCP to identify and distinguish wines according to viticultural or oenological treatments.

Materials and Methods

Experimental wines

A viticulture trial was carried out on a commercial north facing block of 10/5 clone of Pinot noir vines in Waipara, New Zealand. Vines were grown on own roots, irrigated and trained on a Vertical Shoot Positioned (VSP) trellis system. Treatments were applied to both sides of the row and laid out in a completely randomized block design incorporating rows as replicates, with three replicates per treatment. The four leaf removal treatments were: NLR, no leaf removal (control); LR7, leaf removal from the fruiting zone 7 days after flowering; LR30, leaf removal from the fruiting zone 30 days after flowering; and LRV, leaf removal at 5% color change at veraison (by visual assessment). LR7 was applied on 20th December 2008 and 12th December 2009, LR30 was performed on 15th January 2008 and 5th January 2009 the LRV was applied 11th February 2008 and 13th February 2009. Early flowering in the 2008-2009 season resulted in earlier treatment application in December and January compared to the previous season. The winemaking protocol and procedures have been

previously reported in detail in Chapter 5. Duplicates of eight wines from the 2008 vintage and eight from the 2009 vintage were used for sensory analysis which was carried out on 14th December 2009. Bottled wines were stored prior to analysis at approximately 10 °C in a purpose built cellar.

Sensory analysis

Modified free choice profiling

A modified version of free choice profiling was employed with the dual purpose of reducing the number of attributes for data analysis and to allow winemakers to identify traits using their own terminology. To ensure comparison with wine chemical compositional data experts were presented with thirteen generic traits. These were determined prior to formal sensory analysis at an informal tasting session at Lincoln University with a panel that consisted of two oenology lecturers and four oenology postgraduate students. This session was held to identify attributes used to discriminate between treatment wines. Wines were tasted in 20 mL measures and the agreed descriptors (see Table 1) were placed the description generation sheet for each participant to ensure panellists chose additional but alternative descriptors in session one.

Participants

Thirteen Waipara winemakers were recruited for the sensory analysis due to their availability and on the basis of extensive experience of tasting, and working with Pinot noir wines from the region. The eleven males and two females ranged between 25-60 years of age. All panellists were non-smokers and one male winemaker was registered blind. The blind panellist was seated in a private room for both sessions and accompanied by a reader/writer who did not taste the wines and had no influence over descriptors generated or ratings of the wines.

Sensory procedures

Following approval from the Human Ethics Committee at Lincoln University, New Zealand, all invited participants were provided with information about the imminent study and then completed and signed the consent forms. The study was conducted in one day at Pegasus Bay Winery, Waipara in two sessions. One morning session of c. sixty minutes duration and an afternoon session of c. ninety minutes. The room had a mixture of artificial and natural lighting and there was an absence of noise and other stimuli (e.g. olfactory). The ambient

temperature was regulated by an air conditioning unit and held constant at 20 °C throughout both sessions. Panellists were invited to arrive thirty minutes prior to commencement of the sensory study to allow for a full briefing to address any queries that may have arisen. There were six participants seated at each of the two sittings with the registered blind participants located separately. Panellists were seated in separate white booths and sixteen wines (20 mL) were poured into standard ISO (1977) glasses with 3-digit random codes and protected with coverslips (Parr et al. 2007) for session one.

Session one: The first session involved the panellists assessing the sixteen wines for aroma and flavour. Panellists were supplied with the list of previously generated descriptors (Table 1) and asked to attach their own descriptors to the list. Sixteen 20 mL wine samples were provided in ISO glasses in a random order unique to each panellist and three-digit numbers were assigned to each wine. It was emphasised that each panellist would be using both the provided descriptors and those generated by the individual panellist in session two. No discussion between participants regarding the wines or descriptors occurred during or between the sessions. The full list of expert generated descriptors is given in Table 2.

Session Two: Following the session for generating descriptors the panellists were provided with lunch and asked to return in the afternoon to rate the intensity of each descriptor on a 100 mm scale (Vidal et al. 2004). The afternoon session consisted of two flights to ensure wines were tasted in duplicate but in unique random orders for each panellist. As in session one, three digit codes were assigned to the wine glasses and participants vacated the room while the second flights (20 mL) were poured. Using both the descriptors provided and their own individual descriptors participants were asked to place a line on the 100 mm scale thereby rating each wine for that particular attribute.

HS-SPME-GC-MS-SIDA aroma analysis

All treatment wines were analyzed in duplicate according to a method developed using HS-SPME-GC-MS-SIDA described in Chapter 6. A method was developed to analyze fourteen specific aroma compounds chosen for their “fruity” and “green” contribution to red wine aroma. “Fruity” compounds included seven esters which were ethyl acetate, ethyl isobutyrate, ethyl butanoate, ethyl pentanoate, ethyl hexanoate, ethyl cinnamate, ethyl octanoate and two C₁₃ norisoprenoids, β-ionone and β-damascenone. Ethyl decanoate was not determined due to degradation of this compound during analysis. “Green” compounds included the C₆

compounds, hexanal, hexanol, trans-3-hexen-1-ol and cis-3-hexen-1-ol. Additionally, 3-isobutyl-2-methoxypyrazine (IBMP) was analysed by a previously published method by Parr et al. (2007).

Statistical analyses

Data analysis: sensory

Data were quantified by measuring from the left origin of the 100 mm line except for the blind panellist, who was asked to score each wine from 0-100. Analysis of variance (ANOVA) and restricted maximum likelihood (REML) method in GenStat 11, (VSNi, Hemel Hempstead, UK) were used to determine the effect of replicate wines and flights on the panellist attribute ratings. Mean rating values of each treatment wine were calculated and subjected to GPA using the Commandeur algorithm (XLSTAT) to allow for zeros in the data matrix using generic and generic plus panellist-generated terms. The GPA involved Procrustes Analysis of Variance (PANOVA), a permutation test and a plot of the consensus space by principal component analysis (PCA) (Lachnit et al. 2003). The Procrustes Analysis of Variance (PANOVA) gives information about the variability of the transformed data, by analysing the remaining distances between the corresponding product-points. To examine the statistical validity of the GPA results a permutation test is carried out (Lachnit et al. 2003). The resulting consensus is plotted by PCA, which reduces the dimensionality of the data matrix with a minimum loss of information. Additionally, Spearman rank correlation was carried out to determine correlations between sensory and wine chemical analysis using XLSTAT version 2010.2 (Paris, France).

Data analysis: chemical

Analysis of variance (ANOVA) was performed using GenStat 11, (VSNi, Hemel Hempstead, UK).

Results

Results of ANOVA and REML analysis showed no statistical significance of replicate wines or flights on panellist attribute ratings indicating a good reproduction of panellist ratings by duplicate and flight.

Generalised Procrustes Analysis of sensory data

The isotropic scaling factors applied to remove systematic sources of variation between panellists (Grice and Assad, 2009) are given in Table 3, along with residuals for each from the PANOVA of the comparison of rescaled/rotated data grids with the consensus matrix. Results are for GPA of data using only the 13 generic descriptors and that of data utilizing all (generic plus panellist-generated) descriptors. For both datasets, the scaling factors indicated that the ratings for panellists 7 and 10 were stretched and those for expert 5 were compressed compared to the other participants. Additionally, the values of the residuals, which are relatively homogeneous, suggest that no individual panellist deviated markedly from the consensus matrix. For the dataset based on the generic descriptors only, panellist 7 shows the greatest degree of agreement and panellist 3 the greatest deviation. For the dataset based on all descriptors, panellists 13 and 2 show greatest agreement and deviation, respectively.

The consensus proportion (i.e. R_c = the proportion of total variance explained by the consensus matrix) for both datasets was very low, being 0.087 and 0.059 for the generic and generic plus panellist-derived data, respectively. Despite this, permutation tests ($n = 300$) indicated that these consensus proportions were statistically significant ($p < 0.05$). Although, Wakeling et al. (1992) suggests that R_c is a rather conservative measure of consensus. However, it should be noted that these results indicate that there was rather poor agreement among the 13 panellists.

PCA indicated that the first two dimensions (F1 and F2) accounted for 65% of the variance in the generic descriptors consensus matrix (Figure 1) and 59% of the variance in the generic plus panellist-derived consensus matrix (Figure 2). Figure 3 and 4 show the locations of each wine, color coded by panellist, using these two dimensions. The consensus locations for each wine are shown in Figures 5 and 6. Figure 5a is the biplot for dimensions 1 and 2 using generic descriptors. Despite the low consensus proportion values, it appears that there is some degree of agreement between panellists on the relative locations of the different wines.

Relationships between sensory descriptors

PCA of the consensus matrix (Figure 5 and 6) indicated that, for the generic descriptors dataset, the first dimension (F1) was defined by positive values of dark fruit and spice aromas and dark fruit, tannin and weight on the palate, and the negative value with rhubarb aroma. The second dimension (F2) separated the wines primarily by bitter finish on the negative

fraction and red fruit aroma and red fruit on the palate on the positive. PCA of the consensus matrix (Figure 7) for the generic plus panellist-derived dataset indicated that the first dimension (F1) separated the wines on the positive axis by rhubarb aroma (generic descriptor as before), and lees autolysis aroma plus candied, elegant and vegetal palate (panellist-derived). These were negatively correlated to dark fruit aroma and dark fruit, tannin and weight on the palate (generic descriptors as before), plus colour intensity and balanced palate (panellist-derived). Although, spice aroma (generic descriptor) was a contributor to the first PCA dimension in this dataset, a number of other descriptors (cherry, red flowers and ginger aromas, colour, hot finish, and concentration) were highly correlated with this dimension. There was a distinct similarity between the first PCA dimensions (F1) derived from the generic descriptors and the generic plus panellist-derived descriptors datasets. However, the second dimension (F2) for the generic plus panellist-derived descriptors dataset differed from the first dimension, due to the positive values of char aroma and confectionary, rose petal and strawberry on the palate. These were negatively correlated with red fruit and carbonic aromas and red fruit, acid and velvety tannins on the palate. The Spearman rank correlation method revealed correlations between both dimension one and two generic and panellist derived descriptors (Table 4).

Sensory descriptions of wines

Additional descriptors generated by panellists resulted in a similar separation of the treatment wines compared to the separation by generic terms (Figures 5 and 6). However, the additional descriptors provided specific attributes that would otherwise have been unobserved using only generic attributes (Perrin et al. 2007). Furthermore although seventy four additional descriptors were generated by the panellists it should be noted that each was unique to individual panellists. The largest separation between wines based on PCA of the consensus matrices was associated with the first dimension for which three major groups could be distinguished. Some separation also occurred in the second dimension which meant that wines were placed into one of four categories based on sensory analysis.

The NLR wine from 2008 was separated from the other wines and described as rhubarb and lees autolysis aroma with candied palate, vegetal. In contrast, the NLR wine from 2009 together with LR30 from 2009 and LRV from 2008 and 2009 were characterised as dark fruit aroma with dark fruit, balance, tannin and weight on the palate. The LR7 wine from 2008 was also located at this end of the first dimension but was separated in the second dimension

associated with red fruit and carbonic aromas and acid, velvety tannins with a bitter finish. The LR30 wine from 2008 and the LR7 wine from 2009 were intermediate between these. They were defined by char, confectionary aromas and rose petal and strawberry on the palate but also had low consensus rating of rhubarb, vegetal, candied and lees autolysis.

Concentrations of aroma compounds in wines

Concentrations of IBMP were found to be below the limit of detection and therefore have not been reported. Statistical significant differences ($P \leq 0.05$) in wine concentrations of 12 of the other 13 aroma compounds determined were found between leaf removal treatments and/or years (Table 5), the exception being ethyl acetate which, at high concentrations, is normally considered a fault. There was no obvious pattern to the data, except that aroma concentration differences were more frequently due to year than to leaf removal treatment. The interaction between year and leaf removal treatment was not significant for concentrations of ethyl pentanoate, ethyl hexanoate, hexanol, trans-3-hexen-1-ol, β -ionone and β -damascenone. However, concentrations were greater in 2009 for these compounds compared to 2008, except for ethyl isobutyrate which was higher in 2008 compared to 2009. Ethyl hexanoate, hexanol, trans-3-hexen-1-ol and β -ionone showed significant differences due to leaf removal treatments, although no common pattern was apparent. For the other compounds, there was a statistically significant interaction between year and leaf removal treatment: there were higher concentrations of ethyl butanoate and cis-3-hexen-1-ol in 2009 compared to 2008; for ethyl cinnamate there were higher concentrations for the NLR and LR7 treatments in 2008; for ethyl octanoate there was a lower concentration for the LR30 treatment in 2008; and, for hexanal, NLR had the highest concentration in 2008 and the lowest in 2009. Thus, the majority of aroma compounds had higher concentrations in 2009 but there were no dominating trends between leaf removal treatments.

All the “fruity” aroma compounds were found to be above their odor thresholds in all wines (Table 6). In contrast, the “green” aroma compounds were found to be below their odor threshold levels in all wines, although the hexanal level has been determined in water and beer and not in aqueous ethanol solution like the other “green” compounds.

Correlation between wine compositional data and sensory descriptors

The relationship between wine composition (see also Chapter 5) and sensory analysis was investigated by Spearman rank correlation analysis (Table 7). Statistically significant

relationships between two generic descriptors and four panellist derived descriptors that formed factors 1 and 2 of the PCA plots were revealed, but no pattern emerged regarding sensory and wine chemical data.

Discussion

Concentrations of “fruity” and “green” aromas in Pinot noir wines

Whilst it is difficult to compare results obtained from different analytical methods, the majority of the volatile compounds identified and quantified by the HS-SPME-GC-MS-SIDA technique are within the range previously reported in Pinot noir wines (Table 6). Hexanal has not previously been detected in Pinot noir wine and ethyl isobutyrate and ethyl pentanoate have been identified but not quantified. Therefore, the contribution of these aroma compounds to Pinot noir wine aroma is currently unknown (Kalua and Boss 2009).

Concentrations of aroma compounds with “fruity” notes

Ethyl acetate concentrations in our experimental wines were high compared to previous studies (Table 6). Ethyl isobutyrate, ethyl butanoate and ethyl cinnamate decreased in 2009 but ethyl pentanoate, ethyl hexanoate and ethyl octanoate (except NLR) concentrations increased. Reasons for an increase in ethyl ester production during wine fermentation include low fermentation temperatures, yeast strain, lees aging, aeration and nitrogen levels of the must (Saerens et al. 2008, Barbosa et al. 2009, Loscos et al. 2009). Low fermentation temperatures favour ester production responsible for the “fruity” characteristics in wine (Molina et al. 2007). Ethyl isobutyrate was consistently found at higher concentrations in high nitrogen musts (Barbosa et al. 2009). The early leaf removal (LR7 and LR30) in our study may have affected concentrations of ethyl hexanoate and ethyl cinnamate by reducing the levels of precursors in the must. Pereira et al. (2006) found that sun exposed Merlot berries had higher total nitrogen concentrations, which consisted of higher proline, than shaded berries which contained more arginine. Ribereau-Gayon (2000) states that arginine dominates Pinot noir berries compared to Merlot or Cabernet Sauvignon, which are higher in proline. *Saccharomyces cerevisiae* use arginine preferentially and high levels of amino acids in grape must results in quicker fermentations, enhanced aroma compounds and reduces the incidence of stuck fermentations (Hernandez-Orte et al. 2006). LRV had the highest concentration of ethyl octanoate so it is possible that LRV had elevated levels of amino acid precursors, utilized by yeast during fermentation for the manufacture of alcohols for ester production (Keyzers and Boss 2010). Recently, Lohitnavy et al. (2010) found that leaf

removal performed before, during and after flowering on separate grapevines decreased yeast assimilable nitrogen (YAN) in Semillon grape must.

Diáz-Maroto et al. (2005) used artificially aged wines and model wine solutions to investigate the impact of wine aging on ethyl esters. The study found that branched fatty acid ethyl esters (related to yeast nitrogen metabolism) increased compared that of their straight-chain analogues (related to yeast lipid metabolism). This modification of esters during wine aging may account for the differences observed in our study between treatment wines in 2008 and those from 2009. Using a HS-SPME-GC-MS method, Goldner et al. (2009) found that Malbec wines with higher alcohol levels had lower concentrations of ethyl hexanoate and hexanol compared to low alcohol wines. In contradiction, our results show an increase in these compounds in 2009 (14.5%), when the ethanol level was higher than in 2008 (12.5%). Ethyl cinnamate, which decreased in the wines in 2009, has been shown to decrease in Pinot noir grapes during ripening and the 2009 grapes were harvested at higher maturity levels than in 2008 (Fang and Qian 2006).

Previous research has shown that C₁₃ norisoprenoid accumulation increased with cluster exposure and concentrations were much higher in sun-exposed Syrah berries than shaded fruit (Joscelyne et al. 2007, Morrison and Noble 1990, Ristic et al. 2007, Marais et al. 1991, Bureau et al. 2000). Lee et al. (2007) carried out varying degrees of manual leaf removal following fruitset and observed increasing levels of C₁₃ norisoprenoids in Cabernet Sauvignon grapes and resultant wines as sunlight in the fruiting zone increased. Therefore, our results suggest that the increased defoliation in 2009 from partial cane removal and hail damage increased the sunlight and UV radiation in the cluster zones resulting in elevated concentrations of C₁₃-norisoprenoids in wines.

In the study by Lee et al. (2007), the dominating factor for C₁₃ norisoprenoid concentrations in berries from defoliated vines was leaf layer number (LLN). Defoliated vines in our study had a similar mean LLN of 1.5 in 2008 and 1.3 in 2009 but the percentage of cluster exposure was higher across all treatments in 2009 (80%) compared to 2008 (60%) (Chapter 3). When LLN was higher across all treatments in 2008, NLR and LR7 had statistically significantly similar β -ionone concentrations in the wines. However, β -damascenone was highest in LR7 and LR30 supporting the idea that increased cluster exposure is important for β -damascenone concentration but had less impact on β -ionone concentration in our study. Whilst sunlight,

specifically UV radiation, has been shown to influence carotenoid accumulation and degradation to form C₁₃ norisoprenoids, the non-defoliated berries in the study by Lee et al. (2007) also accumulated C₁₃ norisoprenoids in both berries and resultant wines. These results suggest the increased C₁₃ norisoprenoid concentrations found in the 2009 wines in our study are a consequence of increased cluster exposure as opposed to decreased leaf layer number. Accordingly, Kwasniewski et al. (2010) found no statistically significant difference in β -damascenone concentrations in Riesling wines produced from vines that had leaf removal at 2, 33 and 68 days after berry set compared to the non-defoliated control wines. Also, the synthesis of β -damascenone and β -ionone differ. β -Damascenone can be formed either enzymatically or non-enzymatically whereas β -ionone biosynthesis is an enzymatic process only (Pineau et al. 2007, Mendes-Pinto 2009). Therefore cluster exposure may have affected synthesis of these two compounds in a different way in 2008 (Mendes-Pinto. 2009). Similarly, the maturity of the 2009 grapes may have impacted C₁₃ norisoprenoids because Fang and Qian (2006) reported the highest concentrations in late maturity Pinot noir berries. Daniel et al. (2004) reports that β -damascenone consumption by SO₂ increases with increased SO₂ levels. This may explain the lower concentrations in 2008 wines that were higher in total SO₂ levels (see Chapter 5).

Concentrations of aroma compounds with “green” notes

In 2008, early leaf removal wines (LR7 and LR30) had the lowest concentrations of hexanal, hexanol and trans-3-hexen-1-ol while the highest concentrations were found in NLR wines. The evolution of C₆ compounds during ripening has been found to be dependent upon enzyme activity such as alcohol dehydrogenase (ALD) (Kalua and Boss 2009). Our 2008 results suggest increased light and temperature in the fruiting zone from early leaf removal increased fatty acid enzyme activity in LR7 and LR30. Spiers et al. (1998) found that hexanal and trans-3-hexen-1-ol increased with ALD activity in tomatoes, and tomato fruit with higher levels of these alcohols were identified as having “riper fruit” flavours. Alternatively, fatty acid precursors may have been present in higher quantities in NLR compared to berries from defoliated vines (Saerens et al. 2008).

No statistically significant treatment effects were observed in 2009, but C₆ aromatic volatile compound concentrations increased in all treatment wines. It has been reported that C₆ compounds in Cabernet Sauvignon grapes reach their highest concentration towards late maturity (Kalua and Boss 2009). Pinot noir berries were harvested at greater maturity in

2009, 25 Brix, compared to 23 Brix in 2008 which may account for increased concentrations of these compounds in the wines. Trans-3-hexen-1-ol in our wines was found to be higher than previous studies and cis-3-hexen-1-ol was lower than previously reported (Fang and Qian 2006). The difference in the concentrations of these compounds in our research wines could be attributed to environmental growing conditions, climate, yeast strain, wine age or a difference in the analytical methods used. Oliviera et al. (2006 found) that the trans-3-hexenol/cis-3-hexenol ratio discriminated Loureiro wines from those of Alvarinho, Avesso and Trajadura in Portugal. These results suggest a dominance of trans-3-hexen-1-ol compared to cis-3-hexen-1-ol in New Zealand Pinot noir wines.

Sensory analysis of wines by modified-free choice profiling

The use of modified-free choice profiling enabled wines to be analysed using winemaker vocabulary and identified specific traits that would otherwise not have been found using conventional sensory analysis methods (Perrin et al. 2007). Wine produced from non-defoliated vines in 2008 (NLR 08) was distinctly separated due to attributes such as vegetal, red fruit and rhubarb. Similarly, Reynolds et al. (1996) reported that high vigour Pinot noir vines, resulting in increased cluster shading when compared to low vigour vines, produced wines with vegetative overtones. The herbaceous character associated with Pinot noir wines has been attributed in part to excessive vine vigour or yields (Cliff and Dever 1996). The use of the term “vegetal”, which is often associated with “herbaceous”, has connotations in wine tasting as it is associated with methoxypyrazines, as well as used anecdotally to explain the perception of “green tannins” by some winemakers/wine writers (Herderich et al. 2004, Perrin et al. 2007, Preston et al. 2008). Prior to sensory analysis all treatment wines were analysed for methoxypyrazine concentrations (Parr et al. 2007) and found to be below detectable and quantifiable thresholds (Kotseridis et al. 2008) so not reported in the present study. Additionally, NLR wine in 2008 had a higher concentration of hexanal than the defoliated wines and this C₆ aldehyde is associated with “herbal, grassy and green” notes whereas C₆ alcohol compounds have more “fruity and fresh” aromas (Hatanaka 1993, Kalua et al. 2007). However, the odor threshold for hexanal in an ethanol solution is currently unknown so its contribution to “green” characters in the NLR wine in 2008 has yet to be determined. Herderich et al. (2004) state that grape aroma can be affected by grape maturity and that potent aroma compounds such as methoxypyrazines or leaf aldehydes, such as hexanal, could contribute to the “green” and “herbaceous” aromas associated with “green tannins”. Kotseridis et al. (1999) suggest that vegetative notes could also be attributed to

other aldehydes such as decanal and (*E,Z*)-nona-2,6-dienal which were not measured in this present study (Falcao et al. 2007). Furthermore, Arnold and Bledsoe (1990) found that later leaf removal was less effective in reducing the intensity of vegetal aromas than earlier defoliation (Tardaguila et al. 2008).

NLR wines in both years had lower tannin concentrations and phenolics than the leaf removal wines (Chapter 5). NLR wine in 2008 was also defined by panellists as having rhubarb, red fruit and candied nuances that could be attributed to ethyl hexanoate which has been identified as contributing red-berry aromas to red wine (Pineau et al. 2009). NLR wine in 2009 had confectionary and char aromas and rose petal and strawberry palate with higher colour intensity, dark fruit aroma and dark fruit, weight, tannin and balanced palate. This was possibly due to increased cluster exposure from partial cane removal and hail damage in 2009 (Chapter 3). Similarly, the perceived blackberry aroma was higher in Cabernet Sauvignon wines made from highly exposed fruit compared to moderately exposed fruit (Joscelyne et al. 2007). Alternatively, increased β -damascenone from higher cluster exposure in 2009 could have increased the “fruity” notes and may have decreased the “green” perception (Escudero et al. 2007, Mendes-Pintos 2009). Additionally, “fruity” compounds were found above their odor thresholds in the wines and “green” alcohols were below.

Low vigour vines, characterised by high cluster exposure and reduced foliage, produced Pinot noir wines that were found to have earthy, chemical, bitter, sour and astringent qualities determined by sensory analysis (Cortell et al. 2008). In the study, wine tannin (mg/L) and pigmented polymers positively correlated with the wine attributes but in our study few descriptors correlated to wine compositional data.

LR7 in 2008 was defined by high ratings for colour intensity, red fruit, dark fruit, carbonic aromas and acid, weight, tannin, velvety tannins and bitter finish. Importantly, LR7 in 2008 had the highest tannin concentration compared to the other treatment wines (Chapter 5). LR7 wine in 2009 had char and confectionary aromas, and low undertones of lees autolysis aroma and rhubarb, candied and vegetal palate. The combination of the increase in ethyl isobutyrate, ethyl butanoate, ethyl pentanoate, ethyl hexanoate, β -ionone and β -damascenone may have contributed to the overall “fruity” aroma increase in the LR7 and LR30 in both years. In the study by Pineau et al. (2009), ethyl butanoate, ethyl hexanoate and ethyl octanoate conferred red-berry aromas in varietal and blended red wines which may have been enhanced in our

defoliated wines by the presence of β -damascenone. However, wine aroma consists of many different chemical components that influence sensory properties to varying degrees (Jones et al. 2008) and the absence or addition of an individual ester can be masked in the presence of other related esters (Escudero et al. 2004, Sumbly et al. 2010). Additionally, Skinkis et al. (2010) found that increasing sunlight exposure by decreasing leaf layer number resulted in a more intensely coloured and aromatic Traminette wine based on descriptive analysis.

LR30 wine in 2008 was similarly placed on the PCA plots to LR7 2009 and both LR7 and LR30 wines had the highest flavan-3-ol concentrations in both years. Flavan-3-ols are known to impart bitterness and astringency in wine which may have affected the sensory qualities of these early defoliated wines. However, LR30 in 2009 was described as having higher dark fruit flavours, more weight and tannin on the palate, and higher colour intensity with a lack of red fruit and acidity compared to 2008. Grenache wines produced from grapevines that had leaf removal at fruitset (3-4 mm diameter) achieved higher aroma complexity than non-defoliated wines and those defoliated at veraison which is in agreement with our study (Tardaguila et al. 2008). Defoliated treatments in the study displayed low floral aroma compared to non-defoliated and all wines were high in grassiness. However, the study was conducted over only one year and lacked volatile aroma analysis though Grenache wine from the early defoliation was preferred by panellists in agreement with our sensory results. Additionally previous studies found that leaf removal significantly improved wine colour (Hunter et al. 1991, Staff et al. 1997) in accordance with the present study.

The perception of bitterness of tannins can increase, and astringency decrease with increasing concentrations of ethanol and the 2009 wines had higher alcohol (14.5%) than in 2008 (12.5%) (Lea and Arnold 1978, Kutyna et al. 2010). Therefore the bitter finish in LRV 2009 and LR30 2009 may be due to increased alcohol levels. There is evidence that high alcohol content has a negative influence on the sensory attributes of wine (Guth and Sies 2002 as cited by Kutyna et al. 2010). At higher concentrations, ethanol may also increase the perception of hotness, body and viscosity of a wine with lesser effects on sweetness, acidity, aroma, flavour intensity and textural properties (Gawel et al. 2007a, b). Bitter finish and a bitter taste may be elicited by many structurally diverse compounds including flavan-3-ols and ethanol (Jones et al. 2008, Fischer and Noble 1994, Noble 1996, Thorngate 1997, Cortell et al. 2008). Ethanol and flavan-3-ol concentrations increased in all treatment wines in 2009 (see Chapter 5). Leaf removal at veraison in 2008 produced wine that had confectionary and

char aromas and rose petal, strawberry on the palate and was closely placed to NLR 2009 wine.

Wines produced from defoliated vines lacked “green” notes in the sensory analysis but had increased C₆ aroma concentrations in 2009, probably because their concentrations were below odor threshold levels. The 2008 wines had been aging in bottle for one year which may have affected wine composition and sensory characters (Monagas et al. 2006).

Conclusion

Leaf removal produced wines that had more dark fruit aroma, tannin and weight than non-defoliated wines. The current study investigated only fourteen volatile aroma compounds associated with “fruity” or “green” aromas. Therefore changes in aroma profiles of treatment wines by sensory analysis could possibly be attributed to different aroma compounds and aroma interactions not analysed in the present study. Modified-free choice profiling enabled specific wine descriptors to be identified by winemakers that may not have been found by an untrained panel and conventional sensory methods. Further studies could include a wider range of volatile aromatic compounds i.e. trans-2-hexenal, and additional wine chemical analysis during bottle and oak aging. Future work investigating Pinot noir grape maturity, alcohol levels, the trans-3-hexen-1ol/cis-3-hexen-1-ol ratio and the possible role of hexanal in the perception of “green tannins” in red wines is recommended.

Acknowledgements

The authors wish to thank Lincoln University and New Zealand Winegrowers for financial assistance and Pegasus Bay Winery for assistance in kind. The authors would like to thank Jason Breitmeyer for assistance with HS-SPME-GC-MS-SIDA analysis, Richard Sedcole for his help with statistical analysis, the Waipara winemakers who generously donated their valuable time to carry out the sensory analysis, Wendy Parr for her advice and guidance regarding sensory analysis and Sue Blackmore for help in undertaking the sensory experiment.

References

- Antalick, G., Perello, M. and G. de Revel. 2010. Development, validation and application of a specific method for the quantitative determination of wine esters by headspace - solid phase microextraction - gas chromatography - mass spectrometry. *Food Chem.* 121:4:1236-1245.
- Aparicio, J., Toledanao Medina, M. and V. Lauente Rosales. 2007. Descriptive sensory analysis in different classes of orange juice by a robust free choice profile method. *Anal. Chim. Acta.* 595:238–247.
- Arnold, G., Gower, J., Gardner-Lubbe, S. and N. Le Roux. 2007. Biplots of free-choice profile data in generalised orthogonal procrustes analysis. *App. Stat.* 56:4:445-458.
- Aubrey, V., Etievant, P., Ginies, C. and R. Henry. 1997. Quantitative determination of potent flavour compounds in Burgundy Pinot noir using a stable isotope dilution assay. *J. Agric. Food Chem.* 45:6:2120-2123.
- Barcenas, P., Perez Elortondo, F. and M. Albisu. 2003. Comparison of free choice profiling, direct similarity measurements and hedonic data for ewes' milk cheeses sensory evaluation. *Int. Dairy Jour.* 13: 67–77.
- Barbosa, C., Falco, V., Mendes-Faia, A. and A. Mendes-Ferreira. 2009. Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *Saccharomyces cerevisiae* wine strains. *J. Biosci. Bioeng.* 108:2:99-104.
- Binder, S., Knill, T. and J. Schuster. 2007. Branched chain amino acid metabolism in higher plants. *Physio. Plant.* 129:68-68.
- Bureau, S., Baumes, R. and A Razungles. 2000. Effects of bunch shading on glycosylated flavour precursors in grapes of *Vitis vinifera*. L. cv. Syrah. *J. Agric. Food Chem.* 48:1290-1297.
- Chapman, D., Matthews, M. and J. Guinard. 2004. Sensory attributes of Cabernet Sauvignon wines made with different crop yields. *Am. J. Enol. Vitic.* 55:325-334.
- Cliff, M. and M. Dever. 1996. Sensory and compositional profiles of British Columbia Chardonnay and Pinot noir wines. *Food. Res. Int.* 29:34:317-323.
- Cortell, J., Sivertson, H., Kennedy, J. and H. Heymann. 2008. Influence of vine vigor on Pinot noir fruit composition, wine chemical analysis and wine sensory attributes. *Am. J. Enol. Vitic.* 59:1:1-10.
- Daniel, M., Elsey, G., Capone, D., Perkins, M. and M. Sefton. 2004. Fate of Damascenone in Wine: The Role of SO₂. *J. Agric. Food Chem.* 52:8127-8131.
- De Jong, S., Heidema, J. and H.C.M. Van der Knaap. 1998. Generalized procrustes analysis of coffee brands tested by five European sensory panels. *Food Qual. Pref.* 9:3:111-114.
- Diáz-Maroto, C., Schneider, R. and R. Baumes. 2005. Formation pathways of ethyl esters branched short-chain fatty acids during wine aging. *J. Agric. Food Chem.* 53:3503-3509.

- Dolors Guàrdia, M., Aguiar, A., Claret, A., Arnau, J. and L. Guerrero. 2010. Sensory characterization of dry-cured ham using free-choice profiling. *Food Qual. Pref.* 21:148–155.
- Escudero, A., Campo, E., Farina, L., Cacho, J. and V. Ferreira. 2007. Analytical characterisation of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wine. *J. Agric. Food Chem.* 55:11:4501-4510.
- Escudero, A., Gogorza, B., Melus, M., Ortin, N., Cacho, J. and V. Ferreira. 2004. Characterisation of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* 52:11:3516-3524.
- Etiévant, P.X. 1991. Wine. In. *Volatile compounds in food*. Ed. H. Maarse. Food Science and Technology. Marcel Dekker Inc, New York. Pp 483-546.
- Falcao, L., De Revel, G., Perello, M., Moutsiou, A., Zanus, M. and M. Bordignon-Luiz. 2007. A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C₁₃-norisoprenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. *J. Agric. Food Chem.* 55:9:3605-3612.
- Fang, Y. and M. Qian. 2006. Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *J. Agric. Food Chem.* 54:22:8567-8573.
- Ferreira, B., Hory, C., Bard, M., Taisant, C., Olsson, A. and Y. Le Fur. 1995. Effects of skin contact and settling on the level of C18:2, C18:3 fatty acids on C6 compounds in Burgundy Chardonnay musts and wines. *Food Qual. Pref.* 6:35-41.
- Ferreira, V., Lopez, R. and J.F. Cacho. 2000. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* 80:1659-1667.
- Feuillat, M., Charpentier, C. and C. Massoutie, 1997. Enological importance of cryotolerant *Saccharomyces cerevisiae* yeast strains. *Rev Oeno. France.* 24:85:18-21.
- Fischer, U. and A. Noble. 1994. The effect of ethanol, catechin concentration and pH on sourness and bitterness in wine. *Am. J. Enol. Vitic.* 455:1:6-10.
- Fuselgang, K. and B. Zoecklein. 2003. Population dynamics and effects of *Brettanomyces bruxellensis* strains on Pinot noir (*Vitis vinifera* L.) wines. *Am. J. Enol. Vitic.* 54:4:294-300.
- Gawel, R., Francis, L. and E. Waters. 2007a. Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. *J. Agric. Food Chem.* 55:2683–2687.
- Gawel, R., van Sluyter, R. and E. Waters. 2007b. The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines, *Aust. J. Grape. Wine Res.* 13:38–45.
- Goldner, A., Zamora, M., Di Leo, Lira, P., Gianninoto, H. and A. Bandoni. 2009. Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens Studies.* 24:43-257.

Gonzalez-Tomas, L. and E. Costell. 2006. Relation between consumers' perceptions of colour and texture of dairy desserts and instrumental measurements using a generalized procrustes analysis. *J. Dairy Sci.* 89:4511-4519.

Gower, J. C. 1975. Generalised Procrustes analysis. *Psychometrika*, 40:33–50.

Grice, J.W. and Assad. K.K. 2009. Generalized Procrustes Analysis: A tool for exploring aggregates and persons. *Appl. Multivariate. Res.* 13:1:93-112.

Guth, H. 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* 45:3027-3032.

Guth, H. and A. Sies. 2002. Flavour of wines: towards an understanding by reconstitution experiments and an analysis of ethanol's effect on odour activity of key compounds, Proceedings of the eleventh Australian wine industry technical conference, Adelaide. Pp. 4–7.

Guy, C., Piggott, J. and S. Marie. 1989. Consumer profiling of Scotch Whisky. *Food Qual. Pref.* 1:2:69-73.

Hatanaka, A. 1993. The biogenesis of green odour by green leaves. *Phytochem.* 34:5:1120-1218.

Hein, K., Ebeler, S. and H. Heymann. 2009. Perception of fruity and vegetative aromas in red wine. *J. Sens Studies.* 24:441-455.

Herderich. M. Bell. S. Holt. H. Ristic. R. Birchmore. W. Thompson. K. Iland. P. 2004. Grape quality and tannins: the impact of viticultural treatments on grape and wine tannins. Conference Proceedings. Twelfth Australian Wine Industry Technical Conference, Melbourne, Victoria, Australia. 24-29th July, 2009. Pp 79- 84.

Hernandez-Orte, P., Ibarz, M., Cacho, J. and V. Ferreira. 2006. Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake, and aroma generation during alcoholic fermentation. *Food Chem.* 98:300-310.

Heymann, H. 1994. A comparison of free choice profiling and multidimensional scaling of vanilla samples. *J. Sens Studies.* 9:445–453.

Hunter, J.J., De Villiers, O.T. and J.E. Watts. 1991. The effect of partial on quality characteristics of *Vitis vinifera* L. cv. Cabernet-Sauvignon grapes. II. Skin color, skin sugar and wine quality. *Am. J. Enol. Vitic.* 42:13-18.

Jones, P., Gawel, R., Francis, L. and E. Waters. 2008. The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Qual. Pref.* 19:596-607.

Kalua, C. and P. Boss. 2009. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.) *J. Agric. Food Chem.* 57:3818-3830.

- Keyzers, R. and P. Boss. 2010. Changes in volatile compound production of fermentations made from must with increasing grape content. *J. Agric. Food Chem.* 58:1153–1164.
- Kilmartin, P. and L. Nicolau. 2007. Literature survey on aroma and flavour compounds found in Pinot noir and analytical techniques applied. *Wine Science Report*, University of Auckland, New Zealand.
- Kishimoto, T., Wanikawa, A., Kono, K. and K. Shibata. 2006. Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *J. Agric. Food Chem.* 54: 8855-8861
- Kotseridis, Y., Baumes, R. and G. Skouroumounis. 1999. Quantitative determination of β - ionone in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chrom A.* 848:317-325.
- Kotseridis, Y.S., Spinkb, M., Brindlec, I.D., Blakeb, A.J., Searse, M., Chenc, X, Soleasf, D., Inglis, G. and G.J. Pickering. 2008. Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *J. Chrom. A.* 1190: 294–301.
- Kutyna, D., Varela, C., Henschke, P., Chambers, P. and G. Stanley. 2010. A review: Microbiological approaches to lowering ethanol concentration in wine. *Trends. Food Sci. Tech.* 21:6:293-302.
- Kwasniewski, M., Vanden Heuvel, J., Pan, B. and G. Sacks. 2010. Timing of cluster light environment manipulation during grape development affects C₁₃ norisoprenoid and carotenoid concentrations in Riesling. *J. Agric. Food Chem.* 58:6841–6849.
- Lachnit, M., Busch-Stockfisch, M., Kunert, J. and T. Krahl. 2003. Suitability of free choice profiling for assessment of orange-based carbonated soft-drinks. *Food Qual. Pref.* 4: 257-26.
- Lea, A. and G. Arnold. 1978. The phenolics of ciders: bitterness and astringency. *J. Agric. Food Chem.* 29:478–483.
- Lee, S., Seo, M., Riu, M., Cott, J., Block, D., Dokoozlian, N. and S. Ebeler. 2007. Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wine. *Am. J. Enol. Vitic.* 58:291-301.
- Leffingwell, J.C. and D. Leffingwell. 2003. *Gras Flavour Chemicals - detection thresholds.* <http://www.leffingwell.com>.
- Lohitnavy, N., Bastian, S. and C. Collins. 2010. Berry sensory attributes correlate with compositional changes under different viticultural management of Semillon (*Vitis vinifera* L.) *Food Qual. Pref.* doi:10.1016/j.foodqual.2010.05.015.
- Loscos, N., Hernandez-Orte, P., Cacho, J. and V. Ferreira. 2009. Fate of grape flavour precursors during storage of yeast lees. *J. Agric. Food Chem.* 57:5468-5479.

- Louw, C., La Grange, D., Pretorius, S. and P. van Rensburg. 2006. The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and flavour. *J. Biotech.* 125:4:447-461.
- Mamede, M., Cardello, H. and G. Pastore. 2005. Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts. *Food Chem.* 89:1:63-68.
- Marais, J., van Wyk, C. and A. Rapp. 1992. Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin Blanc grapes and Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* 13:23-32.
- Masino, F., Montevercchi, G., Arfelli, G. and A. Antonelli. 2008. Evaluation of the combined effects of enzymatic treatment and aging on lees on the aroma of wine from *Bombino bianco* grapes. *J. Agr. Food Chem.* 56: 9495-9501.
- Massoutier, C., Alexandre, H., Feuillat., M. and C. Charpentier. 1998. Isolation and characterisation of cryrotolerant *Saccharomyces cerevisiae* strains. *Vitis.* 37:1:55-59.
- Meilgaard, M.C. 1975. Flavor chemistry of beer. Part II. 239 aroma volatiles. *Tech Q. Master Brew Assoc. AM.* 12:151-168.
- Mendes-Pinto, M. 2009. Carotenoid breakdown products the – norisoprenoids – in wine aroma. *Arc. Biochem. Biophys.* 483:236-245.
- Meyners, M., Kunert, J. and E.M. Qannari. 2000. Comparing generalized procrustes analysis and statis. *Food Qual. Pref.* 11:77-83.
- Molina, A., Swiegers, J., Varela, C., Pretorius, I. and E. Agosin. 2007. Influence of wine fermentation temperature on the synthesis of yeast derived volatile aroma compounds. *Appl. Microbiol. Biotech.* 77:675-687.
- Monagas, M., Gomez-Cordoves, C. and B. Bartolome. 2006. Evolution of phenolic content of red wines from *Vitis vinifera* L. during aging in bottle. *Food Chem.* 95:405-412.
- Morrison, J. and A. Noble. 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* 41:193-200.
- Murray, J., Delahunty, C. and I. Baxter. 2001. Descriptive sensory analysis: past, present and future. *Food Res Int.* 34:461–471
- Noble, A. 1996. Taste-aroma interactions. *Trends Food Sci. Tech.* 7:439-444.
- Oliveira, J.M., Faria, M., Sa, F., Barros, F. and I.M. Araújo. 2006. C₆-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Acta.* 563: 300–309.
- Parr, W., Green, J., White, G. and R. Sherlock. 2007. The distinctive flavour of New Zealand Sauvignon Blanc: Sensory characterization by wine professionals. *Food Qual. Pref.* 18: 849-861.

- Peleg, H., Gacon, K., Schlich, P. and A. Noble. 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Agric. Food Chem.* 79: 8:1123-1128.
- Pereira, G., Gaudilliere, J., Pieri, J., Hilbert, G., Maucourt, M., Deborde, C., Moing, A. and D. Rolin. 2006. Microclimate influence of mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* 54:6765-6775.
- Perrin, L., Symoneaux, R., Maitre, I., Asselin, C., Jourjon, F. and J. Pages. 2007. Comparison of conventional profiling by a trained tasting panel and free profiling by wine professionals. *Am. J. Enol. Vitic.* 58:4:508-517.
- Peterlunger, E., Celotti, E., Da Dalt, G., Stefanelli, S., Gollino. and R. Zironi. 2002. Effect of training system on Pinot noir grape and wine composition. *Am. J. Enol. Vitic.* 53:1:14-18.
- Pineau, B., Barbe, J., Van Leewen, C. and D. Dubourdieu. 2007. Which impact for β -Damascenone on red wine aroma? *J. Agric. Food Chem.* 55:4103-4108.
- Pineau, B., Barbe, J., Van Leewen, C. and D. Dubourdieu. 2009. Examples of perceptive interactions involved in specific “red” and “black-berry” aromas in red wines. *J. Agric. Food Chem.* 57:3702-3708.
- Preston, L., Block, D., Heymann, H., Soleas, G., Noble, A. and S. Ebeler. 2008. Defining vegetal aromas in Cabernet Sauvignon using sensory and chemical evaluations. *Am. J. Enol. Vitic.* 59:2:137-145.
- Qian, M., Fang, Y. and K. Shellie. 2009. Volatile composition of Merlot wine from different vine water status. *J. Agric. Food Chem.* 56:16:7459-7463.
- Reynolds, A., Schlosser, J., Power, R., Roberts, R., Willwerth, J. and C. de Savigny. 2007. Magnitude and interaction of viticulture and enological effects. I. Impact of canopy management and yeast strain on sensory and chemical composition of Chardonnay Musque. *Am. J. Enol. Vitic.* 58:1:12-24.
- Reynolds, A. and D. Wardle. 1989. Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. *Am. J. Enol. Vitic.* 40:121-129.
- Reynolds, A., Yerle, S., Watson, B., Price, S. and D. Wardle. 1996. Fruit environment and crop level effects on Pinot noir. II. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* 47:3:329-339.
- Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. and A. Lonvaud. 2000. *Handbook of Enology: The microbiology of wine and vinification.* John Wiley and Sons Ltd, Chichester, UK.
- Ristic, R., Downey, M., Iland, P., Bindon, K., Francis, I.L., Herderich, M. and S. Robinson. S. 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Aus. J. Grape Wine Res.* 13:53-65.

- Rubico, S. and M. McDaniel. 1992. Sensory evaluation of acids by free-choice profiling. *Chem Senses*. 17:273-289.
- Saerens, S., Delvaux, F., Verstrepen, K., Van Dijck, P., Thevelein, J. and F. Delvaux. 2008. Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Appl. Environ. Micro.* 74:2:454-461.
- Salinas, R., Zalacain, A., Pardo, F. and G. Alonso. 2004. Stir bar sorptive extraction applied to volatile constituents' evolution during *Vitis vinifera* ripening. *J. Agric. Food Chem.* 52:4821-4827.
- Scheirer, P., Drawert, F. and K. Abraham. 1980. Identification and determination of volatile constituents in Burgundy Pinot noir wines. *Lebensl-Wissen. Tech.* 13:6:318-321.
- Siebert, T., Smyth, H., Capone, D., Neuwohmer, C., Pardon, K., Skouroumounis, G., Herderich, M., Sefton, M. and A. Pollintz. 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Annals. Bio Chem.* 381:937-947.
- Skinkis, P., Bordelon, B. and E. Butz. 2010. Effects of sunlight exposure on berry and wine monoterpenes and sensory characteristics of Traminette. *Am. J. Enol. Vitic.* 61:2:147-156.
- Spiers, J., Lee, E., Holt, K., Yong-Duk, Y., Steele, N., Loveys, B. and W. Schuh. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavour aldehydes and alcohols. *Plant Physi.* 117:1047-1058.
- Staff, S.L., Percival, D.C., Sullivan, J.A. and K.H. Fisher. 1997. Fruit zone leaf removal influences vegetative, yield, disease, fruit composition and wine sensory attributes of *Vitis vinifera* L. Optima and Cabernet franc. *Canadian. J. Plant Sci.* 77:149-153.
- Sumby, K., Grbin, P. and V. Jiranek. 2010. Review: Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chem.* 121:1-16.
- Tardaguila, M., Diago, P., De Toda, M., Poni, S. and M. Vilnova. 2008. Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of cv. Grenache grown under non irrigated conditions. *J. Int. Sci. Vigne Vin.* 42:4:221-229.
- Tesniere, C., Torregrosa, L., Pradal, M., Souquet, J-M., Gilles, C., Dos Santos, K., Chatelet, P. and Z. Gunata. 2006. Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. *J. Exp. Botany.* 57:1:91-99.
- Thorngate, J. 1997. The physiology of human sensory response to wine: A review. *Am. J. Enol. Vitic.* 48:271-279.
- Vidal, S., Courcoux, P., Francis, L., Kwaitkowski, M., Gawel, R., Williams, P., Waters, E. and V. Cheynier. 2004. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual. Pref.* 15:209-217.
- Wakeling, I.N., Raats, M.M. and H. J. H. MacFie. 1992. A new significance test for consensus in generalised procrustes analysis. *J. Sens. Studies.* 7:91-96.

Williams, A. and Arnold, G. 1985. A comparison of the aroma of six coffees characterized by conventional profiling, free-choice profiling and similarity scaling methods. *J. Agric. Food Chem.* 36:204–214.

Williams, A. and S. Langron. 1984. The use of free-choice profiling for the evaluation of commercial ports. *J. Agric. Food Chem.* 35:558-568.

Table 1. Descriptors generated at the preliminary sensory session for the modified-free choice profiling sensory analysis.

Descriptors - Aroma	Descriptors - Palate
Dark fruit	Dark fruit
Red fruit	Red fruit
Medicinal	Acid
Rhubarb	Tannin
Spice	Weight
	Bitter finish
	Drying finish
	Hot finish

Table 2. Descriptors generated by an expert panel of winemakers by free choice profiling.

AROMA	PALATE	PALATE
Aldehydic	Acetone	Juiciness
Berry fruit (Rasp/Straw)	Alcohol	Leather
Carbonic	Astringent	Length
Char	Back palate	Mid palate
Cherry	Balance	Over ripe/jammy
Cornflower	Balanced palate	Primary
Dried Fruit	Broad palate	Raisin
Forest floor	Broad	Raspberry
Ginger	Burnt plastic	Red cherry
Herbal	Candied	Rich caramel
Lees autolysis	Concentration	Rose petal
Musky	Confectionary	Sharp
Perfume	Cosmetic	Simple
Red cherry	Dark chocolate	Softness
Red flower	Chocolate/diacetyl	Sourness
Strawberry	Dimension	Strawberry
Sweet	Dusty tannin	Stewed
Volatile	Edgy	Sulphides
	Elegant body	Sweet finish
VISUAL	Fine tannins	Texture
Colour	Flat	Thin
Colour intensity	Fleshy	Unripe tannin
	Forward palate	Vanilla
	Green fruit	Vegetal
	Glance cherry	Velvety tannin
		Vibrancy
		Viscosity
		Volatile
		White pepper

Table 3. Scaling factors residual variances for each panellist.

Panellist	Generic descriptors		Generic plus panellist-generated descriptors	
	Scaling factor	Residual	Scaling factor	Residual
1	1.080	17.852	1.074	26.573
2	0.806	17.287	0.762	36.823
3	1.041	21.733	1.106	27.645
4	0.977	14.675	0.747	23.999
5	0.725	14.605	0.638	33.075
6	0.927	11.650	0.829	25.796
7	1.388	10.994	1.708	20.587
8	0.920	13.407	1.130	20.568
9	1.006	12.712	1.020	21.138
10	1.356	15.140	1.236	32.004
11	1.155	18.931	1.611	33.332
12	1.162	15.247	1.552	22.325
13	1.085	13.376	1.253	19.703

Table 4. Correlations between descriptors by Spearman rank correlation analysis (significant correlations at $P \leq 0.05$)

PCA Dimension 1 (F1)		
Panellist descriptors	Positive correlated descriptors (generic and panellist derived)	Negative correlated descriptors (generic and panellist derived)
Lees autolysis aroma	Medicinal, herbal.	Perfume, raspberry, alcohol.
Colour intensity	Spice, cherry aroma, red flower, ginger, cornbread, concentration, dark fruit palate and aroma, weight, hot finish, colour, red cherry, dimension, balance, elegant, viscosity, broad palate, vanilla, astringency and sweet finish.	Candied.
Vegetal palate	Astringent, thin, vanilla, sharp, rich caramel, unripe tannins.	Colour, red cherry on the palate.
Candied palate	Primary, cosmetic, vanilla, acetone.	Dark fruit aroma and palate, red cherry aromas, red flower, ginger, cornbread, forest floor, weight, colour, colour intensity, concentration, dimension, balance, red cherry on the palate, elegant palate.
Balanced palate	Raisin, fine tannins , astringency.	Sweet finish.
Elegant body	Dark fruit aroma and palate, cherry aroma, red flower, ginger, cornbread, forest floor, weight, colour, colour intensity, concentration, dimension, balance, sweet finish.	Vanilla, red cherry on the palate, candied, astringency, acetone, vegetal, rich caramel, edgy.
PCA Dimension 1 (F1)		
Generic descriptors	Positive correlated descriptors (generic and panellist derived)	Negative correlated descriptors (generic and panellist derived)
Dark fruit aroma	Spice, cherry aroma, red flower, ginger, cornbread, concentration, dark fruit palate, weight, hot finish, colour, colour intensity, red cherry, dimension, balance, sweet finish.	Vanilla, candied.
Rhubarb aroma	Back palate.	Confectionary, rose petal, texture.
Spice aroma	Cherry aroma, red flower, ginger, cornbread, concentration, dark fruit palate, weight, hot finish, colour, colour intensity, red cherry, dimension, balance.	Sweet
Dark fruit on the palate	Spice, cherry aroma, red flower, ginger, cornbread, concentration, dark fruit aroma, weight, hot finish, colour, colour intensity, red cherry, dimension, balance, sweet finish.	Strawberry, sweet finish.
Tannin on the palate	Palate length, raspberry.	Sourness.

Weight on the palate	Spice aroma, cherry aroma, red flower, ginger, cornbread, concentration, dark fruit palate and aroma, hot finish, colour, colour intensity, red cherry, dimension, balance, elegant, viscosity, broad palate, vanilla, astringency, sweet finish.	Primary, candied.
----------------------	---	-------------------

PCA Dimension 2 (F2) panellist descriptors

Panellist descriptors	Positive correlated descriptors (generic and panellist derived)	Negative correlated descriptors (generic and panellist derived)
Char aroma	Confectionary, rose petal.	Acid on the palate.
Carbonic aroma	Red cherry aroma, colour intensity, dimension, velvety tannins, viscosity.	Musky, drying finish, broad palate, chunky, edgy, rich caramel, rose petal.
Confectionary on the palate	Char aroma, chunky, leather.	Rhubarb, acid on the palate, back palate, velvety tannins.
Rose petal on the palate	Char aroma, broad palate, edgy, rich caramel.	Rhubarb, red cherry aroma, carbonic, viscosity.
Strawberry on the palate	Sourness.	Red fruit aroma, red fruit on the palate, hot finish, green fruit, raspberry.
Velvety tannins	Berry fruit (rasp/straw), thin, sharp, acetone, unripe tannins.	Sourness.

PCA Dimension 2 (F2)

Generic descriptors	Positive correlated descriptors (generic and panellist derived)	Negative correlated descriptors (generic and panellist derived)
Red fruit aroma	Red fruit palate, berry fruit (rasp/straw), unripe tannins.	Strawberry, sourness.
Red fruit on the palate	Red fruit aroma.	Strawberry.
Bitter finish on the palate	Musky, glace cherry.	Red cherry aroma, sulphides.

Table 5. The concentration ($\mu\text{g/L}$) of selected “green” and “fruity” aroma compounds in the treatment wines.

Treatment	Year	Ethyl acetate	Ethyl isobutyrate	Ethyl butanoate	Ethyl hexanoate	Ethyl pentanoate	Ethyl cinnamate	Ethyl octanoate	β -ionone	β -damascenone	Hexanal	Hexanol	Trans-3-hexen-1-ol	Cis-3-hexen-1-ol
NLR	2008	97080	307	178	541	2.0	2.3	424	0.48	3.5	4.5	4860	123	46
LR7	2008	100970	276	178	504	2.5	1.4	399	0.48	4.4	3.3	4166	103	41
LR30	2008	100910	309	154	484	2.3	1.1	347	0.44	4.4	3.8	4187	104	40
LRV	2008	106840	329	186	517	2.3	1.0	434	0.31	4.0	2.9	4686	107	50
NLR	2009	97790	98	219	569	2.6	1.1	393	0.59	13	3.8	5820	154	87
LR7	2009	97720	87	215	530	2.8	1.1	397	0.60	14	4.6	5713	148	96
LR30	2009	99020	81	218	534	2.8	1.0	432	0.53	14	4.6	5917	146	97
LRV	2009	97940	99	213	536	2.9	1.1	457	0.52	14	4.4	5468	145	100
Significance														
Treatment		ns	ns	ns	*	ns	***	**	***	ns	ns	**	***	ns
Year		ns	***	***	***	***	***	ns	***	***	***	***	***	***
Tr x Year		ns	ns	*	ns	ns	***	**	ns	ns	***	ns	ns	*

Significance levels are: ns is $P > 0.05$; * is $P \leq 0.05$; ** is $P \leq 0.01$; *** is $P \leq 0.001$

†All wines were analysed for 3-isobutyl-2-methoxypyrazine (IBMP) using the method from Parr et al. (2007) but were found to be below the detectable and quantifiable concentration of 4.3 ng/L in red wine so are not reported (Kotseridis et al. 2008).

Table 6. The detection and quantification of “fruity” and “green” aroma compounds in Pinot noir wines by different analytical methods. *Pinot noir wine concentrations compiled using data from Schreier et al. (1980), Mamede et al. (2005), Louw et al. (2006), Fang and Qian (2006), Feuillat et al. (1997), Massoutier et al. (1998), Aubrey et al. (1997), Fuselgang and Zoecklein (2003), Antalick et al. (2010), Kotseridis et al. (1999) and Kilmartin and Nicolau (2007). For comparative purposes all mg/L values in previous studies have been converted to µg/L.*

Compound	Aroma	Concentration range found in Pinot noir wines (µg/L)	Microvinification Range (µg/L)	Odor threshold (µg/L)
Ethyl acetate	Blackcurrant/sweet fruity	1800-19,700	97780-100970	12264 ^a
Ethyl isobutyrate	Apple/fruity	0.1-0.8	98-329	15 ^b
Ethyl butanoate	Peach/fruity	100-670	154-219	20 ^b
Ethyl pentanoate	Mint/fruity	Identified but not quantified in Pinot noir wines	2.2–2.9	1.5-5 ^d
Ethyl hexanoate	Blackberry/fruity/ strawberry	120-446	484-569	14 ^b , 5 ^c
Ethyl cinnamate	Cherry/fruit	0.8-6.4	0.95-1.10	1.1 ^c
Ethyl octanoate	Fruity/cooked	170-1800	347-457	5 ^b , 2 ^c
β-ionone	Berry/violet	0.20-0.100	0.31-0.60	0.09 ^c
β-damascenone	Heavy fruity undertone /exotic flowers	3-6	3.5-15	0.05 ^b
Hexanal	Green	Not identified or quantified in Pinot noir wines	2.9-4.6	4.4-5 ^d / 350 ^c
Hexanol	Green/dry grass/toasted	1600-6700	4166-5917	8000 ^b
Trans-3-hexen-1-ol	Green/bitter	10-40	103-154	600 ^a
Cis-3-hexen-1-ol	Fresh cut grass/fruity/green	160-560	40-100	400 ^b

^aEtiévant (1991)

^bGuth et al (1997) Odor thresholds determined in 10% w/w aqueous ethanol

^cFerreira et al. (2000) Odor thresholds determined in 10% w/w aqueous ethanol with 7 g/L glycerol at pH 3.2

^dLeffingwell and Leffingwell (2003) Odor thresholds determined in water only

^eMeilgaard (1975) Odor threshold determined in beer as cited by Kishimoto et al. (2006)

Table 7. Relationship between treatment wine descriptors and wine compositional data by Spearman rank correlation method. Significance level is $P \leq 0.05$

	Generic descriptors	Panellist derived descriptors		Panellist derived descriptors
	PCA F1	Positively correlated	Negatively correlated	PCA F2
	Positively correlated	Positively correlated	Negatively correlated	Positively correlated
Ethyl acetate			Lees autolysis aroma	
Ethyl octanoate			Candied	
Hexanal	Tannin on the palate	Lees autolysis aroma		
TA (g/L)		Vegetal palate		
VA (g/L)				Strawberry
Residual sugar (g/L)		Vegetal palate		
Total phenolics a.u.	Red fruit aroma	Vegetal palate		
Total red pigments		Vegetal palate		

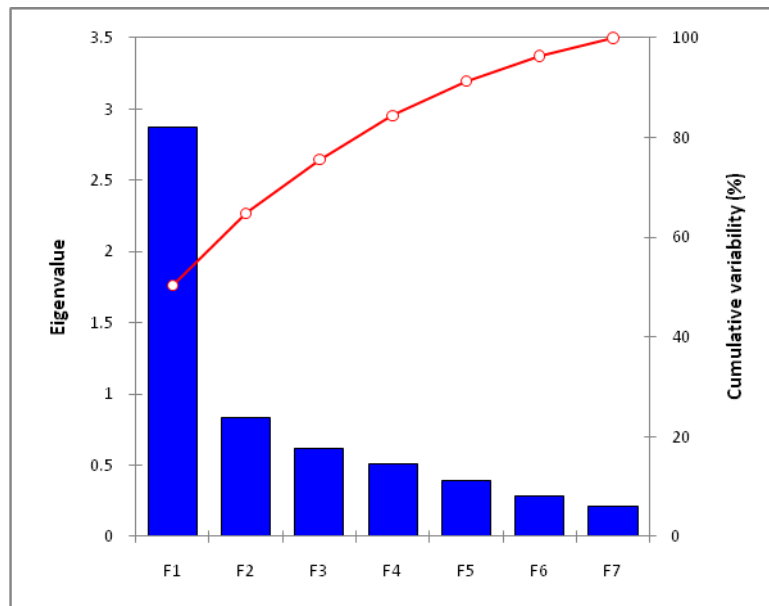


Figure 1. Scree plot from PCA of generic descriptors.

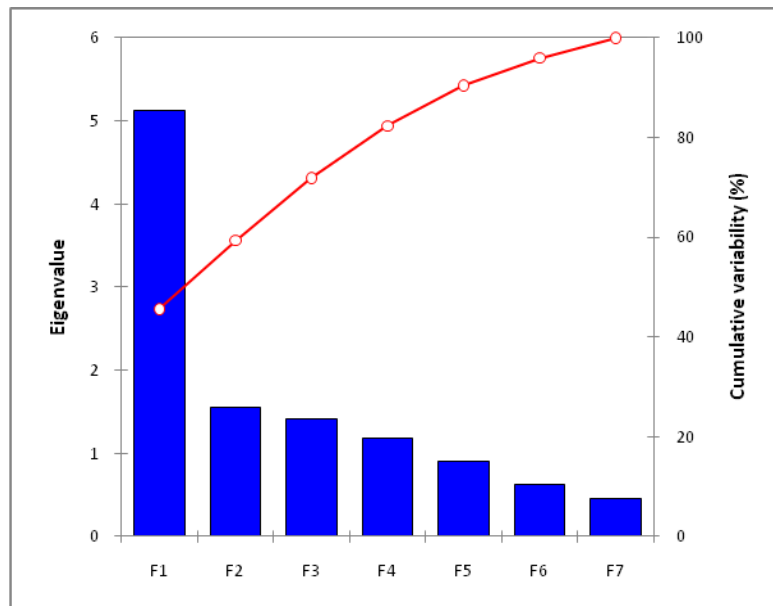


Figure 2. Scree plot from PCA of generic and panellist-generated descriptors.

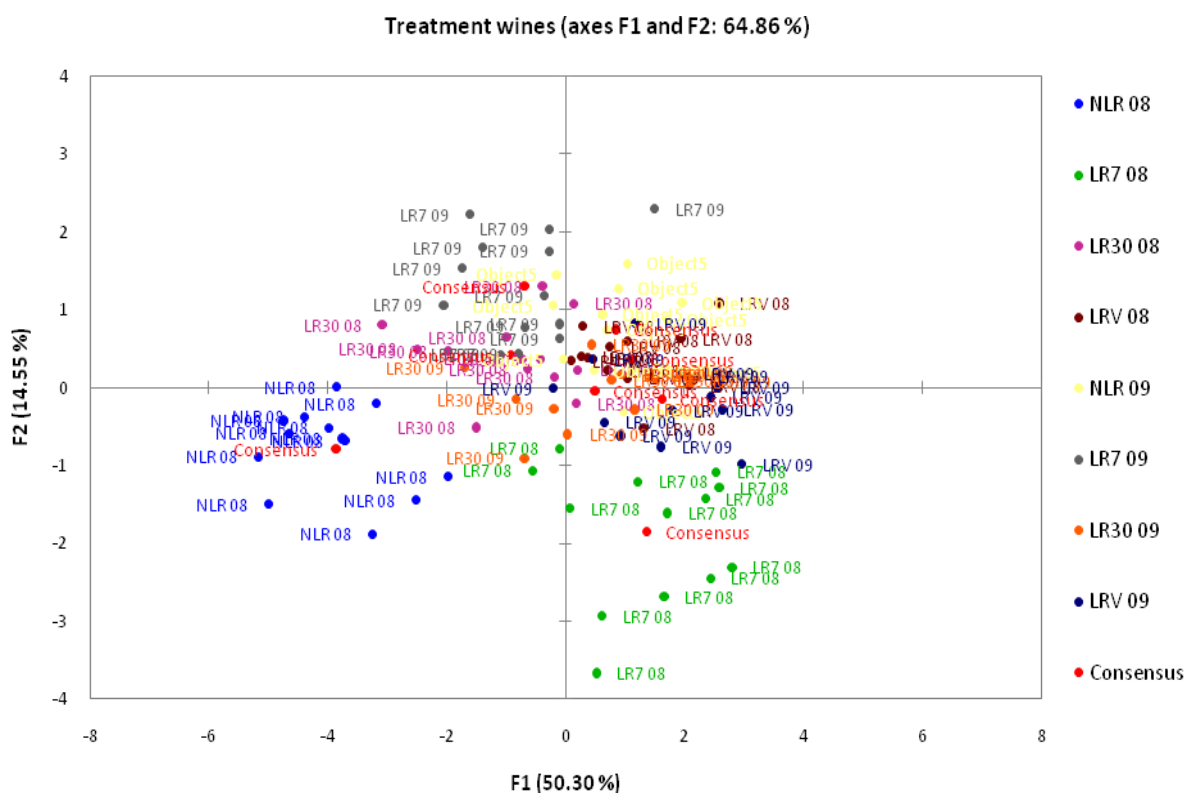


Figure 3. Panellist wine ratings using generic descriptors.

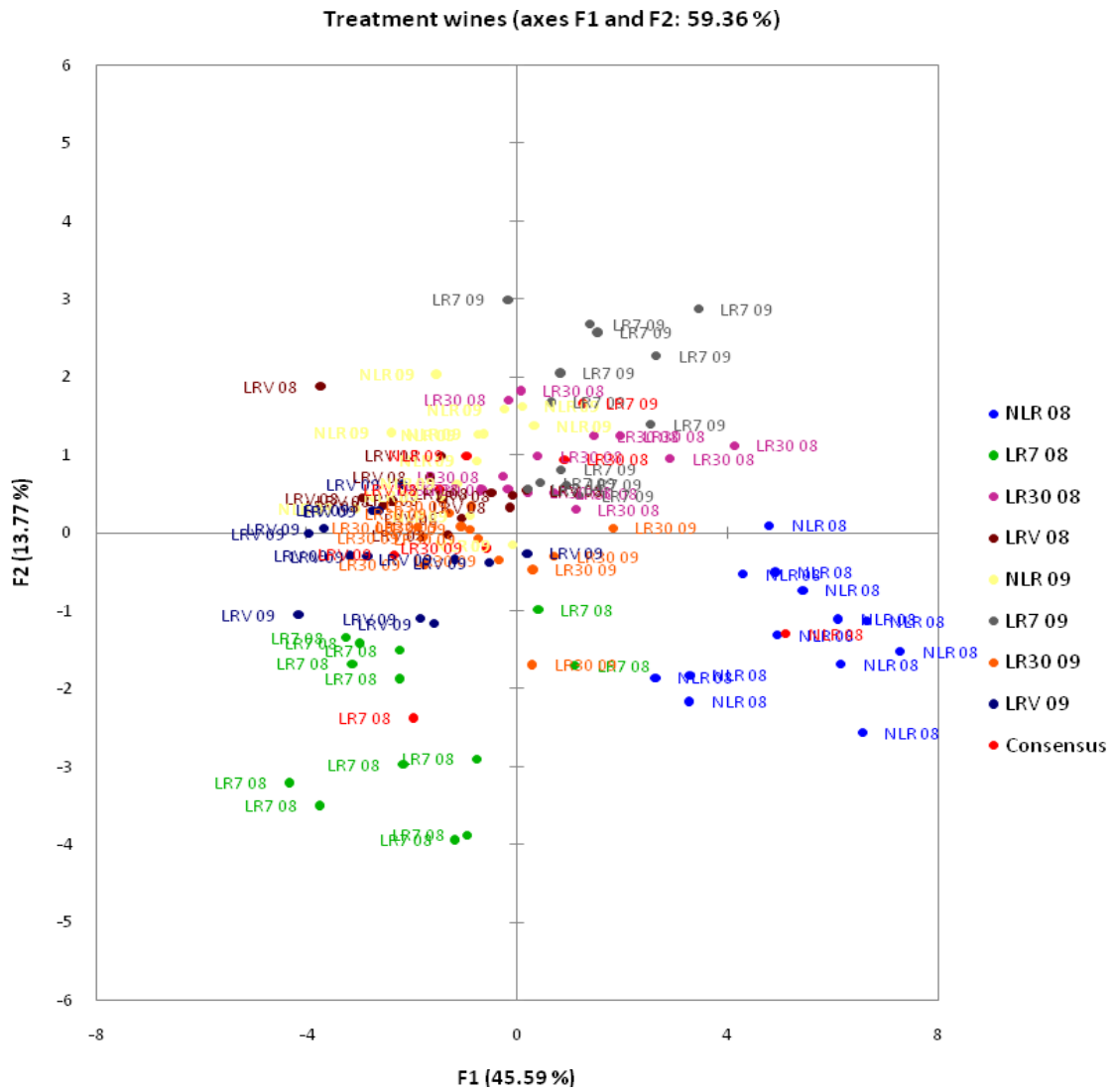


Figure 4. Panellist wine ratings using generic plus expert derived descriptors.

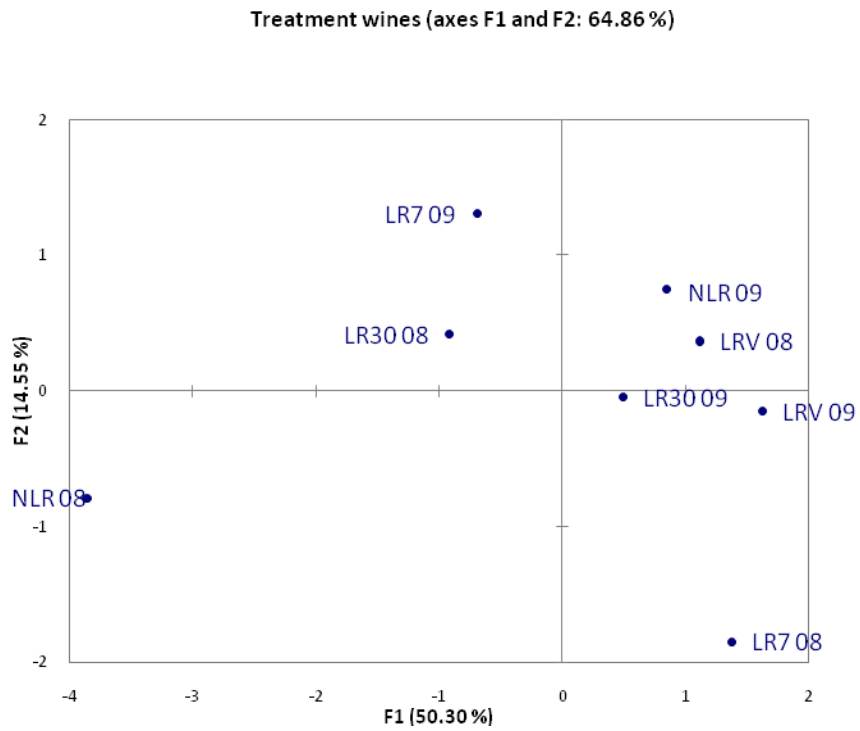


Figure 5. Consensus location of wines using the first two dimensions of PCA of generic descriptors dataset.

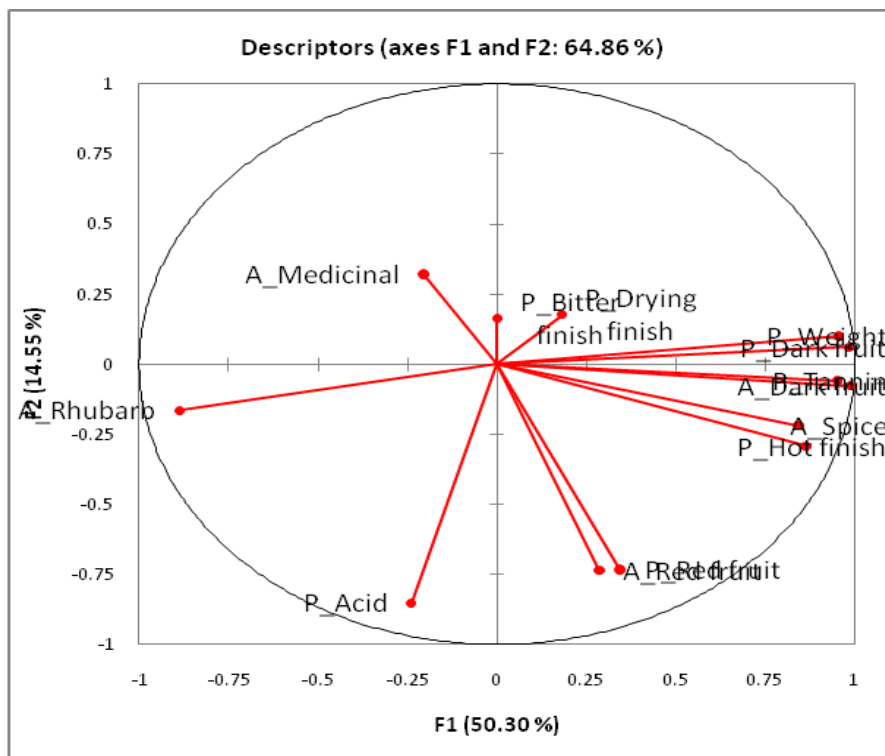


Figure 5a. Consensus location of generic descriptors using the first two dimensions of PCA.

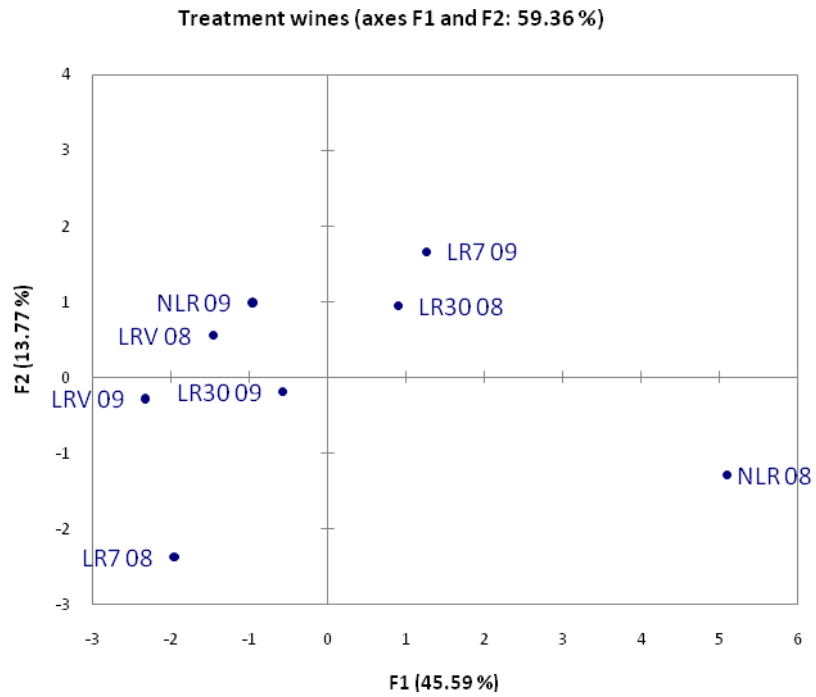


Figure 6. Consensus location of wines using the first two dimensions of PCA of generic plus panellist-generated descriptors dataset.

Chapter 8

General discussion and conclusion

The main objective in this research was to investigate the effect of the timing of a commonly practiced viticultural technique, leaf removal, on Pinot noir berries and the resultant wine. In this study, wines produced from leaf removal treatments carried out at specific times during the growing season in two consecutive years were analysed chemically and sensorially. Sensory analysis using a modified-FCP method was conducted by Waipara winemakers. Wine chemical analyses included standard winemaking protocols in addition to tannin concentration and composition, colour spectral analysis and an investigation into specific “fruity” and “green” volatile aroma compounds. This study required the development of a HS-SPME-GC-MS-SIDA method to analyse the different aroma compounds in the microvinified treatment wines. Additionally, in the vineyard grapevine canopies were characterised, leaf area to fruit weight calculated and berry ripening parameters monitored prior to harvest. In this final thesis chapter the aim is to discuss the wines from the winemakers’ or consumers perspective from the sensory analysis results, back to the vineyard leaf removal treatments. Explanations regarding results, discoveries and the relevance of the current research to viticulture and oenology in New Zealand in combination, with further research suggestions, are included in this chapter.

8.1 Sensory analysis by modified-free choice profiling

The modified-FCP technique revealed that NLR wines in 2008 were described as having rhubarb and lees autolysis aromas and vegetal and candied palate. The same defoliation treatment in 2009 produced wines that had higher intensity of colour, dark fruit aroma, and dark fruit, tannin, weight and balance on the palate than LR7 in 2008 and was also distinctly placed on the PCA plot due to high red fruit and carbonic aromas plus velvety tannins, acid and bitter finish. This could possibly be due to having the highest tannin concentration in 2008. The grouping of NLR 2009, LRV 2008, LRV 2009 and LR30 2009 is attributed to increased perception of dark fruit aroma, colour intensity, and tannin, weight and balanced palate. However, LR7 wine in 2009 is grouped with LR30 wine from 2008 suggesting a component and/or a combination of components that are common to both wines which requires further investigation.

All leaf removal treatments were higher in dark fruit characteristics and tannin, weight and balanced palate in 2009 compared to 2008. Vines that were defoliated at veraison produced wines which have less vintage variation in the sensory analysis compared to earlier leaf removal treatments (LR7 and LR30). Sensory analysis was carried out on both vintages in 2009 which may have affected wine perceptions as the 2009 wines were one year younger. Flavour, aroma and colour differences may have been more prominent in the 2008 treatment wines if winemaker sensory analysis had been carried out a year earlier. The use of winemakers for sensory analysis enabled local producers to taste the effects of the timing of leaf removal on Pinot noir wine and the use of winemakers as expert panelists enabled analysis of unfinished wines. The lack of panel training resulted in a wine sensory method that was less time consuming than conventional methods.

Further analysis of the wines using the same method could be carried out after one, two, three and four years of bottle aging to ascertain whether the flavour and aroma effects remain. Future studies should include oak aging the treatment wines to simulate commercially produced Pinot noir wines. The original hypotheses in Chapter 2 stated that leaf removal reduces the perception of "green" characteristics and increases "fruity" nuances. Unfortunately a disadvantage of free choice profiling is the inability to distinguish by statistical methods, the preferred wines but it does allow for a detailed description of the wines and therefore correlations with chemical compositions.

8.2 "Green" and "fruity" aroma compounds

In this study no discernible pattern was established regarding the concentrations of esters amongst treatment wines. However, the "fruity" C₁₃ norisoprenoids increased sequentially with the timing of leaf removal because the earliest leaf removal, LR7, had the highest concentrations followed by LR30 then LRV. β -Damascenone concentrations were affected more than β -ionone but as their concentration increased more "fruity" nuances were attributed to the early defoliated wines, LR7 and LR30. The increase of C₆ alcohols in all 2009 wines and in wines made from defoliated vines in 2008 may also have contributed to the "fruity" perception. Additionally, all "fruity" aroma compounds were found in concentrations above their odour threshold in all wines and "green" compounds were below.

The role of hexanal in “vegetal” perception requires further investigation as this study indicates that this C₆ aldehyde may play an important role in the “green” perception of wines in the absence of “fruity” aromas. To investigate the role of hexanal in “green tannin” perception, hexanal could be added at varying rates to wines of differing tannin concentrations and the wines subjected to sensory analysis. Additionally, suggestions that hexanal decreases and C₆ alcohols increase with fruit maturity as C₁₃ norisoprenoids increase could be explored during Pinot noir grape ripening prior to harvest (Fang and Qian 2006). Anecdotally, winemakers taste grapes in the vineyard prior to harvest for a decline in “green” flavours and an increase in “fruity” flavours and it could be that they actually taste the change from hexanal to its corresponding alcohol and the simultaneous increase in C₁₃ norisoprenoids without realising it.

Further chemical analysis of additional aroma compounds associated with “fruity” and “green” aromas i.e. trans-2-hexanal, should be studied as wine is a complex medium of aroma compounds and only a small selection were investigated in this research. The “green tannin” perception and a relationship with hexanal concentration in the absence of other wine quality components require further research.

8.3 Wine phenolic composition

NLR wines in 2008 had the lowest tannin concentration, quercetin and total phenolics and exposing clusters early (LR7 and LR30) encouraged synthesis of flavan-3-ol monomers and quercetin. The effects of light on the accumulation of phenolic compounds in plant tissues may not only be explained by providing energy for carbon assimilation and consequently providing carbon resources for biosynthesis. Additionally, the quality of light especially UV radiation is important for the formation and accumulation of certain phenolic compounds in plants (Treutter 2010). Although UV radiation was not measured in the grapevine canopy, the high incidence of UV-B in New Zealand could have elevated synthesis of proanthocyanidins, flavan-3-ol monomers, quercetin and C₁₃ norisoprenoid accumulation and concentration. Interestingly, the wine with the highest quercetin in 2008 was LR30 and in 2009 was LR7, which were grouped in close proximity on the PCA plot (Chapter 7). This similarity could possibly be attributed to quercetin concentration in combination with other wine components that were not analysed in the present study. However, Gawel (1998) states the taste threshold of quercetin is 10 mg/L in 5% ethanol solution and 20 mg/L in beer (Dadic and Belleau 1973). All wines in our study were below these levels but quercetin glycosides, or other

compounds that were not analysed in the present study, may have had an effect on the sensory perception of these wines.

Mean degree of polymerisation (mDP) was unaffected by the timing of leaf removal suggesting that the tannin polymerisation in wine is independent of cluster exposure. Unfortunately, grape derived tannin polymers cannot currently be distinguished from tannin polymers synthesised in the wine (Terrier et al. 2008). LRV in both years was carried out to coincide with the commencement of tannin maturation. The wines made from early leaf removal vines had the highest flavan-3-ol concentrations in both years but this increase did not increase mDP. Interestingly, the greater quantity of flavan-3-ol monomers available in the wines made from early leaf removal vines did not increase the mDP of wine tannins. Additionally, increased wine flavan-3-ols in 2009 across all treatments increased the quantity of proanthocyanidins but did not affect their mDP. The hypothesis in Chapter 2, that early leaf removal, LR7 would result in increased wine colour and tannin concentration was proved correct in 2008 but not 2009. The increased alcohol level in 2009 may have increased phenolic extraction.

Perieria et al. (2006) reported an increase in proline in sun exposed Merlot berries and less arginine compared to naturally shaded fruit. According to Treutter (2010), under stress conditions, the plant is forced to accumulate a large quantity of free proline. Its synthesis is accompanied by the oxidation of NADPH. An increased NADP⁺/NADPH ratio is likely to enhance activity of the oxidative pentose phosphate pathway providing precursors for phenolic biosynthesis via the shikimic acid pathway. The alternating oxidation of NADPH by proline synthesis and reduction of NADP⁺ by the two oxidative steps of the oxidative pentose phosphate pathway serve to link both pathways and thereby facilitate the continuation of high rates of proline synthesis during stress and lead to a simultaneous accumulation of phenolic compounds (Shetty 2004).

Consequently, future studies regarding the timing of leaf removal should measure proline and arginine in grape must prior to fermentation and ascertain whether their concentrations correlate with phenolic concentrations in the resultant wine.

Future research regarding viticultural treatments in New Zealand on phenolic composition should therefore measure the incidence of UV-B within the fruit zone. Early cluster exposure

from early leaf removal (LR7 and LR30) may have activated the flavonoid pathway enzymes in favour of 2,3-*trans* tannin accumulation rather than the 2,3-*cis* configuration in Pinot noir berries suggesting further research regarding enzymes involved in flavan-3-ol synthesis is required. Further research in cool climate northern hemisphere wine regions is required, due to a lower rate of UV radiation than New Zealand, to determine whether tannin concentration increases in wine from early cluster exposure. Altering wine tannin concentration in the vineyard by early leaf removal could reduce the need for exogenous tannin and enzyme additions in the winery.

8.4 Grapevine growth and berry ripening

Traditional berry ripening parameters, °Brix, pH and TA (g/L), showed no difference between treatments but these are not the only physiological ripening parameters used by winemakers to dictate harvest dates. Berry ripeness is also judged using visual and taste tests including berry turgidity, bunch stem lignification, the ease that seeds break from the pulp and seed colour, as well as sensations elicited by tasting berries and chewing seeds (bitterness and astringency). Further research into the effect of the timing of leaf removal on Pinot noir such as alternative physiological ripeness parameters could be investigated in future studies. The effect of the timing and severity of leaf removal on berry cell wall composition, thickness and effects on tannin extraction is recommended. Our study only analysed skins and seeds post fermentation but these could be analysed at harvest for phenolic composition and concentration as well as protein and polysaccharide contents. Results could be compared to phenolic composition of skins and seeds post fermentation. A study such as this could reveal differences from cluster exposure timing that occurred in berry cell wall components and therefore impact tannin binding capacity and extraction. Additionally, it could reveal whether the increase in flavan-3-ols in the early leaf removal wines originated in the skins or seeds. Regarding the current study, it is unclear whether increased flavan-3-ol monomer concentration in the 2009 wines is a direct response to severity of defoliation and cluster exposure or due to increased alcohol in the medium. However, a distinct difference in flavan-3-ol concentrations in LR7 and LR30 wines compared to NLR and LRV wines occurred in the present study. Grapevine regrowth of LR7 and LR30 facilitated the necessary leaf area to ripen the crop. In this study, it is important to take into account that the 2009 wines were produced from grapevines that received greater defoliation than in 2008. Further studies could incorporate the timing of shoot thinning and cluster thinning with leaf removal.

8.5 Conclusion

It is evident that the vines that had leaf removal produced wines with higher tannin concentrations, colour, “fruity” aromas and complexity. LRV wines in both years contained marginally more tannin, colour and flavour than NLR. LR7 and LR30 wines were similar in tannin concentration and colour in both years. The difference in °Brix levels resulted in higher alcohol in 2009 which could have affected tannin extraction and aroma perception. According to sensory analysis, vintage differences were less pronounced by late mechanical leaf removal compared to early leaf removal. Wine chemical and sensory analysis after several years of aging may reveal additional differences in the treatment wines. Tannin and aroma components are important wine quality parameters so research that studies the affects of viticultural practices on final wines is important to winemakers. Revealing the mystery of “green tannin” and the possible role of hexanal in its perception will undoubtedly be a future challenge for wine researchers.

References

- Adams, D. (2006) Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture*. **57**, 249-256.
- Adams, D. and Harbertson, J. (1999) Use of alkaline phosphatase for the analysis of tannins in grape and red wines. *American Journal of Enology and Viticulture*. **50**, 247-252.
- Antalick, G., Perello, M. and de Revel, G. (2010) Development, validation and application of a specific method for the Quantitative determination of wine esters by Headspace-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry. *Food Chemistry*. **121**, 1236-1245.
- Aparicio, J., Toledano Medina, M. and Lauente Rosales, V. (2007) Descriptive sensory analysis in different classes of orange juice by a robust free choice profile method. *Anal. Chim. Acta*. **595**, 238–247.
- Aparicio, R. and Morales, M. (1998) Characterisation of olive ripeness by green aroma compounds of virgin olive oil. *Journal of Agricultural and Food Chemistry*. **46**, 1116-1122.
- Aron, P. and Kennedy, J. (2007) Compositional investigation of phenolic polymers isolated from *Vitis vinifera* L. cv. Pinot noir during fermentation. *Journal of Agricultural and Food Chemistry*. **55**, 5670-5680.
- Arnold, R. and Bledsoe, A. (1990) The effect of various leaf removal treatments on the aroma and flavour of Sauvignon Blanc wines. *American Journal of Enology and Viticulture*. **41**, 74-76
- Arnold, G., Gower, J., Gardner-Lubbe, S. and Le Roux, N. (2007) Biplots of free-choice profile data in generalised orthogonal procrustes analysis. *Applied Statistics*. **56**, 445-458.
- Aronson, J. and Ebeler, S. (2004) Effect of polyphenol compounds on headspace volatility of flavours. *American Journal of Enology and Viticulture*. **55**, 13-21.
- Aubrey, V., Etievant, P., Ginies, C. and Henry, R. (1997) Quantitative determination of potent flavour compounds in Burgundy Pinot noir using a stable isotope dilution assay. *Journal of Agricultural and Food Chemistry*. **45**, 120-2123.
- Barcnas, P., Perez Elortondo, F. and Albisu, M. (2003) Comparison of free choice profiling, direct similarity measurements and hedonic data for ewes' milk cheeses sensory evaluation. *International Dairy Journal*. **13**, 67 – 77.
- Barbosa, C., Falco, V., Mendes-Faia, A. and Mendes-Ferreira, A. (2009) Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *Saccharomyces cerevisiae* wine strains. *Journal of Bioscience and Bioengineering*. **108**, 99-104.

Bavaresco, L., Gatti, M., Pezzutto, S., Fregoni, M. and Mattivi, F. (2008) Effect of leaf removal on grape yield, berry composition and stilbene concentration. *American Journal of Enology and Viticulture*. **59**, 292-298.

Bennett, J., Jarvis, P., Creasy, G. and Trought, M. (2005) Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature chardonnay grapevines. *American Journal of Enology and Viticulture*. **56**, 386-393.

Bergqvist, J., Dokoozlian, N. and Ebisuda, N. (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. *American Journal of Enology and Viticulture*. **52**, 1-7.

Binder, S., Knill, T. and Schuster, J. (2007) Branched chain amino acid metabolism in higher plants. *Physiologia Plantarum*. **129**, 68-68.

Bindon, K., Smith, P. and Kennedy, J. (2010a) Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *Journal of Agricultural and Food Chemistry*. **58**, 2520-2528.

Bindon, K., Smith, P., Holt, H. and Kennedy, J. (2010b) Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinifications. *Journal of Agricultural and Food Chemistry*. **58**, 10736–10746.

Blanchard, L., Darriet, P. and Dubourdieu, D. (2004) Reactivity of 3-mercaptohexanal in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. *American Journal of Enology and Viticulture*. **55**, 115-120.

Bledsoe, A., Kliewer, W. and Marois, J. (1988) Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon Blanc. *American Journal of Enology and Viticulture*. **39**, 49-54.

Bogs, J., Downey, M., Harvey, J., Ashton, A., Tanner, G. and Robinson, S. (2005) Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiology*. **139**, 652-663.

Bogs, J., Ebadi, A., David, D. and Robinson, S. (2006) Identification of the flavonoid hydroxylases from grapevines and their regulation during fruit development. *Plant Physiology*. **140**, 279-291.

Botha, J.J., Ferreira, D. and Roux., D.G. (1981) Synthesis of condensed tannins. Part 4. A direct biomimetic approach to [4,6]- and [4,8]- biflavanoids. *Journal of the Chemical Society. Perkin Translation*. **1**, 1235 – 1245.

Boulton, R. 2001. The copigmentation of anthocyanins and its role in the colour of red wine: A critical review. *American Journal of Enology and Viticulture*. **52**, 2:67-87.

Braidot, E., Zancani, M., Petrusa, E., Peresson, C., Bertolini, A., Patui, S., Macri, F. and Vianello, A. (2008) Transport and accumulation of flavonoids in grapevine (*Vitis vinifera* L.). *Plant Signalling and Behaviour*. **3**, 626–632.

Brander, C., Kepner, R. and Webb, A. (1980) Identification of some volatile aroma compounds of wine in *Vitis vinifera* cultivar Pinot noir. *American Journal of Viticulture and Enology*. **31**, 69-74.

Brooks, L., McCloskey, L., Mckesson, D. and Sylvan, M. (2008) Adams-Harbertson protein precipitation-based wine tannin method found invalid. *Journal of AOAC International*. **91**, 1090-1094.

Broussaud, F., Cheynier, V., Asselin, C. and Moutounet, M. (1999) Flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *American Journal of Enology and Viticulture*. **50**, 277-284.

Broussaud, F., Cheynier, V. and Noble, A. (2001) Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research*. **7**, 33-39.

Bureau, S., Baumes, R. and Razungles, A. (2000) Effects of vine or bunch shading on the glycosylated flavour precursors in grapes of *Vitis vinifera* Cv. Syrah. *Journal of Agricultural and Food Chemistry*. **48**, 1290-1297.

Busse-Valverde, N., Gomez-Plaza, E., Lopez-Roca, J., Gil-Munoz, R., Fernandez-Fernandez, J. and Bautista-Ortin, A. (2010) Effect of different practises on skin and seed proanthocyanidins in three varietal wines. *Journal of Agricultural and Food Chemistry*. **58**, 11333-11339.

Cadot, Y., Minanacastello, M. and Chevalier, M. (2006) Flavan-3-ol compositional changes in grape berries (*Vitis vinifera* L. cv. Cabernet franc) before veraison, using two complementary analytical approaches, HPLC reversed phase and histochemistry. *Analytica Chimica Acta*. **563**, 65-75.

Canals, R., Llaudy, M., Valls, J., Canals, J. and Zamaora, F. (2005) Influence of ethanol concentration on the extraction of colour and phenolic compounds from skins and seeds of Tempanillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry*. **53**, 4019-4025.

Candolfi-Vasconcelos, M.C. and Koblet, W. (1990) Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera* – Evidence of compensation and stress recovering. *Vitis*. **29**, 199–221.

Canuti, V., Conversano, M., Li Calzi, M., Heymann, H., Matthews, M. and Ebeler, S. (2009) Headspace solid phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. *Journal of Chromatography A*. **1216**, 3012-3022.

Carrillo, J., Garrido-Lopez, A. and Tena, M. (2006) Determination of volatile oak compounds in wine by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Chromatography A*. **1102**, 25-36

- Carvalho, E., Mateus, N. and De Freitas, V. (2004) Flow nephelometric analysis of protein-tannin interactions. *Analytica Chimica Acta*. **513**, 97-101.
- Caspari, H.W., Lang, A. and Alspach, P. (1998) Effects of girdling and leaf removal on fruit set and vegetative growth in grape. *American Journal of Enology and Viticulture*. **49**, 359-366.
- Caven-Quantrill, D. and Buglass, A.J. (2008) Seasonal variation of flavour content of English vineyard grapes, determined by stir-bar sorptive extraction-gas chromatography-mass spectrometry. *Flavour and Fragrance Journal*. **23**, 239-248.
- Cerpa-Calderon, F. and Kennedy, J. (2008) Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. *Journal of Agricultural and Food Chemistry*. **56**, 9006-9014.
- Chapman, D., Matthews, M. and Guinard, J. (2004) Sensory attributes of Cabernet Sauvignon wines made with different crop yields. *American Journal of Enology and Viticulture*. **55**, 325-334.
- Chellemi, D. and Marois, J. (1992) Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew. *American Journal of Enology and Viticulture*. **43**, 53-57.
- Cheynier, V. (2005) Polyphenols in foods are more complex than often thought. *The American Journal of Clinical Nutrition* **81**, 223S-229S.
- Cheynier, V., Duenas-Paton, M., Salas, E., Maury, C., Souquet, J-M. Sarni-Manchado, P. and Fulcrand, H. (2006) Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture*. **3**, 298-305.
- Chorti, E., Guidoni, S., Ferrandino, A. and Novello, V. (2010) Effect of different cluster sunlight exposure levels to ripening and anthocyanin accumulation in Nebbiolo grapes. *American Journal of Enology and Viticulture*. **61**, 1:23-30.
- Cliff, M. and Dever, M. (1996) Sensory and compositional profiles of British Columbia Chardonnay and Pinot noir wines. *Food Research International*. **29**, 317-323.
- Cohen, S., Tarara, J. and Kennedy, J. (2008) Assessing the impact of temperature on grape phenolic metabolism. *Analytica Chimica Acta*. **621**, 57-67.
- Conde, C., Silva, P., Fontes, N., Dias, A., Tavares, R., Sousa, M., Agasse, A. Delrot, S. and Geros, H. (2007) Biochemical changes throughout grape berry development and fruit and wine quality. *Global Science Books*. Pp1-22.
- Coombe, B., G. (1960) Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. *Plant Physiology*. **35**, 241-250.

Cortell, J. and Kennedy, J. (2006) Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L) Pinot noir fruit and extraction in a model wine system. *Journal of Agricultural and Food Chemistry*. **54**, 8510-8520.

Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2005) Influence of vine vigour on grape (*Vitis vinifera* L. Cv. Pinot noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry*. **53**, 5798-5808.

Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2007a) Influence of vine vigour on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *Journal of Agricultural and Food Chemistry*. **55**, 6575-6584.

Cortell, J., Halbleib, M., Gallagher, A., Righetti, T. and Kennedy, J. (2007b) Influence of Vine vigour on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins. 2. Anthocyanins and Pigmented polymers in wine. *Journal of Agricultural and Food Chemistry*. **55**, 6585-6595.

Cortell, J., Silvertsen, H., Kennedy, J., Heymann, H. (2008) Influence of vine vigour on Pinot noir fruit composition, wine chemical analysis, and wine sensory analysis. *American Journal of Enology and Viticulture*. **59**:1:1-10.

Crippen, D. and Morrison, J. (1996) The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. *American Journal of Enology and Viticulture*. **37**, 243-247.

Crozier, A., Clifford, M. and Ashihara. I. (2006) *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*. Blackwell Publishing, Oxford, UK. Pp 16-18.

Dadic, M. and Belleau, G. (1973) Polyphenols and beer flavor. *Proceedings of the American Society of Brewing Chemists* **4**, 107-114.

Daniel, M., Elsey, G., Capone, D., Perkins, M. and Sefton, M. (2004) Fate of Damascenone in wine: The role of SO₂. *Journal of Agricultural and Food Chemistry*. **52**, 8127-8131.

Deaville, E., Green, R., Mueller-Harvey, I., Willoughby, I. and Frazier. R. (2007) Hydrolysable tannin structures influence relative globular coil protein binding strengths. *Journal of Agricultural and Food Chemistry*. **55**, 4554-4561.

De Freitas, V. and Mateus, N. (2002) Nephelometric study of salivary protein-tannin aggregates. *Journal of the Science of Food and Agriculture*. **82**, 113-119.

De la Calle Garcia, D., Reichenbacher, M., Danzer, K., Hurlbeck, C., Bartsch, C. and Feller, K. (1998) Analysis of wine bouquet components using headspace solid-phase microextraction-capillary gas chromatography. *Journal of High Resolution Chromatography*. **21**, 373-377.

De Jong, S., Heidema, J. and Van der Knaap, H.C.M. (1998) Generalised procrustes analysis of coffee brands tested by five European sensory panels. *Food Quality and Preference*. **9**, 111-114.

Delcour, J.A., ferreira, D. and Roux, D. G. (1983) Synthesis of condensed tannins. Part 9. The condensation sequence of leucoanthocyanidin with (+)-catechin and with the resultant procyanidins. *Journal of the Chemical Society. Perkins Translation*. **1**, 1711–1717.

Deluc, L., Barrieu, F., Marchive, C., Lauvergeat, V., Decendit, A. Richard, T., Carde, J., Merillon, J. and Hamdi, S. (2006) Characterisation of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. *Plant Physiology*. **140**, 499-511.

Des Gachons, C.P. and Kennedy, J. (2003) Direct method for determining seed and skin proanthocyanidin extraction into red wine. *Journal of Agricultural and Food Chemistry*. **51**, 5877-5881.

Dey, P. and Harbourne. J. 1997. *Plant Biochemistry*. Academic Press, San Diego, California, USA.

Diáz-Maroto, C., Schneider, R. and Baumes, R. (2005) Formation pathways of ethyl esters branched short-chain fatty acids during wine aging. *Journal of Agricultural and Food Chemistry*. **53**, 3503-3509.

Dixon, R., Xie, De-Yu. and Sharma, S. (2005) Tansley Review: Proanthocyanidins-a final frontier in flavonoid research? *New Phytologist*. **165**, 9-28.

Dokoozlian, N.K. and Kliewer, W.M. (1995) The light environment within grapevine canopies. I. Description and seasonal changes during fruit development. *American Journal of Enology and Viticulture*. **46**, 209-218.

Dokoozlian, N.K. and Kliewer, W.M. (1996) Influence of light on grape berry growth and composition varies during development. *Journal of American Society of Horticulture Science*. **121**, 869-874.

Dolors Guàrdia, M., Aguiar, A., Claret, A., Arnau J. and Guerrero, L. (2010) Sensory characterisation of dry-cured ham using free-choice profiling. *Food Quality and Preference*. **21**, 148–155.

Downey, M., Harvey, J. and Robinson, S. (2003a) Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research*. **9**, 110-121.

Downey, M., Harvey, J. and Robinson, S. (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research*. **10**, 55-73.

Downey, M., Harvey, J. and Robinson, S. (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*. **9**, 15-27.

Downey, M., Dokoozlian, N., Krstic, K. and Mark, P. (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *American Journal of Enology and Viticulture*. **57**, 257-268.

Dufour, C. and Bayonove, C.L. (1999) Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *American Journal of Enology and Viticulture*. **47**, 678-684.

Du Toit, W., Marais, J., Pretorius, I. and Du Toit, M. (2006) Oxygen in must and wine: A review. *South African Journal of Enology and Viticulture*. **27**, 76-94.

Ebadi, A., May, P. and Coombe, B. (1996) Effect of short-term temperature and shading on fruit-set, seed and berry development in model vines of *Vitis vinifera*, cvs Chardonnay and Shiraz. *Australian Journal of Grape and Wine Research*. **2**, 1-8.

Ebeler, S. (2001) Analytical chemistry: Unlocking the secrets of wine flavour. *Food Reviews International*. **17**, 45-64.

Eglinton, J., Griesser, M., Henschke, P., Kwiatowski, M., Parker, M. and Herderich, M. (2004) Yeast-mediated formation of pigmented polymers in red wine. Red wine colour: Revealing the mysteries. ACS Symposium series 886, Washington DC, USA. Pp 7-12.

Escudero, A., Campo, E., Farina, L., Cacho, J. and Ferreira, V. (2007) Analytical characterisation of the aroma of five premium red wines. Insights into the role of odour families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*. **11**, 4501-4510.

Escudero, A., Gogorza, B., Melus, M., Ortin, N., Cacho, J. and Ferriera, V. (2004) Characterisation of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agricultural and Food Chemistry*. **52**, 3516-3524.

Esti, M. and Tamborra, P. (2006) Influence of winemaking techniques on aroma precursors. *Analytica Chimica Acta*. **563**, 173-179.

Etiévant, P.X. (1991) Wine. In. *Volatile compounds in food*. Ed. H. Maarse. Food Science and Technology. Marcel Dekker Inc, New York. Pp 483-546.

Falcao, L., De Revel, G., Perello, M., Moutsiou, A., Zanus, M. and Bordignon-Luiz, M. (2007) A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C₁₃-norisoprenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. *Journal of Agricultural and Food Chemistry*. **55**, 3605-3612.

Fan, W. and Qian, M. (2005) Headspace solid phase microextraction and gas chromatography-olfactory dilution analysis of young and aged Chinese “Yanghe Daqu” liquors. *Journal of Agricultural and Food Chemistry*. **53**, 7931-7938.

Fan, W., Xu, Y., Jiang, W. and Li, J. (2010) Identification and quantification of impact aroma compounds in four non-floral *Vitis vinifera* varieties of grapes. *Journal of Food Science*. **75**, 81-88.

Fang, Y. and Qian, M. (2005) Aroma compounds in Oregon Pinot noir wine determined by aroma extract dilution analysis (AEDA). *Flavour and Fragrance Journal*. **20**, 22-29.

Fang, Y. and Qian, M. (2006) Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *Journal of Agricultural and Food Chemistry*. **54**, 8567-8573.

Ferreira, B., Hory, C., Bard, M., Taisant, C., Olsson, A. and Le Fur, Y. (1995) Effects of skin contact and settling on the level of C18:2, C18:3 fatty acids on C6 compounds in Burgundy Chardonnay musts and wines. *Food Quality and Preference*. **6**, 35-41.

Ferreira, V., Lopez, R. and Cacho, J.F. (2000) Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*. **80**, 1659-1667.

Feuillat, M., Charpentier, C. and Massoutie, C. (1997) Enological importance of cryotolerant *Saccharomyces cerevisiae* yeast strains. *Revue Francaise d'Oenologie, France*. **24**, 85:18-21.

Fischer, U. (2007) *Flavours and fragrances: Chemistry, bioprocessing and sustainability Wine aroma*. Springer Publishers, Berlin, Germany. **11**, 241-267.

Fischer, U. and Noble, A. (1994) The effect of ethanol, catechin concentration and pH on sourness and bitterness in wine *American Journal of Enology and Viticulture*. **455**, 6-10.

Flamini, R. (2003) Mass spectrometry in grape and wine chemistry. Part 1: Polyphenols. *Mass Spectrometry Reviews* **22**, 218-250.

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J., Moutounet, M. and Cheynier, V. (2006) Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry*. **54**, 7331-7338.

Francis, I. and Newton, J. (2005) Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*. **11**, 114-126.

Frivik, S. and Ebeler, S. (2003) Influence of sulfur dioxide on the formation of aldehydes in white wine. *American Journal of Enology and Viticulture*. **54**, 31-38.

Frietas, V., Glories, Y. and Monique, A. (2000) Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *American Journal of Enology and Viticulture*. **51**, 397-403.

Fulcrand, H., Morel-Salmi, C., Mane, C., Poncet-Legrand, C. and Cheynier, V. (2005) Tannins: from reactions to complex supramolecular structures. Advances in Tannin and Tannin Management. ASVO Seminar Proceedings. Adelaide, South Australia .Pp 11-19.

Fujita, A., Soma, N., Goto-Yamamoto, N., Mizuno, A., Kiso, K. and Hashizume, K. (2006) Effect of shading on proanthocyanidin biosynthesis in the grape berry. Journal of the Japanese Society for Horticultural Science. **76**, 112-119.

Fujita, A, Akiko, S., Noriko, G., Nami, S., Hitoshi, K., Toshitak, A., Koizumi, T. and Hashizume, K. (2005) Anthocyanidin reductase gene expression and accumulation of flavan-3-ols in grape berry. American Journal of Enology and Viticulture. **56**, 336-342.

Fuselgang, K. and Zoecklein, B. (2003) Population dynamics and effects of *Brettanomyces bruxellensis* strains on Pinot noir (*Vitis vinifera* L.) wines. American Journal of Enology and Viticulture. **54**, 294-300.

Gagne, S., Lacampagne, S., Claisse, O. and Geny, L. (2009) Leucoanthocyanidin reductase and anthocyanidin reductase gene expression and activity in flowers, young berries and skins of *Vitis vinifera* L. cv. Cabernet Sauvignon during development. Plant Physiology and Biochemistry. **47**, 282-290.

Gawel, R. (1998) Red wine astringency: A review. Australian Journal of Grape and Wine Research. **4**, 74-95.

Gawel, R., Francis, L. and Waters, E. (2007a) Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. Journal of Agricultural and Food Chemistry. **55**, 2683–2687.

Gawel, R., van Sluyter, R. and Waters, E. (2007b) The effects of ethanol and glycerol on the body and other sensory characteristics of Riesling wines, Australian Journal of Grape and Wine Research. **13**, 38–45.

Girard, B., Kopp, T., Reynolds, A. and Cliff, M. (1997) Influence of vinification treatments on aroma constituents and sensory descriptors of Pinot noir wines. American Journal of Enology and Viticulture. **48**, 198-206.

Giovanelli, G. and Brenna, O. (2007) Evolution of some phenolics components, carotenoids and chlorophylls during ripening of three Italian grape varieties. European Food Research and Technology. **225**, 145-150.

Goldberg, D., Karumanchiri, A. Tsang, E. and Soleas, G. (1998) Catechin and epicatechin concentrations of red wines: Regional and cultivar-related differences. American Journal of Enology and Viticulture. **49**, 23-33.

Goldner, A. Zamora, M. Di Leo Lira, P. Gianninoto, H. and Bandoni, A. (2009) Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. Journal of Sensory Studies. **24**, 43-257.

Gonzalez-Tomas, L. and Costell, E. (2006) Relation between consumers' perceptions of colour and texture of dairy desserts and instrumental measurements using a generalized procrustes analysis. *Journal of Dairy Science*. **89**, 4511-4519.

Gower, J., C. (1975) Generalised procrustes analysis. *Psychometrika*. **40**, 33–50.

Grice, J.W. and Assad. K.K. (2009) Generalized Procrustes Analysis: A tool for exploring aggregates and persons. *Applied Multivariate Research*. **13**, 93-112.

Guadalupe, Z., Soldevilla, A., Saenz-Navajas, M.A. and Yestarian, B. (2006) Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation. *Journal of Chromatography A*. **1112**, 112-120.

Gubler, W., Bettiga, L. and Heil, D. (1991) Comparisons of hand and machine leaf removal for Botrytis bunch rot. *American Journal of Enology and Viticulture*. **42**, 233-236.

Guth, H. (1997) Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry*. **45**, 3027-3032.

Guth, H. and Sies, A. (2002) Flavour of wines: towards an understanding by reconstitution experiments and an analysis of ethanol's effect on odour activity of key compounds, *Proceedings of the eleventh Australian wine industry technical conference, Adelaide*. Pp. 4–7.

Guy, C., Piggott, J. and Marie, S. (1989) Consumer profiling of scotch whisky. *Food Quality and Preference*. **1**, 69-73.

Hagerman, A.E. and Butler, L.G. (1978) Protein precipitation method for quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*. **26**, 809-812.

Hanlin, R.L., Hrmova, M., Harbertson, J. and Downey, M. (2009) Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. *Australian Journal of Grape and Wine Research*. **16**, 173–188.

Harbertson, J.F. and Downey, M.O. (2009) Investigating differences in tannin levels determined by methylcellulose and protein precipitation. *American Journal of Enology and Viticulture*. **60**, 246-249.

Harbertson, J.F., Hodgins, R.E., Thurston, L.N., Schaffer, L.J. Reid, M.S., Landon, J.L., Ross, C.F. and Adams, D.O. (2008) Variability of tannin concentration in red wines. *American Journal of Enology and Viticulture*. **59**, 210-214.

Harbertson, J., Kennedy, J. and Adams, D. (2002) Tannins in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *American Journal of Enology and Viticulture*. **53**, 54-59.

Harbertson, J.F. Picciotto, E.A. and Adams, D.O. (2003) Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *American Journal of Enology and Viticulture*. **54**, 301-306.

Harbertson, J.F. and Spayd, S.E. (2006) Measuring phenolics in the winery. *American Journal of Enology and Viticulture*. **57**, 280-288.

Hardie, W.J. and Aggenbach, S.J. (1996) Effects of site, season and viticultural practices on grape seed development. *Australian Journal of Grape and Wine Research*. **2**, 21-24.

Hatanaka, A. (1993) The biogenesis of green odour by green leaves. *Phytochemistry*. **34**, 1120-1218.

He, F., Pan, Q., Shi, Y. and Duan, C. (2008a) Chemical synthesis of proanthocyanidins in vitro and their reactions in aging wines. *Molecules*. **13**, 3007-3032.

He, F., Pan, Q., Shi, Y. and Duan, C. (2008b) Review: Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules*. **13**, 2674-2703.

Hein, K., Ebeler, S. and Heymann, H. (2009) Perception of fruity and vegetative aromas in red wine. *Journal of Sensory Studies*. **24**, 441-455.

Herderich, M., Bell, S., Holt, H., Ristic, R., Birchmore, W., Thompson, K. and Iland, P. (2004) Grape maturity and tannins: the impact of viticultural treatments on grape and wine tannins. Conference proceedings, Twelfth Australian Wine Industry Technical Conference, Melbourne, Victoria. 24th – 29th July. 2004. Pp 79-84.

Herderich, M., Birse, M., Damberg, R., Holt, H., Iland, P., Lattey, K. and Smith, P. (2005) Grape and wine tannins – an overview on current research, emerging applications, and future challenges. *ASVO Seminar Proceedings. Advances in Tannin and Tannin Management*. Adelaide, Australia.

Herderich, M. and Smith, P., (2005) Analysis of grape and wine tannins: Methods, applications and challenges. *Australian Journal of Grape and Wine Research*. **11**, 205-214.

Hernandez-Orte, P., Ibarz, M., Cacho, J. and Ferreira, V. (2006) Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake, and aroma generation during alcoholic fermentation. *Food Chemistry*. **98**, 300-310.

Herrman, N.K. (1995) The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. *The Plant Cell* **7**, 907-919.

Heymann, H. (1994) A comparison of free choice profiling and multidimensional scaling of vanilla samples. *Journal of Sensory Studies*. **9**, 445–453.

Howard, K.L., Mike, J.H. and Riesen, R. (2005) Validation of a solid-phase micro extraction method for headspace analysis of wine aroma components. *American Journal of Enology and Viticulture*. **56**, 37-45.

Huang, X., Huang, H. and Wang, H. (2005) Cell walls of loosening skin in post-veraison grape berries lose structural polysaccharides and calcium while accumulating structural proteins. *Scientia Horticulturae*. **104**, 249-263.

- Hunter, J. (2000) Implications of seasonal canopy management and growth compensation in grapevine. *South African Journal of Enology and Viticulture*. **21**, 81–91.
- Hunter, J.J., De Villiers, O.T. and J.E. Watts, J.E. (1991) The effect of partial on quality characteristics of *Vitis vinifera* L. cv. Cabernet-Sauvignon grapes. II. Skin colour, skin sugar and wine quality. *American Journal of Enology and Viticulture*. **42**, 13-18.
- Hunter, J. and Visser, J. (1990) The effect of defoliation on growth characteristics of *Vitis vinifera* Cabernet Sauvignon II. Reproductive growth. *South African Journal of Enology and Viticulture*. **11**, 26-32.
- Hunter, J., Volscheck, J., Marais, J. and Fouche, G. (2004) Composition of Sauvignon Blanc grapes as affected by pre-veraison canopy manipulation and ripeness level. *South African Journal of Enology and Viticulture*. **25**, 13–17.
- Ibern-Gómez, M., Andrés-Lacueva, C., Lamuela-Raventós, R. and Waterhouse, A. (2002) Rapid HPLC analysis of phenolic compounds in red wines. *American Journal of Enology and Viticulture*. **53**, 218-221.
- Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000) *Techniques for Chemical Analysis and Quality Monitoring During Winemaking*. Patrick Iland Wine Promotions, South Australia.
- Intrieri, C., Filippetti, I., Allegro, G., Centinari, M. and Poni, S. (2008) Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research*. **14**, 25-32.
- Jaakola, L. and Hohtola, A. (2010) Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell and Environment*. doi: 10.1111/j.1365-3040.2010.02154.x
- Jackson, R. 2008. *Wine Science: Principals and Applications*. Third Edition. Academic Press, San Diego, California, USA.
- Jackson, D. and Lombard, P. (1993) Environmental practices affecting grape composition and wine quality – A review. *American Journal of Enology and Viticulture*. **44**, 409-430.
- Jensen, J.S., Werge, H.M., Egrbo, M. and Meyer, A.S. (2008) Effect of wine dilution on the reliability of tannin analysis by protein precipitation. *American Journal of Enology and Viticulture*. **59**, 103-105.
- Johnson, L.F. and Pierce, L.L. (2004) Indirect measurement of leaf area index in California north coast vineyards. *Horticultural Science*. **39**, 236-238.
- Jones, P., Gawel, R., Francis, L. and Waters, E. (2008) The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Quality and Preference*. **19**, 596-607.

- Jorgensen, E., Marin, A. and Kennedy, J. (2004) Analysis of the oxidative degradation of proanthocyanidins under basic conditions. *Journal of Agricultural and Food Chemistry*. **52**, 2292-2296.
- Joscelyne, V., Downey, M., Mazza, M. and Bastian, S. (2007) Partial shading of Cabernet Sauvignon and Shiraz vines altered wine colour and mouthfeel attributes but increased exposure had little impact. *Journal of Agricultural and Food Chemistry*. **55**, 10888-10896.
- Jung, D., M. and Ebeler, S., E. (2003) Headspace solid-phase microextraction method for the study of the volatility of selected flavour compounds. *Journal of Agricultural and Food Chemistry*. **51**, 200-205.
- Kallithraka, S., Garcia-Viguera, C., Bridle, P. and Bakker, J. (1995) Survey of solvents for the extraction of grape seed phenolics. *Phytochemical Analysis*. **6**, 265-267.
- Kalua, C. and Boss, P. (2008) Sample preparation optimisation in wine and grapes, dilution and sample/headspace volume equilibrium theory for headspace solid-phase microextraction. *Journal of Chromatography A*. **1192**, 25-35.
- Kalua, C.M. and Boss, P.K. (2009) Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L). **57**, 3818-3830.
- Keller, M. (2009) Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research*. **16**, 56-69.
- Kennedy, J.A., Ferrier, J., Harbertson, J.F. and Peyrot Des Gachons, C. (2006) Analysis of tannins in red wines using multiple methods: correlation with perceived astringency. *American Journal of Enology and Viticulture*. **57**, 481-485.
- Kennedy, J., Hayasaka, Y., Vidal, S., Waters, E. and Jones, G. (2001) Composition of grape skin proanthocyanidins at different stages of berry development. *Journal of Agricultural and Food Chemistry*. **49**, 5348-5355.
- Kennedy, J. and Jones, G. (2001) Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry*. **49**, 1740-1746.
- Kennedy, J., Saucier, C. and Glories, Y. (2006) Grape and wine phenolics: History and perspective. *American Journal of Enology and Viticulture*. **57**, 239-248.
- Kennedy, J. and Waterhouse, A. (2000) Analysis of pigmented high-molecular-mass grape phenolics using ion-pair, normal-phase high performance liquid chromatography. *Journal of Chromatography A*. **866**, 25-34.
- Keyzers, R. and Boss, P. (2010) Changes in volatile compound production of fermentations made from must with increasing grape content. *Journal of Agricultural and Food Chemistry*. **58**, 1153-1164.

- Kielhorn, S. and Thorngate, J. III. (1999) Oral sensations associated with flavan-3-ols (+)-catechin and (-)-epicatechin. *Food Quality and Preference*. **10**, 109-116.
- Kilmartin, P. and Nicolau, L. (2007) Literature survey on aroma and flavour compounds in Pinot noir and analytical techniques applied. Wine Science Programme and New Zealand Winegrowers Report, University of Auckland, New Zealand.
- Kirkenmeyer, S. and Tepper, B. (2003) Understanding creaminess perception of dairy products using free-choice profiling and generic responsivity to 6-n-Propylthiouracil. *Chemical Senses*. **28**, 527–536.
- Kishimoto, T., Wanikawa, A., Kono, K. and Shibata, K. (2006) Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *Journal of Agricultural and Food Chemistry*. **54**, 8855-8861
- Kliwer, W. and Fuller, R. (1973) Effect of time and severity of defoliation on growth of roots, trunk, and shoots of “Thompson Seedless” grapevines. *American Journal of Enology and Viticulture*. **24**, 59–64.
- Kliwer, W. and Dokoozlian, N. (2005) Leaf area/crop weight ratios of grapevines: Influence of fruit composition and wine quality. *American Journal of Enology and Viticulture*. **56**, 170–180.
- Koblet, W., Candolfi-Vasconcelos, C., Zxeifel, W. and Howell, S. (1994) Influence of leaf removal, rootstock, and training system on yield and fruit composition of Pinot noir grapevines. *American Journal of Enology and Viticulture*. **45**, 181-187.
- Koerner, J., Hsu, V., Lee, J. and Kennedy, J. (2009) Determination of Proanthocyanidin A2 content in Phenolic Isolates by Reverse Phase High Performance Liquid Chromatography. *Journal of Chromatography A*. **9**, 1403-1409.
- Kolb, C., Kopeck, Y.J., Riederer, M. and Pfundel, E.E. (2003) UV Screening by phenolics in berries of grapevines (*Vitis vinifera*). *Functional Plant Biology*. **30**, 1177-1186.
- Kotseridis, Y., Baumes, R. and Skouroumounis, G., K. (1998) Synthesis of labelled [²H₂]2-methoxy-3-isobutylpyrazine, [²H₃]α-ionone, and [²H₃]β-ionone, for quantification in grapes, juices and wines. *Journal of Chromatography A*. **824**, 71-78.
- Kotseridis, Y., Baumes, R. and Skouroumounis, G. (1999) Quantification of free and hydrolytically liberated β-damascenone in red grapes and wines using a stable isotope dilution assay. *Journal of Chromatography A*. **849**, 245-254.
- Kotseridis, Y., Baumes, R. and G. Skouroumounis. (1999) Quantitive determination of β-ionone in red wines and grapes of Bordeaux using a stable isotope dilution assay. *Journal of Chromatography A*. **848**, 317-325.
- Kotseridis, Y. and Baumes, R. (2000) Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *Journal of Agricultural and Food Chemistry*. **48**, 400-406.

Kotseridis, Y.S., Spink, M., Brindley, I.D., Blakeb, A.J., Searse, M., Chenc, X, Soleasf, D., Inglis, G. and Pickering, G.J. (2008) Quantitative analysis of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *Journal of Chromatography A*. **1190**, 294–301.

Koyama, K., Goto-Yamamoto, N. and Hashizume, K. (2007) Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grapes (*Vitis vinifera*). *Bioscience, Biotechnology and Biochemistry*. **71**, 958-965.

Kutyna, D., Varela, C., Henschke, P., Chambers, P. and Stanley, G. (2010) A review: Microbiological approaches to lowering ethanol concentration in wine. *Trends Food Science and Technology*. **21**, 293-302.

Kwasniewski, M., Vanden Heuvel, J., Pan, B. and Sacks, G. (2010) Timing of cluster light environment manipulation during grape development affects C₁₃ norisoprenoid and carotenoid concentrations in Riesling. *Journal of Agricultural and Food Chemistry*. **58**, 6841–6849.

Lacampagne, S., Gagne, S. and Geny, L. (2009) Involvement of abscisic acid in controlling the proanthocyanidin biosynthesis pathway in grape skin: New elements regarding the regulation of tannin composition and leucoanthocyanidin reductase (LAR) and anthocyanin reductase (ANR) activities and expression. *Journal of Plant Growth Regulation*. **29**, 81–90

Lachnit, M., Busch-Stockfisch, M., Kunert, J. and Krahl, T. (2003) Suitability of free choice profiling for assessment of orange-based carbonated soft-drinks. *Food Quality and Preference*. **4**, 257-26.

Lattanzio, V., Kroon, P.A., Quideau, S. and Treutter, D. (2008) Introduction: Plant Phenolics–Secondary Metabolites with Diverse Functions. In *Recent Advances in Polyphenol Research*; Daayf, F., Lattanzio, V., Eds.; Wiley-Blackwell: Chichester, UK. Volume 1, pp. 1–35.

Lawless, H.T. and Heymann, H. (1998) Descriptive analysis in sensory evaluation of food: Principles and Practices. C. Hall (Ed.) Plenum Publishing, New York. Pp 1–13.

Lazarus, S., Adamson, G., Hammerstone, J. and Schmitz, H. (1999) High-Performance liquid chromatography/mass spectrophotometry analysis of proanthocyanidins in foods and beverages. *Journal of Agricultural and Food Chemistry*. **47**, 3693-3701.

Lea, A. and Arnold, G. (1978) The phenolics of ciders: bitterness and astringency. *Journal of Agricultural and Food Chemistry*. **29**, 478–483.

Lee, A., Seo, M., Riu, M., Cotta, J., Block, D., Dokoozilian, N. and Ebeler, S. (2007) Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wine. *American Journal of Enology and Viticulture*. **58**, 291–301.

Leffingwell, J.C. and Leffingwell, D. (2003) Gras Flavour Chemicals - detection thresholds. <http://www.leffingwell.com>.

Lohitnavy, N., Bastian, S. and Collins, C. (2010) Berry sensory attributes correlate with compositional changes under different viticultural management of Semillon (*Vitis vinifera* L.) Food Quality and Preference. doi:10.1016/j.foodqual.2010.05.015.

Lopes, C. and Pinto, P.A. (2005) Easy and accurate estimation of grapevines leaf area with simple mathematical models. *Vitis*. **44**, 255- 61.

Loscos, N., Hernandez-Orte, P., Cacho, J. and Ferreira, V. (2009) Fate of grape flavour precursors during storage of yeast lees. *Journal of Agricultural and Food Chemistry*. **57**, 5468-5479.

Louw, C., La Grange, D., Pretorius, S. and Van Rensburg, P. (2006) The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and flavour. *Journal of Biotechnology*. **125**, 447-461.

Makker, H.P.S. (1989) Protein precipitation methods for quantification of tannins: a review. *Journal of Agricultural and Food Chemistry*. **37**, 1197-1202.

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, L. (2004) Polyphenols: Food sources and bioavailability. *Journal of Clinical Nutrition*. **79**, 727-747.

Mamede, M., Cardello, H. and Pastore, G. (2005) Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts. *Food Chemistry*. **89**, 63-68.

Marais, J., Van Wyck, C. and Rapp, A. (1991) Carotenoid levels in maturing grapes as affected by climatic regions, sunlight and shade. *South African Journal of Enology and Viticulture*. **12**, 64-69.

Marais, J., Van Wyk, C. and Rapp, A. (1992) Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin Blanc grapes and Weisser Riesling wines. *South African Journal of Enology and Viticulture*. **13**, 23-32.

Martí, M. P., Mestres, M., Sala, C., Busto, O. and Guasch, J. (2003) Solid-phase micro-extraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *Journal of Agricultural and Food Chemistry*. **51**, 7861-7865.

Masino, F., Monteverchi, G., Arfelli, G. and Antonelli, A. (2008) Evaluation of the combined effects of enzymatic treatment and aging on lees on the aroma of wine from *Bombino bianco* grapes. *Journal of Agricultural and Food Chemistry*. **56**, 9495-9501.

Massoutier, C., Alexandre, H., Feuillat., M. and Charpentier, C. (1998) Isolation and characterisation of cryrotolerant *Saccharomyces cerevisiae* strains. *Vitis*. **37**, 55-59.

Mattivi, F., Vrhovsek, U., Masuero, D. and Trainotti, D. (2009) Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. *Australian Journal of Grape and Wine Research*. **15**, 27-35.

Maugé, C., Granier, T., Langlois d'Estaintot, B., Gargouri, B., Manigand, C., Schmitter, J., Chaudière, J. and Gallois, B. (2010) Crystal structure and catalytic mechanism of leucoanthocyanidin reductase from *Vitis vinifera*. *Journal of Molecular Biology*. **397**, 1079-1091.

Mazauric, J. and Salmon, J. (2006) Interactions between yeast lees and wine polyphenols during simulation of wine aging: II. Analysis of desorbed polyphenol compounds from yeast lees. *Journal of Agricultural and Food Chemistry*. **54**, 3876–3881.

McRae, J., Falconer, R. and Kennedy, J. 2010. Thermodynamics of Grape and Wine Tannin Interaction with Polyproline: Implications for Red Wine Astringency. *Journal of Agricultural and Food Chemistry*. **58**, 12510-12518.

Meagher, L., Lane, G., Sivakumaran, S., Tavendale, M. and Fraser, K. (2004) Characterization of condensed tannins from *Lotus* species by thiolytic degradation and electrospray mass spectrometry. *Animal Feed Science and Technology*. **117**, 151–163.

Meilgaard, M.C. (1975) Flavor chemistry of beer. Part II. 239 aroma volatiles. *Tech Q. Master Brew Assoc. AM*. **12**, 151-168.

Mendes-Pinto, M.M. (2009) Carotenoid breakdown products: norisoprenoids in wine aroma. *Archives of Biochemistry and Biophysics*. **483**, 236-245.

Mercurio, M., Damberg, R., Herderich, M. and Smith, P. (2007) High throughput analysis of red wine and grape phenolics – adaption and validation of methylcellulose precipitable tannin assay and modified Somers colour assay to a rapid 96 well plate format. *Journal of Agricultural and Food Chemistry*. **55**, 4651-4657.

Mercurio, M.D. and Smith, P.A. (2008) Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. *Journal of Agricultural and Food Chemistry*. **56**, 5528-5537.

Meyners, M., Kunert, J. and Qannari, E.M. (2000) Comparing generalized procrustes analysis and statis. *Food Quality and Preference*. **11**, 77-83.

Miranda-Lopez, R., Libbey, L. and Watson, B. (1992) Identification of additional odor-active compounds in Pinot noir wines. *American Journal of Enology and Viticulture*. **43**, 90-92.

Monagas, M., Begona, B. And Gomez-Cordoves, C. (2005) Updated knowledge about the presence of phenolic compounds in wine. *Critical Review. Food Science and Nutrition*. **45**, 85-118.

- Monagas, M., Gomez-Cordoves, C. and Bartolome, B. (2006) Evolution of phenolic content of red wines from *Vitis vinifera* L. during aging in bottle. *Food Chemistry*. **95**, 405-412.
- Moreno-Arribas, M. and Polo, C. (2009) *Wine Chemistry and Biochemistry*. Published by Springer Science and Business, New York, USA. Pp 425–426.
- Morrison, J. and Noble, A. (1990) The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *American Journal of Enology and Viticulture*. **41**, 193-200.
- Murray, J., Delahunty, C. and Baxter, I. (2001) Descriptive sensory analysis: past, present and future. *Food Research International*. **34**, 461–471.
- Noble, A. 1996. Taste-aroma interactions. *Trends in Food Science and Technology*. **7**, 439-444.
- Noble, A. (1999) Why do wines taste bitter and feel astringent? *Chemistry of wine flavor*. American Chemical Society Symposium Series (ed). Waterhouse. A. L. and Ebeler. S. E. Washington DC, USA. American Chemical Society. Pp 156-165.
- Noguerol-Pato, R., Gonzalez-Barreiro, C., Cancho-Grande, B. And Simal-Gandra, J. (2009) Quantitative determination and characterisation of the main odourants on Mencia monovarietal red wines. *Food Chemistry*. **117**, 473-484.
- Nuzzo, V. (2004) Crop load effects on leaf area evolution and light interception in 'Montepulciano' grapevines (*Vitis vinifera* L.) trained to 'Tendone' system. Proc. 1st IS on Grapevine. *Acta Horticulturae*. **652**, ISHS.
- Obradovic, D. (2005) Grape derived tannins and their application. *Advances in tannin and tannin management*. Twelfth Australian Wine Industry Technical Conference, Melbourne, Victoria, Australia. ASVO Proceedings. Pp 23-27.
- Oliveira, J., Sá, F., Barros, F. and Araújo, I. (2006) C₆-alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta*. **563**, 300–309.
- Ollat, N. and Gaudillere, J. (1998) The Effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *American Journal of Enology and Viticulture*. **49**, 251-258.
- Paolocci, F., Bovone, T., Tosti, N., Arciono, S. and Damiani, F. (2005) Light and exogenous transcription factor qualitatively and quantitatively affect biosynthetic pathway of condensed tannins in *Lotus corniculatus* leaves. *Journal of Experimental Botany*. **56**, 1093-1103.
- Parr, W., Green, J., White, G. and Sherlock, R. 2007. The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. *Food Quality and Preference*. **18**, 849-861.

Pascual-Teresa, A., Rivas-Gonzalo, J. and Santos-Buelga, C. (2000) Prodelphinidins and related flavanols in wine. *International Journal of Food Science and Technology*. **35**, 33-40.

Passos, C., Cordoso, S., Domingues, M., Domingues, P., Silva, C. and Coimbra, M. (2007) Evidence for galloylated type-A procyanidins in grape seeds. *Food Chemistry*. **195**, 1457-1467.

Pastor Del Rio, J. and Kennedy, J. (2006) Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *American Journal of Enology and Viticulture*. **57**, 125-132.

Peleg, H., Gacon, K., Schlich, P. and Noble, A. (1999) Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture*. **9**, 1123-1128.

Percival, D.C., Fisher, K.H. and Sullivan, J.A. (1994) Use of fruit zone leaf removal with *Vitis vinifera* L. cv. Riesling grapevines. II. Effect on fruit composition, yield, and occurrence of bunch rot (*Botrytis cinerea*). *American Journal of Enology and Viticulture*. **45**, 133-140.

Pereira, G., Gaudillere, J., Pieri, P., Hilbert, G., Maucourt, M., Deborde, D., Moing, A. and Rolin, D. (2006) Microclimate influence on mineral and metabolic profiles of grape berries. *Journal of Agricultural and Food Chemistry*. **54**, 6765-6775.

Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J. and Camara, J. (2006) Analytical characterisation of the aroma of Tinto Negra Mole red wine: Identification of the main odorant compounds. *Analytica Chimica Acta*. **563**, 154-164.

Perret, C., Pezet, R. and Tabacchi, R. (2003) Fractionation of grape tannins and analysis by matrix-assisted laser desorption/ionisation time of flight mass spectrometry. *Phytochemical Analysis*. **14**, 202-208.

Perrin, L., Symoneaux, R., Maitre, I., Asselin, C., Jourjon, F. and Pages, J. (2007) Comparison of conventional profiling by a trained tasting panel and free choice profiling by wine professionals. *American Journal of Enology and Viticulture*. **58**, 508-517.

Petit, P., Granier, T., Langlois D'Estaintot, B., Manigand, C., Bathany, K., Schmitter, J.M., Lauvergeat, V., Hamdi, S. and Gallois, B. (2007) Crystal structure of grape dihydroflavonol 4-reductase, a key enzyme in flavonoid biosynthesis. *Journal of Molecular Biology*. **368**, 1345-1357.

Petrie, P.R., Trought, M.C.T. and Howell, G.S. (2000a) Fruit composition and ripening of Pinot noir (*Vitis vinifera* L.) in relation to leaf area. *Australian Journal of Grape and Wine Research*. **6**, 46-51.

Petrie, P.R., Trought, M.C.T. and Howell, G.S. (2000b) Growth and dry matter partitioning of Pinot noir (*Vitis vinifera* L.) in relation to leaf area and crop load. *Australian Journal of Grape and Wine Research*. **6**, 40-45.

- Petrie, P.R., Trought, M.C.T. and Howell, G.S. and Buchan, G. (2003) The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. *Functional Plant Biology*. **30**, 711-717.
- Peterlunger, E., Celotti, E., Da Dalt, G., Stefanelli, S., Gollino. and Zironi, R. (2002) Effect of training system on Pinot noir grape and wine composition. *American Journal of Enology and Viticulture*. **53**, 14-18.
- Phelps, G. (1999) The influence of site, crop load and cluster exposure on Pinot noir fruit composition in Canterbury. M.Appl.Sc.Thesis, Lincoln University, Lincoln.
- Pineau, P., Barbe, J.C., Van Leeuwen, C. and Dubourdieu, D. (2007) Which impact for β -Damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry*. **55**, 4103-4108.
- Pineau, B., Barbe, J., Van Leewen, C. and Dubourdieu, D. (2009) Examples of perceptive interactions involved in specific “red-“ and “black-berry” aromas in red wines. *Journal of Agricultural and Food Chemistry*. **57**, 3702-3708.
- Polaskova, P., Herszage, J. and Ebeler, S. (2008) Wine flavour: chemistry in a glass. *Chemical Society Review*. **37**, 2478-2489.
- Poni, S., Bernizzoni, F., Civardi, S. and Libelli, N. (2009) Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red *Vitis vinifera* L. cultivars. *American Journal of Enology and Viticulture*. **15**, 185–193.
- Poni, S., Casalin, L., Bernizzon, F., Civardi, S. and Intrieri, C. (2006) Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *American Journal of Enology and Viticulture*. **57**, 397-407.
- Poni, S., Intrieri, C. and Silvestroni, O. (1994) Interactions of leaf age, fruiting and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. I. Gas exchange. *American Journal of Enology and Viticulture*. **45**, 71-78.
- Preston, L., Block, D., Heymann, H., Soleas, G., Noble, A. and Ebeler, S. (2008) Defining vegetal aromas in Cabernet Sauvignon using sensory and chemical evaluations. *American Journal of Enology and Viticulture*. **59**, 137-145.
- Price, S., Breen, P., Valladao, M. and Watson, B. (1995) Cluster sun exposure and quercetin in Pinot noir grapes and wine. *American Journal of Enology and Viticulture*. **46**, 187-194.
- Prigent, S., Voragen, A., Van Koningsveld, G., Baron, A., Renard, C. and Gruppen, H. (2009) Interactions between globular proteins and procyanidins of different degrees of polymerization. *Journal of Dairy Science*. **92**, 5843–5853
- Qian, M., Fang, Y. and K. Shellie. 2009. Volatile composition of Merlot wine from different vine water status. *Journal of Agricultural and Food Chemistry*. **56**, 7459-7463.

Raven, P., Evert, R. and Eichorn, S. (1999) *Biology of Plants*. Sixth Edition. W.H. Freeman and Co. New York, USA.

Remy, S., Fulcrand, H., Labarbe, B., Cheynier, V. and Moutounet, M. (2000) First confirmation in red wine of products resulting from direct anthocyanin–tannin reactions. *Journal of the Science of Food Agriculture*. **80**, 745–751.

Reynolds, A., Schlosser, J., Poer, R., Roberts, R., Willwerth, J. and Desavigny, C. (2007) Magnitude and interaction of viticultural and enological effects. I. Impact of canopy management and yeast strain on sensory and chemical composition of Chardonnay Musque. *American Journal of Enology and Viticulture*. **58**, 12-24.

Reynolds, A. and Wardle, D. (1989) Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. *American Journal of Enology and Viticulture*. **40**, 121-129.

Reynolds, A., Yerle, S., Watson, B., Price, S. and Wardle, D. 1996. Fruit environment and crop level effects on Pinot noir. II. Composition and descriptive analysis of Oregon and British Columbia wines. *American Journal of Enology and Viticulture*. **47**, 329-339.

Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. and Lonvaud, A. (2000) *Handbook of Enology: The microbiology of wine and vinification*. John Wiley and Sons Ltd, Chichester, UK.

Ribereau-Gayon, P. and Glories, Y. (1986) Phenolics in Grapes and Wine. *Proceedings of Sixth Australian Wine industry Technical Conference, Adelaide, South Australia*. Pp 247-256.

Ricardo Da Silva, J.M. (2009) The evolution of grape phenolic compounds during maturation of the grape cluster: The particular case of condensed tannins. *Fourth International SASEV Conference on Enology and Viticulture Beyond 2010, Cape Town, South Africa*.

Richards, A. (2001) *Alien vision: exploring the electromagnetic spectrum with imaging technology*. Published by the International Society for Optical Engineering, Washington, USA. **1**, 1-6.

Ristic, R., Bindon, K., Francis, L., Herderich, M. and Iland, P. (2010) Flavonoids and C₁₃-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. *Australian Journal of Grape and Wine Research*. **16**, 369 – 388.

Ristic, R., Downey, M., Iland, P., Bindon, K., Francis, L., Herderich, M. and Robinson, S. (2007) Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research*. **13**, 53-65.

Ristic, R. and Iland, P. (2005) Relationships between seed and berry development of *Vitis vinifera* L. Shiraz: Developmental changes in seed morphology and phenolic composition. *Australian Journal of Grape and Wine Research*. **11**, 43-58.

Roberts, M. and Paul, N. (2006) Tansley Review. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytologist* **170**, 677-699.

Robinson, A., Ebeler, S., Heymann, H., Boss, P., Solomon, P. and Trengrove, R. (2009) Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry*. **57**, 10313-10322.

Roby, G., Harbetson, J., Adams, D. and Matthews, M. (2004) Berry size and vine water deficits as factors in wine grape composition: anthocyanins and tannins. *Australian Journal of Grape and Wine Research*. **10**, 100-107.

Rocha, S., Ramalheira, V., Barros, A., Delgadillo, I. and Coimbra, M. (2001) Headspace solid phase microextraction (SPME) analysis of flavour compounds in wines. Effect of the matrix volatile composition in the relative response factors in a wine model. *Journal of Agricultural and Food Chemistry*. **49**, 5142–5151.

Rodrigues, F., Caldeira, M. and Camara, J. (2008) Development of a dynamic headspace solid-phase microextraction procedure coupled to GC-q-MSD for evaluation of the chemical profile of alcoholic beverages. *Analytica Chimica Acta*. **609**, 82-104.

Rubico, S. and McDaniel, M. (1992) Sensory evaluation of acids by free-choice profiling. *Chemical Senses*. **17**, 273-289.

Sacchi, K., Bisson, L. and Adams, D. (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture*. **56**, 197-206.

Saerens, S.M.G., Delvaux, F., Verstrepen, K.J., Van Dijck, P., Thevelein, J.M. and Delvaux, F.R. (2008) Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Applied and Environmental Microbiology*. **74**, 454-461.

Salas, E., Fulcrand, H., Meudec, E. and Cheynier, V. (2003) Reactions of anthocyanins and tannins in model solutions. *Journal of Agricultural and Food Chemistry*. **51**, 7951-7961.

Salinas, R., Zalacain, A., Pardo, F. and Alonso, G. (2004) Stir bar sorptive extraction applied to volatile constituents evolution during *Vitis vinifera* ripening. *Journal of Agricultural and Food Chemistry*. **52**, 4821-4827.

Santos-Buelga, C. and Scalbert, A. (2000) Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *Journal of Agricultural and Food Chemistry*. **80**, 1094-1117.

Santos-Buelga, C. and Williamson, G. (2003) *Methods in polyphenol analysis*. The Royal Society of Chemistry. Cambridge, UK.

Sarneckis, C., Damberg, R., Jones, P., Mercurio, M., Heiderich, M. and Smith, P. (2006) Quantification of condensed tannins by precipitation with methylcellulose: development

and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*. **12**, 39-49.

Saunders, C. (2009) Research article: The anti-proliferative effect of different tomato varieties on the human colon adenocarcinoma cells. *Bioscience Horizons*. **2**, 172-179.

Schreier, P. (1980) Wine aroma composition: Identification of additional volatile constituents of red wine. *Journal of Agricultural and Food Chemistry*. **28**, 926-928

Scheirer, P., Drawert, F. and Abraham, K. (1980) Identification and determination of volatile constituents in Burgundy Pinot noir wines. *Lebensl-Wissen. Tech*. **13**, 318-321.

Schofield, P., Mbugua, D. and Pell, A. (2001) Analysis of condensed tannins: A review. *Animal Feed Science and Technology*. **91**, 21-40.

Seddon, T. and Downey, M. (2008) Comparison of analytical methods for the determination of condensed tannins in grape skin. *Australian Journal of Grape and Wine Research*. **14**, 54–61.

Serafini, M., Mainia, G. and Ferro-Luzzi, A. (1997) Effect of ethanol on red wine tannin–protein (BSA) interactions. *Journal of Agricultural and Food Chemistry*. **45**, 3148–3151.

Shetty, K. (2004) Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: A review. *Process Biochemistry*. **9**, 789–803.

Silber, M.L., Davitt, B.B., Khairutdinov, R.F. and Hurst, J.K. (1998) A mathematical model describing tannin-protein association. *Analytical Biochemistry*. **263**, 46-50.

Siebert, K.J. (1999) Protein-polyphenol hazes in beverages. *Food Technology*. **53**, 54-57.

Siebert, K.J. (2006) Haze formation in beverages. *Lebensmittel Wissenschaft und Technologie*. **39**, 987-994.

Siebert, K.J., Carrasco, A. and Lynn, P.Y. (1996) Formation of protein-polyphenol haze in beverages. *Journal of Agricultural and Food Chemistry*. **44**, 1997-2005.

Siebert, T., Smyth, H., Capone, D., Neuwohner, C., Pardon, K., Skouroumounis, G., Herderich, M., Sefton, M. and Pollnitz, A. (2005) Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Analytical and Bioanalytical Chemistry*. **381**, 937-947.

Siebert, T., Wood, C., Elsey, G. and Pollnitz, A. (2008) Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *Journal of Agricultural and Food Chemistry*. **56**, 3745–3748

Skinkis, P., Bordelon, B. and Butz, E. (2010) Effects of sunlight exposure on berry and wine monoterpenes and sensory characteristics of Traminette. *American Journal of Enology and Viticulture*. **61**, 147-156.

Silva Ferreira, A. and Guedes De Pinho, P. (2003) Analytical method for determination of some aroma compounds in white wines by solid phase microextraction and gas chromatography. *Journal of Food Science*. **68**, 2817-2820.

Smart, R. (1985) Principles of grapevine canopy microclimate manipulation with implications for yield and quality. *American Journal of Enology and Viticulture*. **36**, 230-239.

Smart, R. and Robinson, M. (1991) *Sunlight into Wine*. Winetitles Ltd, Ashford. South Australia.

Smith, S. and Codrington, C. (1988) Viticultural and oenological implications of leaf removal for New Zealand vineyards. *In: Proc. Sec. International Symposium Cool Climate Viticulture and Oenology, Auckland, New Zealand*. R. Smart, R. Thornton, S. Rodriguez, J. Young (Eds). Pp 127-133.

Smith, P., Sarnrckis, C., Jones, P., Dambergs, R. and Herderich, M. (2005) Development and application of a simple and robust assay for quantitation of tannins in grape and wine samples. . ASVO Seminar Proceedings. *Advances in Tannin and Tannin Management*. Adelaide, Australia.

Soleas, G., Diamandis, E. and Goldberg, D. (1997) Wine as a biological fluid: History, production, and role in disease prevention. *Journal of Clinical Laboratory Analysis*. **11**, 287-313

Somers, C. and Evans, M. (1977) Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO₂, "chemical age". *Journal of the Science of Food and Agriculture*. **28**, 279-287.

Spayd, S., Tarara, J., Mee, D. and Ferguson, J. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*. **53**, 171-182.

Spiers, J., Lee, E., Holt, K., Yong-Duk, Y., Steele, N., Loveys, B. and Schuh, W. (1998) Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavour aldehydes and alcohols. *Plant Physiology*. **117**, 1047-1058.

Spranger, I.M., Climaco, C.M., Sun, B., Eiriz, N., Fortunato, C., Nunes, A., Leandro, M.C., Avelar, M.L. and Belchior, P.A. (2004) Differentiation of red wine making technologies by phenolic and volatile composition. *Analytica Chimica Acta*. **513**, 151-161.

Staff, S.L., Percival, D.C., Sullivan, J.A. and Fisher, K.H. (1997) Fruit zone leaf removal influences vegetative, yield, disease, fruit composition and wine sensory attributes of *Vitis vinifera* L. Optima and Cabernet franc. *Canadian Journal of Plant Science*. **77**, 49-153.

Strong, D. and Azarenko, A. (2000) Relationship between trunk cross-sectional area, harvest index, total tree dry weight and yield components of "Starkspur Supreme Delicious" apple trees. *Journal of American Pomological Society*. **51**, 1:22-27.

- Sun, B., Leandro, C., Da Silva, R.J. and Spranger, I. (1998) Separation of grape and wine proanthocyanidins according to their degree of polymerisation. *Journal of Agricultural and Food Chemistry*. **46**, 1390-1396.
- Sun, J., Liang, F., Bin, Y., Li, P. and Duan, C. (2007) Screening non-coloured phenolics in red wines using liquid chromatography/Ultraviolet and mass spectrometry/mass spectrometry libraries. *Molecules* **12**, 679-693.
- Sumby, K., Grbin, P. and Jiranek, V. (2010) Review: Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry*. **121**, 1-16.
- Svobodova, S., Psotova, J. and Walterova, D. (2003) Natural phenolics in the prevention of UV-induced skin damage: A review. *Biomedical Papers*. **147**, 137-145.
- Tao, J., Dykes, S. and Kilmartin, P. (2007) Effect of SO₂ concentration on polyphenol development during red wine micro-oxygenation. *Journal of Agricultural and Food Chemistry*. **55**, 6104-6109.
- Tarara, J., Lee, J., Spayd, S. and Scagel, C. (2008) Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture*. **59**, 235-247.
- Tardaguila, J., Diago, M., Martinez-de Toda, F., Poni, S. and Vilanova, M. (2008) Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of CV. Grenache grown under non-irrigated conditions. *Journal International de la Vigne et du Vin*. **42**, 221-229.
- Tat, L., Comuzzo, P., Stolfo, I. and Battistutta, F. (2005) Optimization of wine head space analysis by solid-phase micro-extraction capillary gas chromatography with mass spectrometric and flame ionisation detection. *Food Chemistry*. **93**, 361-369.
- Terrier, N., Poncet-Legrand, C. and Cheynier, V. 2008. Flavanols, flavonols and dihydroflavonols. *Wine chemistry and Biochemistry*. Eds: M. Victoria Moreno-Arribas and M. Carmen Polo. Springer Science and Business Media, New York, USA. Pp 463-507.
- Tesniere, C., Torregrosa, L., Pradal, M., Souquet, J-M., Gilles, C., Dos Santos, K., Chatelet, P. and Gunata, Z. (2006) Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. *Journal of Experimental Botany*. **57**, 91-99.
- Thorngate, J. (1997) The physiology of human sensory response to wine: A review. *American Journal of Enology and Viticulture*. **48**, 271-279.
- Thorngate, J. and Singleton, V. (1994) Reactions of monomeric and polymeric flavan-3-ols with monomeric pigment in model wine solutions. *American Journal of Enology and Viticulture*. **45**, 349-352.

Treutter, D. (2006) Significance of flavonoids in plant resistance: A review. *Environmental Chemistry Letters*. **4**, 147-157.

Treutter, D. (2010) Review: Managing phenol contents in crop plants by phytochemical farming and breeding—visions and constraints. *International Journal of Molecular Sciences*. **11**, 807-857.

Vaquero, I., Marcobal, A. and Muñoz, R. (2004) Tannase activity by lactic acid bacteria isolated from grape must and wine. *International Journal of Food Microbiology*. **96**, 199-204.

Vasconcelos, S.C. and Castagnoli, S. (2000) Leaf canopy structure and vine performance. *American Journal of Enology and Viticulture* . **51**, 390–396.

Versari, A., Boulton, R. and Parpinell, G. (2007) Analysis of SO₂-resistant polymeric pigments in red wines by high-performance liquid chromatography. *American Journal of Enology and Viticulture*. **58**, 523-525.

Versari, A., Boulton, R. and Parpinello, G. (2008) A comparison of analytical methods for measuring the colour components of red wines. *Food Chemistry*. **106**, 397-402.

Vidal, S., Courcoux, P., Francis, L., Kwaitkowski, M., Gawel, R., Williams, P., Waters, E. and Cheynier, V. (2004) Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference*. **15**, 209-217.

Vidal, S., Francis, L. and Guyot, S. (2003) The mouthfeel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of Agricultural and Food Chemistry*. **83**, 564-573.

Vidal, S., Francis, L., Noble, A., Kwiatowski, M. and Cheynier, V. (2004) Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta*. **513**, 57-65.

Wakeling, I.N., Raats, M.M. and MacFie, H. J. H. (1992) A new significance test for consensus in generalised procrustes analysis. *Journal of Sensory Studies*. **7**, 91-96.

Waterhouse, A. (2002) Wine Phenolics. *Annals of New York Academy of Science* **957**, 21-36.

Williams, A. and Arnold, G. (1985) A comparison of the aroma of six coffees characterised by conventional profiling, free-choice profiling and similarity scaling methods. *Journal of the Science of Food and Agriculture*. **36**, 204–214.

Williams, A. and Langron, S. (1984) The use of free-choice profiling for the evaluation of commercial ports. *Journal of the Science of Food and Agriculture*. **35**, 558-568.

Winkler, A.J., Cook, J.A., Kliewer, W.M. and Lider, L.A. (1974) *General Viticulture*. University of California Press, Berkeley, USA.

Yu Xie, D. and Dixon, R. (2005) Tannins and Related Polyphenols (Part 2) Review: Proanthocyanidin biosynthesis – still more questions than answers? *Phytochemistry*. **66**, 2127-2144.

Zimman, A., Joslin, W., Lyon, M., Meier, J. and Waterhouse, A. (2002) Maceration variables affecting phenolic composition in commercial-scale Cabernet Sauvignon winemaking trials. *American Journal of Enology and Viticulture*. **53**, 93-98.

Zoecklein, B., Wolf, T., Duncan, N. and Judge, J. (1992) Effects of fruit zone leaf removal on yield, fruit composition and fruit rot incidence in Chardonnay and Riesling (*Vitis vinifera* L.) grapes. *American Journal of Enology and Viticulture*. **43**, 139-148.

Zoecklein, B., Wolf, T., Duncan, S., Marcy, J. and Jasinski, Y. (1998) Effect of fruit zone leaf removal on total glyconjugates and conjugate fraction concentration of Riesling and Chardonnay (*Vitis vinifera*) grapes. *American Journal of Enology and Viticulture*. **49**, 259-265.

Zhang, Z., Yang, M. J. and Pawliszyn, J. (1994) Solid-phase microextraction. A solvent-free alternative for sample preparation. *Analytical Chemistry*. **66**, 844-854.