VITICULTURAL MANAGEMENT OF GRAPE TANNIN AND ANTHOCYANIN LEVELS TO ACHIEVE DESIRED WINE QUALITY SPECIFICATIONS



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Front cover: The research tannin team and visiting collaborators from the USA, (from left) Dr. Jim Harbertson (Research/Extension Enologist, Washington State University), Dr. Mark Kelm (Senior Research Chemist, Constellation Wines US), Marica Mazza (Research Scientist, Viticulture and Oenology, DPI Mildura), Nardia Baker (Research Officer, Viticulture and Oenology, DPI Mildura), Dr. John Thorngate (Research Manager & Applications Chemist, Constellation Wines US), Dr. Mark Downey (Senior Research Scientist, Viticulture and Oenology, DPI Mildura) and Rachel Hanlin (PhD student, University of Adelaide and DPI Mildura).

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Abstract

This project report summarises the results of over five years research on the extent of variation in winegrape tannins and the viticultural and environmental drivers of that variation. Condensed tannins were found in all of the 40 wine and table grape varieties examined and considerable variation in tannin content, composition and polymer length distribution was observed between varieties, seasons and growing sites as well as within those sites. Despite observing substantial variation in the tannin polymer subunit composition and in some cases not detecting individual terminal or extension subunits, the four key flavan-3-ols, catechin, epicatechin, epicatechin-gallate or epigallocatechin were detected in all varieties. Within site variation was frequently as great as that between sites or seasons indicating that underlying site features are one of the greatest drivers of tannin variability in winegrapes. The marked seasonal variation observed indicates that another major driver is climate.

A significant observation of the project was the length of condensed tannin polymers determined in grape skin, particularly of table grape varieties. In many cases these were greater than in winegrapes and were several times greater than previously reported polymer lengths. The project also examined the extractability of tannins from grapes, concluding that grape berry cell walls play an important role in tannin extraction and that tannin interactions with cell walls is an area requiring further research. Other areas for future investigation include the impact of anthocyanins on tannin solubility, the sensory character of individual tannin fractions and the development of rapid, high-throughput analytical methods.

Executive Summary

This project, Viticultural management of grape tannin and anthocyanin levels to achieve desired wine quality specifications, was developed in response to the industry trend towards reward for quality based payment systems within the Australian wine sector. While a number of parameters have been used historically to assess grapes prior to harvest, the shift towards reward for quality had been based on relatively few parameters, most notably colour. As the limitations of colour as a key indicator of quality became apparent it was envisaged that the inclusion of additional parameters would ensure a more robust payment system.

In grapes and wine, the single biggest group of compounds that contribute to wine quality are phenolics. This group includes the coloured compounds (anthocyanins) as well as other diverse compounds. The largest class of phenolics are the condensed tannins, which are extracted from the grapes during winemaking and contribute to long term colour stability and mouthfeel in the wine. The grape and wine sector has long recognized this and several methods were developed for the assessment of tannins and phenolics. However, none of these found widespread acceptance, largely because it is difficult to interpret the data generated.

There are two main reasons for this. The methods fail to differentiate between important classes of phenolics and the body of knowledge on tannins and other phenolics in grapes is relatively limited. While some of the structures are known and many have been identified in grapes and wine, their distribution across varieties, relative abundance with respect to each other and the agronomic and environmental factors that influenced their accumulation is largely unknown.

The project sought to address this knowledge gap for tannins and to a lesser extent anthocyanins with an aim to communicate a general understanding of winegrape tannins to the Australian wine sector.. This was achieved by collating extant knowledge, re-analysing and re-interpreting samples from recent research projects and collecting and analysing a wide array of samples from different sites, across seasons and from different varieties.

Reported here is a summary of that work, It represents the major findings and identifies remaining gaps in current knowledge and research opportunities arising from this work. Briefly, the research conducted has shown that all grape varieties tested to date contain tannins and that all varieties contain all of the common tannin subunits, catechin, epicatechin, epicatechin-gallate and epigallocatechin. In some cases, the levels are low and there are instances where subunits can not be detected as either terminal or extension subunits. In no instance is a particular subunit completely absent.

In examining polymer length, the research presented here reported considerably longer polymers than have been previously reported and in many cases these were found in table grape rather than winegrape varieties. This may indicate opportunities for blending, co-fermentation and breeding to develop new or unique wine tannin profiles or wines with tannin profiles to satisfy specific consumer segments. Significant variation in polymer lengths between varieties may also provide an opportunity for investigating the mechanism of polymer formation.

To increase the sample number and minimise costs, this project sourced samples from other projects and field trials wherever possible. While initially the project may have sought to examine particular varieties or regions, this sometimes wasn't possible because there were no trials available with those varieties or at those sites that could be readily accessed. In the interest of maximising the overall project impact, some of these were not pursued further.

One of the largest components of the project reported here was the contribution to the GWRDC funded Soil and Water Initiative Five Sites Project (GWRDC Project No. DPI 04/04). Fruit from the five sites was collected at harvest and shipped from each of the sites to the DPI facility at Mildura and analysed for anthocyanin and tannin content. This data showed significant variation across some vineyards, while others were much more uniform. Data from these sites also showed significant seasonal variation, although in some cases there were clear patterns that remained stable from year to year. Both of these observations indicate underlying site phenomena that are driving anthocyanin and tannin accumulation, although for all these sites there were seasons where this pattern was not apparent indicating an overriding seasonal or climatic effect. In attempting to define the exact environmental, seasonal or viticultural influence a large number of site, season and physiological parameters were measured and management data collated. From these data we were unable to identify a single parameter that was a consistent driver at all sites for all seasons, although on many occasions anthocyanin and tannin content was negatively correlated with vine vigour, which itself frequently reflected soil water holding capacity or irrigation. The overriding seasonal influence appears to be temperature, however this analysis is incomplete.

As data from various sites and trials was collated and in many cases wine was made and analysed for both the chemistry and sensory character, ideas about the relationship between final wine character and the composition of the fruit used began to coalesce around the ratio of anthocyanins to tannins in the fruit and how this impacted final wine composition. Previous work in this area has generally focussed on the ratios in the wine, not the fruit, with the hypothesis that this ratio is a driver of the formation of pigmented polymers. Here we considered similar ideas but they were not clearly supported by the data. What became apparent is that the relationship between tannins and anthocyanins in

wine relates to the influence of anthocyanins on the solubility of tannins in the wine matrix. Testing of this hypothesis is underway.

An element of developing a knowledge base to inform grower and winemaker decision-making is the measurement of perceived quality parameters, consistent with the philosophy; you can't change what you can't measure. Thus, part of this project was to identify and develop, where necessary, methodologies for routine analysis of tannins and other phenolics. Prior to the commencement of the project a method for assaying tannin in grapes and wine by protein precipitation was published and this was adopted as the mainstay of the analyses reported here. In addition, high-performance liquid chromatographic and rapid spectral methods were utilised and as new or alternative methods were published, they were evaluated. To date, both a protein and a methylcellulose precipitation method have been published and both are robust and accessible to the industry. A direct comparison of these methods and high-performance liquid chromatography (HPLC) identified significant discrepancies between the methods. That three analytical methods purporting to measure the same thing gave significantly different results suggests the methods in fact measure somewhat different tannin components extracted from grape skin. Further investigations of the precipitation methods have eliminated the solvent used and method of quantification as factors causing the observed differences. This indicates a fundamental difference in the nature of the binding interactions between tannin and protein and tannin and methylcellulose. These differences continue to be investigated. Methods that yield different data about the same samples offer an opportunity to increase our knowledge of grape and wine tannins.

From an industry perspective, both methods are robust and data generated by either method is comparable. However, for widespread industry adoption, issues remain regarding laboratory capacity to undertake the analysis and proficiency of the laboratory staff. Both will contribute to the value of the data generated. As in many other instances, a rapid analytical method was perceived as an attractive option. Early attempts to apply near infrared (NIR) technology to the determination of grape and wine tannins showed only limited success and it was perceived that addition of the UV and visible spectra to the analysis would strengthen the predictive models used to determine phenolic classes using this approach. The research undertaken in this project demonstrated that in fact the UV-vis spectra were sufficient to predict anthocyanins, tannins and other phenolics in wine. This approach has been adopted and is being refined by several groups. We have subsequently extended this approach to whole berry homogenates and this work is ongoing with the support of our collaborators.

While the samples from the five sites and from many of the larger data-sets were analysed by the protein precipitation method, smaller scale investigations using a range of analytical techniques were directed at dissecting the structure of tannin polymers and their subunit composition. As this work progressed the interaction of tannin with

components of the grape that were affecting extractability, both for analysis and into the wine became apparent and a portion of our effort was focussed in this area. Informed by the differential interaction of tannins with protein and methylcellulose, we proposed a model for tannin interaction with the cell walls in grape berries and published a comprehensive review of research to date in this area. This work is ongoing. Early results indicate that in addition to tannins and anthocyanins, the tannin binding capacity of cell walls may also be a parameter that requires routine analysis to optimise winemaking and wine quality.

This project has produced 13 research papers to date, 18 industry journal articles and 28 conference posters. The project team has made 15 presentations to industry, stakeholder and research fora. Additional research papers are in preparation for publication.

Background

The Australian Wine Industry has enjoyed strong growth over the last 15 years, mainly through growth in export markets such as the UK and USA. Australia's competitive advantage in the global wine market has been in the production of high quality wines, relative to their competitors, at a range of different price points. To maintain this 'quality at a price point' position in an ever-changing marketplace, it is essential that the Australian Wine Industry continually improves 'quality' in each price segment of the market. Part of the strategy to maintain this advantage must include Australian producers consistently managing winegrape composition.

In such a competitive market environment, sugar and grape colour are no longer sufficient as a measure of fruit quality. Grape colour is only one of the many components of wine colour, albeit one of the easiest to measure. Furthermore, with the shift in the grape and wine industry towards quality based payment systems, additional objective measures of quality are required. Tannins are the largest class of compounds in the grape that make a direct contribution to wine quality, both in terms of wine colour stability and mouthfeel (astringency). While tannins themselves are a much more complex group of compounds than the anthocyanins (coloured compounds), analysis of total tannins is relatively straightforward (Downey and Adams 2005). More detailed analysis, such as HPLC, is also possible although currently the data from this analysis is primarily limited to composition and average polymer length (Downey *et al.* 2003a; Kennedy and Jones 2001). Although determination of structure and polymer length distribution is rapidly becoming more accessible (Kelm *et al.* 2006; Kennedy and Taylor 2003).

However, before any of these technologies can be usefully applied to viticultural management of winegrape tannins, some knowledge of the range of tannin content and composition in the major Australian winegrape varieties is needed. A body of anecdotal evidence exists that suggests considerable variability in tannin content between seasons as well as between sites and cultivars. However, very little of this information has been quantified and little of that has filtered through to the winegrowing and winemaking community. Such knowledge is essential for the development of strategies to manage tannins in the vineyard with a view to improving fruit and wine quality to ensure the long-term sustainability of the Australian wine industry.

Over the past 5 years, a substantial body of research on the phenolic composition of grapes and wine has been generated though GWRDC funded research projects including the Flavonoid Project (CSIRO, CRCV 3.3.1), the Tannin Project (AWR 96/1, CRCV 1.2) and the Bunch Exposure Project (DNR 02/09). These projects have examined both the content and composition of various phenolic components such as tannins (Downey *et al.* 2003a), flavonols (Downey *et al.* 2003b) and anthocyanins (Downey 2004), primarily in Shiraz grapes. In addition, these projects have examined biosynthesis of phenolic

compounds and the influence of environmental factors on their accumulation (Bogs *et al.* 2005; Downey *et al.* 2006; Downey *et al.* 2004b).

While these projects have generated a valuable body of research, much of which has been communicated to industry (Downey and Adams 2005; Downey *et al.* 2004a; Downey *et al.* 2004d; Downey *et al.* 2004e; Downey and Krstic 2004a; b; 2005) they have also identified rich opportunities for further research. In addition, these recent projects have exposed substantial gaps in our current knowledge.

Particularly in warm irrigated vineyards, the relationship between the grape and wine colour density of finished wine is not related to anthocyanin content and composition alone (Njegovan 2004). Research suggests that some other component is a significant determinant of final wine colour. The most likely grape constituents contributing to wine colour, apart from anthocyanins, are the condensed tannins due to their involvement in the formation of pigmented polymers (Vidal *et al.* 2004). Furthermore, tannins comprise around 80% of the total phenolic load of the grape berry (Ribereau-Gayon and Glories 1986). The availability of comprehensive tannin content and composition data in concert with similar information for anthocyanins has the potential to develop a predictive model for wine colour based on the phenolic composition of the grape. Such a model, coupled with effective real-time analytical methods, would be an invaluable industry outcome.

Project Aims and Performance Targets

1. To examine the extent and magnitude of variation (between-vineyard and temporal) in grape tannin content within Australia's major winegrape varieties (eg. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot) and within selected regions (eg. Sunraysia, Barossa, Coonawarra and Yarra Valley).
2. To examine the extent of spatial variability (within-vineyard) in tannins and anthocyanins within selected vineyards and understand the temporal stability in these patterns.
3. To determine how changing phenolic composition impacts upon wine colour, wine sensory characteristics and wine score in Australia's major red varieties (eg. Shiraz, Cabernet Sauvignon and Merlot).
4. To determine the major viticultural management practices (eg. crop load, bunch exposure, variety/rootstock/clone, irrigation management and pest and disease pressure) that effect tannin, flavonol and anthocyanin levels in the major Australian winegrape varieties (eg. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot).
5. Development of standardised industry methods for the sampling and analysis of grape samples for the determination of total tannin (spectrophotometer/NIR/FTIR), tannin composition (HPLC) and anthocyanin composition (HPLC).

6. Develop and implement a targeted extension and evaluation strategy to facilitate the adoption of research findings throughout the Australian Wine Industry, using various change agents, ie Grapecheque, Viticare, industry grower groups, industry trade journals (eg. Australian Viticulture) and scientific publications (eg. Australian Journal of Grape and Wine Research).

2005-2006

Outputs	**Performance Targets**
1. Formation of project Industry Reference Group.	Industry Reference Group (IRG) agreed to in consultation with GWRDC and formed by 30th November 2005.
2. Formalisation of research agreement with E&J Gallo winery, California	Formal agreement for collaborative funding over this 5 year project documented and signed by DPI and E&J Gallo delegates by 30th November 2005. This will include travel to California Aug-Sep 2005.
3. Appointment of PhD student.	PhD student appointed by 31st January 2006.
4. 1st Honours student appointed and thesis submitted.	1st Honours student appointed 30th August 2005. Thesis completed by 30th June 2006.
5. Scientific paper on the effect of temperature on phenolic accumulation in winegrapes.	Scientific paper submitted to international peer reviewed journal by 30th June 2006.
6. 1st Industry Reference Group Meeting held.	1st Industry Reference Group Meeting held by 31st May 2006. Project plan outlined for duration of GWRDC funded project by 30th June 2006.
7. Purchase of second hand HPLC for Tannin analysis.	Second hand HPLC acquired, updated and modified to perform tannin polymer length separation by 30th December 2005.
8. Development of a formal project extension and evaluation strategy.	Extension and evaluation strategies documented and agreed to by project supervisor and chief investigator by 20th June 2006.
9. Industry trade journal article on project produced	General article which outlines aims and objectives of project written and submitted for publication to Australian Viticulture by 30th June 2006.

2006-2007

Outputs	**Performance Targets**
1. 2nd Honours student appointed and thesis submitted.	2nd Honours student appointed 30th August 2006. Thesis completed by 30th June 2007.
2. 2nd Industry Reference Group Meeting held.	2nd Industry Reference Group Meeting held by 31st May 2007. Project updates and future plans outlined. This includes presentations from Honours and PhD students.
3. Scientific paper/s on tannin polymer separation submitted.	Collaboration with Jim Kennedy, Oregon State University and DPI State Chemistry Laboratories to develop HPLC technique. Scientific paper submitted containing information about various grape cultivars, sites and seasons by 30th June 2007.
4. Purchase of new NIR rapid grape analysis equipment.	Purchase of new InfraXact (Foss) equipment for the rapid analysis of grape homogenate for tannins and anthocyanins by 30th June 2007.
5. Annual exchange with E&J Gallo researchers in California.	Mark Downey to travel to E& J Gallo in California to continue research activities on experimental sites in California by 30th November 2006.
6. Development of grower extension package for delivery to grower/industry groups.	Annual extension package developed and delivered to various target grower/industry groups around Australia by 30th June 2007.

2007-2008

Outputs	Performance Targets
1. 3rd Honours student appointed and thesis submitted.	3rd Honours student appointed 31st August 2007. Thesis completed by 30th June 2008.
2. 3rd Industry Reference Group Meeting held.	3rd Industry Reference Group Meeting held by 31st May 2008. Project updates and future plans outlined. This includes presentations from Honours and PhD students.
3. Scientific paper/s on spatial variation in tannin content and sampling implications submitted.	Scientific paper produced in collaboration with Dr Doug Adams from UC Davis on the spatial variation in grape tannins in a vineyard and implications for sampling submitted for publication by 30th June 2008.
4. Annual exchange with E&J Gallo researchers in California.	Mark Downey to travel to E& J Gallo in California to continue research activities on experimental sites in California by 30th November 2007.
5. Updated grower extension package for developed for delivery to grower/industry groups.	Annual extension package developed and delivered to various target grower/industry groups around Australia by 30th June 2008.

2008-2009

Outputs	Performance Targets
1. 4th Honours student appointed and thesis submitted.	4th Honours student appointed 31st August 2008. Thesis completed by 30th June 2009.
2. 4th Industry Reference Group Meeting held.	4th Industry Reference Group Meeting held by 31st May 2009. Project updates and future plans outlined. This includes presentations from Honours and PhD students.
3. Scientific paper/s on viticultural management practices that influence tannin levels in wine grapes.	Scientific paper produced in collaboration with Dr Doug Adams from UC Davis and Dr Nick Dokoozlian on the viticultural management practices that influence tannin levels in wine grapes sampling submitted for publication by 30th June 2009.
4. Annual exchange with E&J Gallo researchers in California.	Mark Downey to travel to E& J Gallo in California to continue research activities on experimental sites in California by 30th November 2008.
5. Updated grower extension package for developed for delivery to grower/industry groups.	Annual extension package developed and delivered to various target grower/industry groups around Australia by 30th June 2009.

2009-2010

Outputs	Performance Targets
1. 5th Honours student appointed and thesis submitted.	5th Honours student appointed 31st August 2009. Thesis completed by 30th June 2010.
2. Last Industry Reference Group Meeting held.	Last (5th) Industry Reference Group Meeting held by 31st May 2010. Project outcomes highlighted and new project plans highlighted. This includes presentation from Honours student.
3. Scientific paper/s on viticultural management practices that influence tannin levels in wine grapes.	Scientific paper produced on how changing phenolic composition impacts upon wine colour, wine sensory characteristics and wine score in Australia's major red varieties submitted for publication by 30th June 2010.
4. Annual exchange with E&J Gallo researchers in California.	Mark Downey to travel to E& J Gallo in California to continue research activities on experimental sites in California by 30th November 2009.

4. Updated grower extension package for developed for delivery to grower/industry groups.	Final extension packages developed for future delivery to various target grower/industry groups around Australia and project evaluation completed by 30th June 2010.
6. PhD student completed	PhD student completed and thesis submitted by 30th June 2010
7. Development of standardised industry methods for grape sampling and analysis of total tannin composition and anthocyanin composition.	The development and production of standardised industry methods for the sampling and analysis of grape samples for the determination of total tannin (spectrophotometer/NIR/FTIR), tannin composition (HPLC) and anthocyanin composition (HPLC). To be published in relevant industry manuals and texts by 30th June 2010.
8. Final report	Final project reported submitted by 30th June 2010

Methodology

Objective 1. To examine the extent and magnitude of variation (between-vineyard and temporal) in grape tannin content within Australia's major winegrape varieties (eg. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot) and within selected regions (eg. Sunraysia, Barossa, Coonawarra and Yarra Valley).

1.1 Variation in tannin concentration during berry development and between growing seasons.

1.1.1 Sample Collection

Fruit was collected from *Vitis vinifera* L. cvs Shiraz, Cabernet Sauvignon, Merlot and Chardonnay throughout berry development at weekly intervals from a vineyard located in Iraak, near Mildura, Victoria. Fruit was collected for between one to three growing seasons (2002-2003, 2003-2004, and 2004-2005). A representative sample of approximately 12 bunches were removed at random and a subsample of three replicates of 30 berries were collected for berry weight. Subsequently skins were removed and skin weight recorded. Skin samples were then rapidly frozen in liquid nitrogen, ground to a fine powder and stored and -80°C until analysed. Samples were then weighed to approximately 1.0 g (+/- 5%) in three replicates and weight recorded to four decimal places.

1.1.2 Extraction, Purification and Analysis of Tannins from Grapes Skins.

Weighed out samples was extracted with 10 mL of 70% acetone/water (v/v) that contained 0.1% ascorbic acid to prevent oxidation of tannin. Samples were then vortex mixed for one minute and sonicated for 20 minutes. Following sonication samples were centrifuged for 10 minutes (4,000 x g). Tannin extracts were then purified utilising liquid-liquid separation to remove any non-tannin material as described by Seddon and Downey (2008).

Following the removal of non-tannin material 100 µL of purified sample was transferred to 1.5 mL microfuge tube and dried down under vacuum. Dried down samples then underwent acid catalysed cleavage in the presence of excess phloroglucinol as described by Kennedy and Jones (2001). Samples were then analysed by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) as reported by Hanlin and Downey (2009).

Terminal subunit composition was determined utilising standard curves of catechin, epicatechin, and epicatechin-gallate. Whilst, extension subunits were quantified by

utilising the catechin standard curve and conversion factors as reported by Kennedy and Jones (2001).

1.2 Regional differences in tannin content.

1.2.1 Sample Collection and Preparation

For the 2003-2004 growing season Fruit was collected from *Vitis Vinifera* L. Shiraz, Cabernet Sauvignon, and Pinot Noir from vineyards located in Iraak, near Mildura, Victoria; Yarra Valley . and Mornington Pennisula. A representative sample of approximately 12 bunches were removed randomly at commercial harvest and a subsample of 3 replicates of 30 berries were collected for berry weight. Subsequently skins were removed and skin weight recorded. Skin samples were then rapidly frozen in liquid nitrogen, ground to a fine powder and stored and -80℃ until analysis. Samples were then weight to approximately 1.0 g (+/- 5%) in three replicates and weight recorded to four decimal places.

1.2.2 Tannin Analysis of Whole Berry Homogenate

Total tannin concentration was determined utilising a protein precipitation method described by Harbertson et al. (2003). Sample extract was precipitated with Bovine Serum Albumin (1 mg/mL) and centrifuged to form a pellet. The tannin-protein pellet was then resuspended into solution and absorbance measured at 510nm with and without the addition of a ferric chloride solution. Tannin content was quantified using a standard curve of ferric chloride-catechin and expressed as milligrams of catechin equivalents per gram fresh weight of whole berries.

Objective 2. To examine the extent of spatial variability (within-vineyard) in tannins and anthocyanins within selected vineyards and understand the temporal stability in these patterns.

2.1 Vineyard sites

The study sites were drip irrigated commercial Shiraz (*Vitis Vinifera* L.) vineyards in the wine regions of regions of Langhorne Creek (South Australia), Sunraysia, located in Iraak near Mildura (Victoria), Riverina, in Griffith (New South Wales), Central Victoria, near Tatura (Victoria) and Great Southern (Western Australia) (Table 1). Sites varied in size from 6.4 to 16 Ha. Target vines were selected using a statistical method developed to select sampling locations, this method used k-means standardised clustering (SAS 2002) to summarise soil and plant variation concurrently from EM38 and aerial imagery data.

Table 1: Basic description and location of vineyard sites.

Site	Size (ha)	Location (°)	Row spacing (m)	Vine spacing (m)	Row Orientation
LC	6.8	35.33º S 138.98º E	3	1.8	N – S
MIL	6.4	34.42º S 142.28º E	3	2.4	E – W
GRI	12.3	34.25º S 146.21º E	3.3	1.8	NE – SW
CV	16	Location confidential	3	1.8	N – S
GS	6.8	34.39º S 116.95º E	3.6	1.8	E – W

2.2. Sample Collection

At commercial harvest, a section of the vine was harvested from each of the target vines, and bunch number and weight recorded. For Mildura and Griffith a 50cm section of the vine was harvested, 180cm was harvested for each target vine from the Great Southern site, and the entire target wine was harvested in Tatura and Langhorne Creek. A sub-sample of 30 bunches was then collected and berries removed from rachis. Berry weight was determined for 100 berries, with these berries utilised for determination of Total Soluble Solids (°Brix) (see 2.4). Finally 150 berries were collected and stored at -80°C for analysis of anthocyanins and condensed tannins.

2.3 Sample Preparation and Sample Extraction

Whole berries stored at -80ºC were ground under cryogenic conditions into a powdered form. Approximately 1.0 g (+/- 5%) of ground sample was then weighed into a 10 mL polypropylene centrifuge tube and weight recorded. To the weighed sample, 10 mL of 50% ethanol/water (v/v) was added, the tube was then vortexed and sonicated for 20 minutes. Following sonication, samples were once again vortexed and centrifuged (10 minutes at 4,000 x g). Supernatant was then utilised for tannin and anthocyanin determination (see section 2.5 & 2.6).

2.4 Measurement of Total Soluble Solids

Berry juice Total Soluble Solids (TSS) (expressed as ºBrix; grams of sucrose equivalents/gram of berry juice expressed as %) was measured after crushing and centrifuging the fresh berry sample at harvest. TSS was measured using a digital refractometer.

2.5 Tannin Analysis of Whole Berry Homogenate

Refer to section 1.2.2.

2.6 Analysis of Total Anthocyanins of whole berry homogenate.

A 100µL sample of whole berry extract prepared with 50% ethanol in water (v/v) was added to test tube containing 1 M HCL. Following an incubation period of 3 hours, the absorbance was recorded at 520 nm (Iland et al, 2004). Total anthocyanin concentration was calculated utilising information recorded during analysis (weight of homogenate, extract volume and dilution factors) as well as the reported value for the extinction coefficient of malvidin-3-glucoside (Somers & Evans, 1974).

2.7 Mapping and spatial analysis

For each year and site, maps were interpolated into 2 m grid by global point kriging of the vine data using VESPA (Minasny et al. 2005) following the protocol of Bramley and Williams (2001), the analyses were carried out in ArcGIS (version 9.3: ESRI 2009) using the SPATIAL ANALYST extension.

Objective 3. To determine how changing phenolic composition impacts upon wine colour, wine sensory characteristics and wine score in Australia's major red varieties (eg. Shiraz, Cabernet Sauvignon and Merlot).

3.1 Variation in anthocyanin and tannin concentration in Shiraz fruit and the Impact on final wine.

3.1.1 Fruit Collection and Sample Preparation

Fruit from *Vitis vinifera* L. Shiraz was harvested from a vineyard located in Mildura, Victoria for 100 targeted vines (refer to section 2.1.1) during the 2008/09 growing season at commercial harvest. A one metre section was harvested from each of the vines, bunch number and weight recoded. A subsample of 20 bunches had the berries removed and 150 berries collected and stored at -80°C for anthocyanin and condensed tannin analysis.

3.1.2 Sample Preparation and Extraction

Refer to section 2.3.

3.1.3 Tannin Analysis of Whole Berry Homogenate

Refer to section 1.2.2.

3.1.4 Anthocyanin Analysis of Whole Berry Homogenate

Refer to section 2.6

3.1.5 Fruit for winemaking.

Following analysis for tannin and anthocyanin concentration of at fruit harvest from each of the 100 targeted vines, the fruit was categorised as containing low levels of anthocyanins (0.50 mg/g – 1.12 mg/g fwt whole berry) or high anthocyanins (1.50 mg/g – 2..00 mg/g fwt whole berry). In addition fruit was categorised as containing either low levels of tannin (1.40 mg/g – 1.70 mg/g fwt whole berry) or high tannin (2.10 mg/g – 2.75 mg/g fwt whole berry). Grapes were then separated according to their anthocyanin and tannin content to produce parcels of fruit containing the following combinations of anthocyanin and tannin: Low Anthocyanin/Low Tannin (Wine 1), High Anthocyanin/Low Tannin (Wine 2), Low Anthocyanin/High Tannin (Wine 3), and High Anthocyanin/High Tannin (Wine 4). Fruit harvested from the 100 targeted vines which fell into one of the 4 wine combinations was amalgamated for winemaking.

In order to determine the overall anthocyanin and tannin concentration of the amalgamated fruit, weight of fruit (from each relevant targeted vine) as well as overall weight of the fruit designated for each of the winemaking treatments was utilised to determine proportional contribution of anthocyanins and tannins from each target vine.

3.1.6 Winemaking

Following segregation of fruit to meet the combination of anthocyanin and tannin concentrations required fruit was transported to the Provisor™ experimental winery located in Merbein, Victoria. Wine was made according to the standard winemaking practice of Provisor™ (Gong *et al* 2010). Fruit from each treatment was crushed and divided between three fermentation vessels. Wines underwent seven days of maceration during fermentation. Following fermentation to dryness, the wine underwent as series of steps including racking, cold stabilization prior to bottling. Wines were then stored at 18℃ where they will remain until 1 year post-bottling before sensory and descriptive analysis.

3.1.7 Spectrophotometric Analysis of wines

At various time points during the winemaking process and post bottling, 20 mL of wine was collected for spectrophotometric analysis. Wines were analysed utilising methods outlined by Iland et al (2004) for analysis of wine colour density and wine hue. In addition tannin, anthocyanin, iron reactive phenolics, and polymeric pigment concentration was

determined utilising methods described by Harbertson *et al.* (2003). Prior to analysis wine were centrifuge at 4,000 x g to remove any solid material, this was particularly important for samples collected prior to bottling.

3.2 Influence of bunch exposure on final wine phenolic and sensory parameters

3.2.1. Bunch Exposure Treatments

Viticultural canopy management practices were employed to alter bunch exposure for three successive vintages 2003, 2004, and 2005. During 2003/2004 growing season three exposure treatments were applied to vines of *Vitis vinifera* L. cvs Cabernet Sauvignon, and Shiraz. The treatments used to modify bunch exposure included a foliage wire that lifted the canopy above the existing trellis (exposed), extensive leaf removal in the fruit zone (highly exposed), and a treatment where 70% shade cloth was applied to both sides of the canopy (shaded). The control was current commercial practice. The treatments were applied to a commercial vineyard located in Iraak, near Mildura, Victoria.

3.2.2 Fruit for Winemaking

At commercial harvest approximately 100 kg of fruit was collected from each of the exposure treatments and the control vines. Wine was subsequently made from each of treatments and the control, according to the standard winemaking practice of the Experimental Winery, CSIRO Plant Industry, Merbein, Victoria (Walker *et al.* 1998). Fruit from each treatment was crushed and divided between three fermentation vessels. Wines underwent three days of maceration during fermentation. Upon completion of fermentation wines were stored for three months at 1°C prior to bottling. Wines were stored at 18°C prior to descriptive sensory and wine chemical analysis at two years post-bottling.

3.2.3 Spectrophotometric analysis of wines

Refer to section 3.1.7.

3.2.3 Sensory Analysis

Descriptive sensory analysis of wines was performed in order to characterise differences in the perceived organoleptic profile between wine treatments and between the wine making replicates. Wine replicates were evaluated by a panel of 11 and 10 people for Cabernet Sauvignon and Shiraz wines respectively over a period of three months. Sensory evaluation of wines involved a process of panellist training in aroma, taste and

mouthfeel sensation detection and evaluation prior to formal sensory evaluation described by Joscelyne *et al,* (2007).

Objective 4. To determine the major viticultural management practices (eg. crop load, bunch exposure, variety/rootstock/clone, irrigation management and pest and disease pressure) that effect tannin, flavonol and anthocyanin levels in the major Australian winegrape varieties (eg. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot).

4.1 Influence of bunch exposure on tannin, anthocyanin, and flavonol levels:

4.1.1 Bunch Exposure Treatments

Viticultural canopy management practices were employed to alter bunch exposure on *Vitis Vinifera* L. cvs Cabernet Sauvignon, Shiraz, Chardonnay, Merlot, Pinot Noir, and Petit Verdot. over three successive vintages 2003, 2004, and 2005. There were up to four exposure treatments applied. The treatments used to modify bunch exposure included a foliage wire that lifted the canopy above the existing trellis (exposed), extensive leaf removal in the fruit zone (highly exposed), and a treatment where 70% shade cloth was applied to both sides of the canopy (shaded). In addition to canopy manipulation a light exclusion treatment was applied. The control was current commercial practice. The treatments were applied to a commercial vineyard located in Iraak, near Mildura, Victoria.

4.1.2 Sample Collection and Preparation

Refer to section 1.1.1

4.1.3 Extraction, Purification, and Analysis of Tannins in Grape Skins

Refer to section 1.1.2

4.1.4 Anthocyanin and Flavonol Analysis in Grape Skin

Anthocyanin and flavonol composition was determined by High Performance Liquid Chromatography (HPLC). Grape skins samples (1.0g) were extracted with 10mL 50% methanol in water (v/v) (Downey *et al.* 2007). Each tube was then vortexed and sonicated for 20 minutes at room temperature (approximately 24℃). Following sonication each tube was vortexed again and centrifuged at 4,000 x g for 10 minutes. Subsequently 1.5ml of the supernatant was transfer to a 2mL screw tube and centrifuged at 13,000 x g to remove any remaining cell debris prior to analysis.

Following centrifugation, 200 µL of grape skin extract was transferred to a HPLC autosampler vial for analysis using a Wakosil™ analytical column (150 mm x 4.6 mm, 3µm packing). The HPLC separation utilised a binary solvent gradient as described by Downey and Rochfort (2008). Anthocyanins are expressed as milligrams of malvidin-3-*O*-glucoside equivalents per gram of fresh berry skin, and flavonols are expressed as milligrams of quercetin-3-*O*-glucoside equivalents per gram of fresh berry skin.

4.2 Influence of differing rootstock on tannin, anthocyanin, and flavonol levels:

4.2.1 Rootstocks samples

Grape samples of from nine rootstocks from the 2007-2008 and 2009-2010 growing season were. Vines were grown in a research vineyard located at Koorlong, near Mildura, Victoria. Rootstocks included 1103 Paulsen, 140 Ruggeri, Dodridge, Freedom, MG5512, MG6262, MS1010, MS3769, and Ramsey (Table 2)

Table 2: Parentage of rootstocks analysed for tannins, anthocyanins and flavonols.

Abbrev.	**Rootstock**	**Parentage**
1103 Paulsen	1103 Paulsen	*Vitis berlandieri* X *Vitis rupestris*
140 Ruggeri	140 Ruggeri	*Vitis berlandieri* X *Vitis rupestris*
Dog Ridge	Dog Ridge	Hybrids of *Vitis candicans*
Freedom	Freedom	1613 (Solonis X Othello) X Dogridge
Ramsey	Ramsey	*Vitis champini*
MG5512	Merbein 5512	*Vitis berlandieri* X *Vitis berlandieri* cross
MG6262	Merbein 6262	*Vitis cinerea* X *Vitis cinerea* cross
MS1010	Merbein 1010	*Vitis champini* X *Vitis vinifera* cross
MS3769	Merbein 3769	*Vitis longii* X *Vitis vinifera*

4.2.2 Sample Collection and Preparation

At commercial harvest six bunches were selected at random from each of the nine rootstocks. Berries were removed from the rachis, and three replicates of 30 berries selected at random and weighed to determine berry weight. The skins were then removed and skin weight determined. Skins were then rapidly frozen with liquid nitrogen at stored -80°C. Skins were homogenised under nitrogen, and approximately 1.0g (+/- 5%) of skin sample was weighed into a 10 mL centrifuge tube and the weight recorded to four decimal places. Samples were stored at -80°C until analysis for anthocyanins and condensed tannin.

4.2.3 Extraction, Purification, and Analysis of Tannins in Grape Skins

Refer to Section 1.1.2

4.2.4 Anthocyanin and Flavonol Analysis

Refer to section 4.1.4.

4.3 Influence of sustained deficit irrigation on tannin, anthocyanin, and flavonol levels:

4.3.1 Irrigation Treatments

Field trials were conducted on *Vitis Vinifera* L. cultivars, Cabernet Sauvignon and Shiraz, located in Irymple, Victoria, and Dareton New South Wales respectively. The experimental layout was a row-column design with four irrigation treatments replicated eight times across four rows, each consisting of four vines, the to out vine being buffer vines (Chalmers 2007). Each row consisted of four irrigation treatments (Table 3).

Table 3 Irrigation regimes established for the Cabernet Sauvignon and Shiraz trial sites (Chalmers 2007).

Variety	Dripper Flow Rates (L/h) at 0.5 m spacings	Irrigation regimes (% of Et_c)
Cabernet Sauvignon	2.3, 1.6, 1.2*, 1.2	100%, 70%, 52%, 43%
Shiraz	3.5, 2.3, 1.6, 1.2	100%, 65%, 45%, 34%

** Drippers were spaced at 0.5 m and 0.6 m to create 52% and 43% deficit SDI treatments*

4.3.2 Sample Collection and Preparation

At commercial maturity, a 1.0 m segment of the canopy was harvested, bunches counted and weight recorded. A sub-sample of ten bunches were randomly selected and berries removed. For each replicated a 30 berry sample was collected weighed and skins removed. Skins were then weighed and rapidly frozen with liquid nitrogen, ground into a fine powder and stored at -80°C until analysed for anthocyanin content . In addition a sample of 100 berries was collected and stored at -20°C prior to grinding and analysis for total tannin by protein precipitation.

4.3.3 Tannin Analysis of Whole Berry Homogenate

Refer to section 1.2.2.

4.3.4 Anthocyanin and Flavonol Analysis of Grape Skin

Refer to section 4.1.4.

4.4 Varietal differences in tannin and anthocyanin levels:

4.4.1 Sample Collection

Samples were collected from vineyards across California. Samples were collected immediately prior to commercial harvest over three growing seasons. Berries were removed from bunches and bulked then sub-sampled. Berries were then homogenised in the winery assessment lab in California, frozen at -20°C, and shipped to Mildura, Australia for analysis. In total, 22 cultivars were studied however for several of the cultivars there were only a few samples, mainly as a result of the timing of collection.

4.4.2 Tannin Analysis

Refer to section 2.1.5

4.4.3 Anthocyanin and Flavonol Analysis of Whole Berry Homogenate

Refer to Section 2.1.6

4.5 Yield influence on tannin and anthocyanin content:

4.5.1 Sample Collection and Yield Determination

Fruit from *Vitis vinifera* L. Shiraz was harvested from a vineyard located in Mildura, Victoria for 100 targeted vines (refer to section 2.1) for three growing seasons. A 50 cm transect of the canopy was harvested, bunches counted and weighed. Yield was expressed as kg/m^2 and determined using the following formula:

Weight of fruit harvested (kg) ÷ ((transect harvested (m) x row spacing (m))

4.5.2 Sample Preparation and Extraction

Refer to section 2.3.

4.5.3 Tannin Analysis of Wine Berry Homogenate

Refer to section 2.1.5

4.5.4 Anthocyanin Analysis of Whole Berry Homogenate

Refer to Section 2.1.6

4.6 Regional differences in tannin, anthocyanin and flavonol composition.

4.6.1 Sample Collection and Preparation

For the 2003-2004 growing season fruit was collected from *Vitis vinifera* L. cvs Shiraz, Cabernet Sauvignon, Merlot, and Pinot Noir from vineyards located in Iraak, near Mildura (Victoria); Yarra Valley (Victoria) and Mornington Peninsula (Victoria). A representative sample of approximately 12 bunches were removed randomly at commercial harvest and a subsample of three replicates of 30 berries were collected for berry weight. Subsequently skins were removed and skin weight recorded. Skin samples were then rapidly frozen in liquid nitrogen, ground to a fine powder and stored and -80°C until analysed. Samples were then weighed to approximately 1.0 g (+/- 5%) in three replicates and weight recorded to four decimal places.

4.6.2 Extraction, Purification, and Analysis of Tannins in Grape Skins

Refer to section 1.1.2

4.6.3 Anthocyanin and Flavonol Analysis of Grape Skins

Refer to section 4.1.4.

Objective 5. Development of standardised industry methods for the sampling and analysis of grape samples for the determination of total tannin (spectrophotometer/NIR/FTIR), tannin composition (HPLC) and anthocyanin composition (HPLC).

At the beginning of this project, there were published methods for most of the fundamental research, although several issues had been identified with all of these methods in previous work. Thus, several of these methods needed to be revisited and revised. While some existing methods were in widespread industry use, for example the methods for determination of colour (wine colour density, wine hue and total grape anthocyanins) as published by Iland *et al.* (2004), others had only been recently developed or were unsuitable for routine analysis because of the expertise required and the cost of instrumentation, thus a rapid high-throughput analytical method that could be

adapted to routine analysis would be a valuable tool for the industry and necessary to facilitate a route-to-market for other innovations arising from this project. Here we describe the revision of methods as well as the development of new methods, the state of current methods and the remaining gaps or barriers to adoption.

5.1 Vineyard sampling of winegrapes

Methods and outcomes against this objective are presented together in the Results and Discussion section.

5.2 Grape Tissue Extraction for Analysis

Methods and outcomes against this objective are presented together in the Results and Discussion section.

5.3 Total Tannin Analysis of Whole Berry Homogenate by Protein Precipitation

Methods and outcomes against this objective are presented together in the Results and Discussion section.

5.4 Rapid determination of phenolics components in red wines from UV-visible spectra

Phenolic components; total tannins, iron-reactive phenolics, anthocyanins, large and small polymeric pigments were determined according to the Harbertson-Adams Assay (Harbertson *et al.* 2002; Harbertson *et al.* 2003) (see Methods Sections 1.2.2, 2.3, 2.5 & 2.6).

For wines; UV-visible spectra were collected with a Metertech SP8001 bench-top spectrophotometer using 2mm pathlength, disposable UVettes™ (Eppendorf). Samples were scanned from 230-900nm at approximately 0.17nm intervals. Water was used for the reference scan. Spectral data was collected on all fermentation samples at both a 5-fold dilution with water and undiluted. Finished wines were diluted 3-fold with water and spectra were collected on both diluted and undiluted samples.

For grape samples, UV-visible spectra were collected using a microtitre plate reader (SpectraMax Plus384, Molecular Devices, Sunnyvale, USA) and 96-well plates. Grape extracts (see Methods Section 2.3) were diluted with water and spectra were collected on diluted and undiluted extracts (Skogerson *et al.* 2007).

NIR spectra were also collected for all samples using the FOSS InfraXact™ unit equipped with the small sample cup (60mm diameter) and 0.2mm gold reflector. Samples were

scanned from 570-1098nm at 2nm intervals and 1100-1848nm at 2nm intervals. NIR spectra were collected on undiluted samples only (Skogerson *et al.* 2007).

Data analysis on the wine samples was performed using Unscrambler® (Version 9.0, CAMO ASA, Norway) as described in Skogerson et al. (2007). Data analysis (Unscrambler®) on the grape extracts is ongoing as part of a current collaboration with Constellation Wines US and the Californian wine industry Gold Standards Technical Group.

Objective 6. Develop and implement a targeted extension and evaluation strategy to facilitate the adoption of research findings throughout the Australian Wine Industry, using various change agents, ie Grapecheque, Viticare, industry grower groups, industry trade journals (eg. Australian Viticulture) and scientific publications (eg. Australian Journal of Grape and Wine Research).

Methods and outcomes against this objective are presented together in the Results and Discussion section.

Results/Discussion

Objective 1. To examine the extent and magnitude of variation (between-vineyard and temporal) in grape tannin content within Australia's major winegrape varieties (eg. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot) and within selected regions (eg. Sunraysia, Barossa, Coonawarra and Yarra Valley).

1.1 Variation in tannin concentration during berry development and between growing seasons.

Samples were collected from fruit-set through to commercial harvest for three growing seasons. For Cabernet Sauvignon the developmental period ranged from 15 weeks (2002/03) to 18 weeks (2003/04). Total tannin concentrations were highest at around fruit-set, with maximum concentrations around 19.40 mg/g of skin 6 weeks pre-veraison in 2002/03, 22.72 mg/g of skin 7 weeks pre-veraison in 2003/04 and 7.19 mg/g of skin 6 weeks pre-veraison in 2004/05, after which total tannin concentrations declined (Figure 1.1 A). By veraison, tannin concentration in Cabernet Sauvignon had decreased almost 50% for all seasons, after which tannin concentration continued to decline at a slower

rate. Tannin concentration at harvest was similar in 2003/04 (4.62 mg/g of skin) and 2004/05 (4.18 mg/g of skin), in spite of the maximum tannin concentration measured for the 2003/04 season being three times higher during berry development. Tannin concentration at harvest in 2002/03 was considerably lower than the other two seasons at 2.67 mg/g of skin.

In Shiraz the developmental period from fruit-set through to harvest varied between seasons from 14 weeks in 2004/05 to 20 weeks in 2003/04. Like Cabernet Sauvignon, Shiraz maximum concentrations of tannin were measured at around fruit-set. In 2003/03 the highest measured tannin concentration was 11.65 mg/g of skin 6 weeks pre-veraison, in 2003/04 13.70 mg/g of skin 7 weeks pre-veraison, and 7.37 mg/g of skin 5 weeks pre-veraison in 2004/05 (Figure 1.1 B). Tannin concentration at harvest varied considerably between seasons. Levels were 6.57 mg/g of skin in 2002/03; 4.89 mg/g of skin in 2003/04 and 2.88 mg/g of skin in 2004/05. In addition, there was considerable variation in the rate of decline of tannin concentration over the developmental period in each season.

In Merlot the duration of the growing season for the two seasons studied was simila, at 14 and 13 weeks for 2002/03 and 2003/4 respectively But the accumulation of tannins was different between the two seasons. In 2002/03, the maximum tannin concentration was measured at 5.23 mg/g of skin 4 weeks pre-veraison, slowly declining to 2.23 mg/g of skin at harvest. In contrast, maximum tannin concentration in 2003/04 was measured at 14.42 mg/g of skin 8 weeks pre-veraison, declining to 4.38 mg/g of skin at harvest (Figure 1.1 C).

In Cabernet Sauvignon, Shiraz and Merlot, tannins accumulated to a maximum concentration, declined rapidly prior to veraison, followed by a slower rate of decline post-veraison.

Tannin levels in Chardonnay were measured in the 2002/03 growing season. The highest concentration of 14.79 mg/g of skin was measured at fruit-set 6 weeks pre-veraison (Figure 1.1 D). After fruit-set, tannin concentration declined rapidly to veraison with a reduction of almost 50%. However, following veraison, rather than the rate of decline slowing, tannins declined at the same rate reaching a final tannin concentration of 2.29 mg/g of skin at harvest.

This data shows that variation in tannin concentration exists between cultivars, particularly over the course of berry development and there is considerable variation in the way tannins accumulate and the rate of decrease of extractability over the developmental period within cultivars from the same vineyard between seasons.

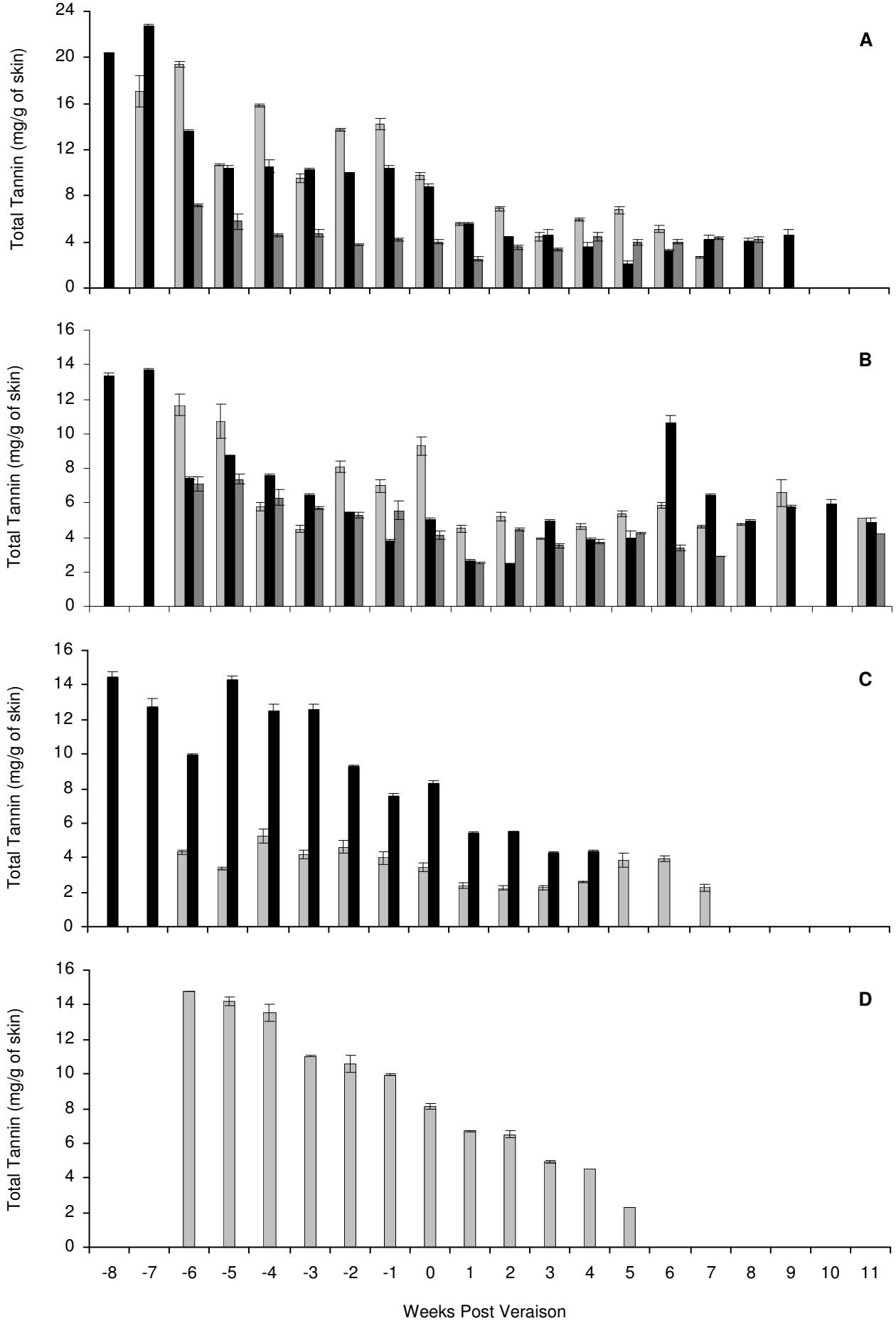


Figure 1.1: Tannin mg/g fresh weight of skin expressed as catechin equivalents for 3 growing seasons (2002/03 □, 2003/04 ■ , and 2004/05 ▩) from fruit set through to harvest, pre- and post-veraison for **A** Cabernet Sauvignon, **B** Shiraz, **C** Merlot and **D** Chardonnay.

Previous research on Shiraz and Pinot Noir has indicated that the peak in tannin accumulation occurred around the time of veraison and declined post-verasion towards harvest. The data reported here was not consistent with those previous observations. However, these observations were consistently observed across multiple seasons and varieties and the analysis was conducted using the same analytical methods and by a number of different operators. Thus, the data presented here demonstrates a real difference from previous observations. In comparing this data with the previous research in search of a logical explanation for this difference it is apparent that there is a clear climatic difference between the data collected from Sunraysia and the data collected previously. The previous data was collected from cool production regions; Pinot Noir in Oregon and Shiraz from Adelaide and McLaren Vale, south of Adelaide. Climatically these regions are significantly cooler than than the Sunraysia region and it is likely that this difference in temperature accounts for the observed difference in the pattern of tannin accumulation and in the patterns of decreasing tannin extractability observed in Shiraz, Cabernet Sauvignon, Merlot and Chardonnay grapes grown in the Sunraysia region.

1.2 Regional differences in tannin content.

Samples of Shiraz, Cabernet Sauvignon and Pinot Noir grapes were collected from three vineyards in the 2003/04 growing season. Vineyards were located in the Sunraysia region near Mildura (Vic.), the Yarra Valley in the hills north east of Melbourne and the Mornington Peninsula, south east of Melbourne (Vic.).

The concentration of total tannin in Shiraz was similar in berries from the vineyards located in Sunraysia and Mornington Peninsula at 2.39 mg/g and 2.15 mg/g fresh weight of whole berry respectively (Figure 1.2). Total tannin in Shiraz berries from the Yarra Valley was higher at 3.84 mg/g fresh weight of whole berry.

Likewise for Cabernet Sauvignon, concentration of tannin at Sunraysia and the Mornington Peninsula were similar at 3.83 mg/g and 3.72 mg/g fresh weight of whole berry respectively (Figure 1.2). Total tannin content of berries from the Yarra Valley was higher at 4.44 mg/g fresh weight of whole berry.

In similar fashion, tannin concentration of Pinot Noir grapes from Sunraysia and Mornington Peninsula was similar and higher in the Yarra Valley with levels of 4.27 mg/g, 4.35 mg/g, and 5.01 mg/g fresh weight of berry respectively.

As the three sites are geographically and climatically quite distinct, a greater range of tannin levels could be expected between varieties as well sites for each variety. Whilst this particular data set shows a smaller than expected difference in tannins between sites, the small data set may not be representative of the differences between the regions or varieties, particularly given the variation that can exist for a variety from the same site

between growing seasons (Figure 1.1). Regional variability is explored more in Objective 2.

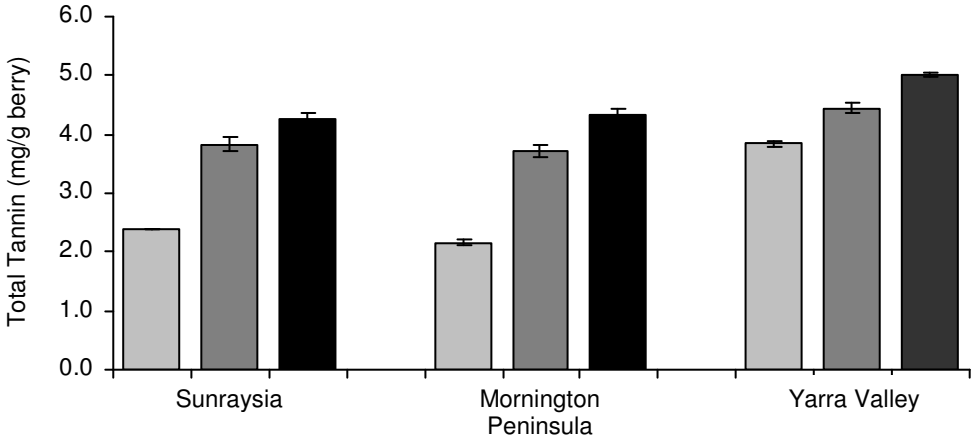


Figure 1.2: Tannin mg/g fresh weight of berry expressed as catechin equivalents for Shiraz □, Cabernet Sauvignon ■, and Pinot Noir ■ grown in Sunraysia, Mornington Peninsula and the Yarra Valley, in the 2003/04 growing season.

Objective 2. To examine the extent of spatial variability (within-vineyard) in tannins and anthocyanins within selected vineyards and understand the temporal stability in these patterns.

This component of the project was delivered in conjunction with the GWRDC-funded Soil and Water Initiative Five Sites Project (GWRDC PROJECT No. DPI 04/04) (Goodwin 2009). Anthocyanins and tannins were determined at harvest for each of the 100 target vines at each site. Fruit was collected from each site at harvest in 2006, 2007 and 2008 and shipped to DPI Mildura for processing and analysis. All samples were analysed in triplicate using the published protein precipitation assay of Harbertson et al. (2003). Total soluble solids (°Brix) was also determined for each vine.

Seasonal differences in quality parameters have long been recognised and are reflected in the truly great vintages that have elevated winemaking to the status of art. Consequently, it is little surprise that there are marked differences in grape tannins observed between seasons. Similarly, there are regions that are recognised as consistently producing higher quality wines than others and certainly, both the regional and seasonal differences have been borne out here. What is not immediately apparent, are the underlying causes of the site and seasonal variation. Generally, we can attribute seasonal difference to changes in climate variables from year-to-year and no doubt some

interactions between those and management practices. However, the real climate driver has not been unequivocally established. Although many assert it is temperature (Downey *et al.* 2006). While there are studies that support this and our own data lends credence to this hypothesis, managing temperature in the vineyard is no small undertaking. While climate affects regions differently and each site differently each year, it should affect a site in a relatively consistent fashion. As a consequence climate is not likely the sole driver of within site variation. It most likely interact with driver(s) of tannin variation but this had not been adequately explored.

The possible drivers of within vineyard variability is partially examined in GWRDC PROJECT No. DPI 04/04, where these samples were sourced from. This project collected a large amount of other data with the aim of describing the extent of variation within vineyards and of identifying the drivers of within vineyard variation.

2.1 Within site variation

2.1.1 Total tannin concentration variation

Whole berry tannin concentration was determined by a protein precipitation method described by Harbertson et al (2003). Total tannin was quantified using a standard curve of catechin with ferric chloride and expressed as milligrams of catechin equivalents per gram fresh weight of whole berries.

Langhorne Creek (LC) showed considerable within site variation for all three seasons, with the difference in tannin concentrations being up to 1.55 mg/g berry across the site. The 2005/06 and 2007/08 seasons had considerably lower tannin concentrations than the 2006/07 season, which ranged from 1.69 to 3.24 mg/g berry. Although the majority of the Langhorne Creek (LC) site had higher tannin values in 2007/08 than in 2005/06, the highest observed tannin concentrations for Langhorne Creek (LC) occurred in 2006/07, ranging from 2.47 to 4.02 mg/g berry. The final season (2007/08) was observed to have tannin concentrations between 1.69 and 3.24 mg/g berry. The pattern of tannin accumulation for this site was similar for the final two seasons, with generally higher concentrations observed on the outskirts of the site with tannin concentration decreasing towards the centre of the site. This pattern was not observed in 2005/06. This pattern of accumulation may reflect vine vigour and underlying soil type. That this pattern was not observed in 2005/06 suggests another factor, such as environmental drivers had an overriding influence.

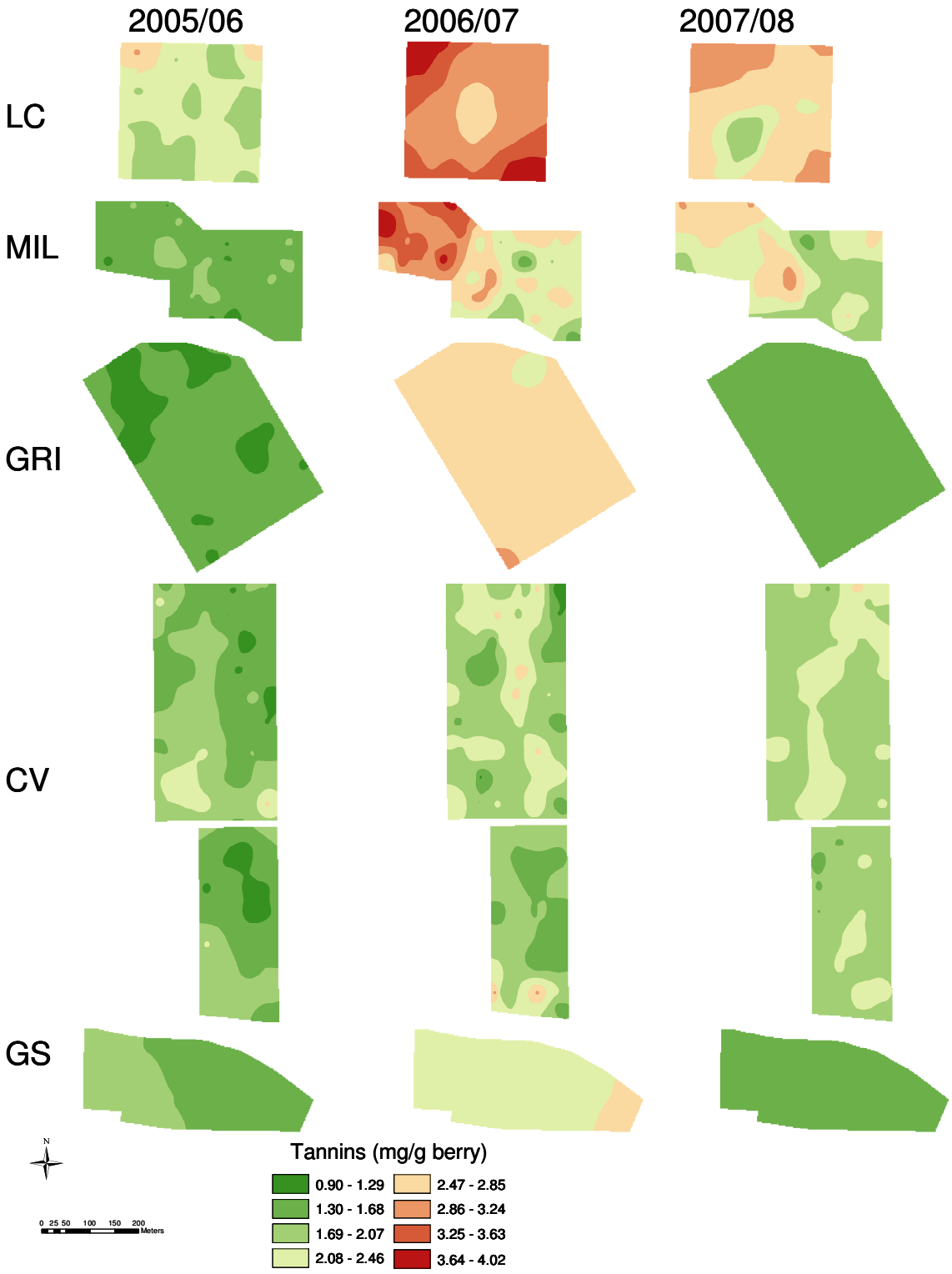


Figure 2.1: Spatial variation maps for total tannin concentration of grapes from vines at Langhorne Creek (LC) Griffith (GRI),Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08 seasons. Grape samples for tannin analyses were collected from 100 vines and expressed in mg/g berry before kriging was used to derive the plotted values.

The range of measured tannin concentrations observed for the Mildura (MIL) site were similar between the last two seasons, ranging from 1.30 to 4.02 mg/g berry in 2006/07 and 1.30 to 3.24 mg/g berry for the 2007/08 season. The least variation in tannin concentration for this site occurred in 2005/06, with tannin ranging from 0.90 to 2.07 mg/g berry. This site was observed to show higher tannin concentrations in 2006/07 and 2007/08 in the north-west corner of the site although the concentration in this area differed between seasons. Tannin concentrations also showed a gradual decline to the south-eastern section of the vineyard for both 2006/07 and 2007/08. Knowledge of this site shows the western end to be at the top of a slight rise on lighter soil while the south-eastern part of the vineyard had heavier soil and greater water holding capacity with vines tending to be more vigorous.

The Central Victoria (CV) site showed some of the largest spatial variation for all three seasons. While the level of tannin in berries from this site appeared similar between seasons, the 2006/07 season had a greater range of tannin concentrations than other seasons, ranging from 0.90 to 3.63 mg/g berry. This range was considerably bigger than that in 2005/06 (0.90 to 2.85 mg/g berry) or 2007/08 (1.30 to 2.85 mg/g berry).

Like Griffith (GRI), the within site variation observed for the Great Southern (GS) site was small, with this site being relatively uniform across all three seasons. The range of measured tannin concentrations for the Great Southern (GS) site was similar between seasons, ranging from 1.30 to 2.07 mg/g berry in 2005/06 and 1.30 to 1.68 mg/g berry in 2007/08. In 2005/06 and 2007/08 the majority of the Great Southern (GS) site had similar tannin levels (1.30 - 1.68 mg/g berry) while in 2006/07 the majority of the site had higher tannin levels (2.08 – 2.46 mg/g berry).

2.1.2 Anthocyanin Variation

Total anthocyanin concentration was determined using a method developed by Iland et al. (Iland *et al.* 2004). Anthocyanin concentrations are expressed as milligrams anthocyanin per gram fresh weight of whole berry.

Similar to what was observed for tannin, the highest concentrations of anthocyanins were observed at Langhorne Creek (LC) in all three seasons, with anthocyanin concentrations as high as 3.10 mg/g berry (Figure 2.2). Overall, the highest concentrations of anthocyanins for all of the sites occurred in 2007/08, whilst 2005/06 generally had lower concentrations of anthocyanins at all sites.

The Griffith (GRI) site had the lowest observed concentrations of anthocyanins for all three seasons with the majority of the site having anthocyanin concentrations of 0.66 to 1.00 mg/g fresh weight of berry (2005/06) and 1.01 to 1.70 mg/g berry (2006/07 and 2007/08) (Figure 2.2). Compared to other sites the Griffith (GRI) and Great Southern (GS) sites had greater uniformity across the sites.

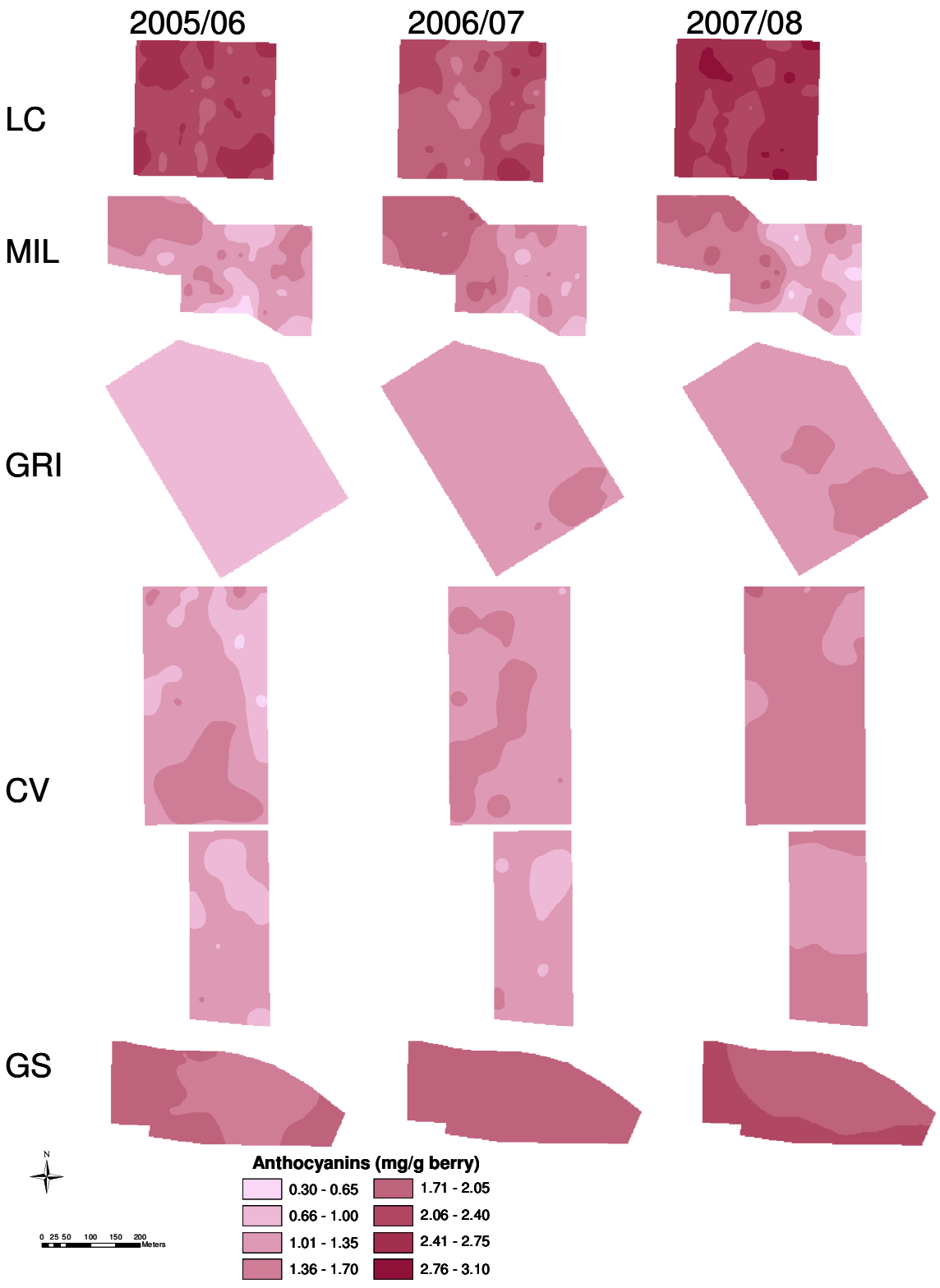


Figure 2.2: Spatial variation maps of total anthocyanin concentration in grapes from vines at Langhorne Creek (LC) Griffith (GRI),Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08 seasons. Grape samples for anthocyanin analyses were collected from 100 vines and expressed in mg/g berry before kriging was used to derive the plotted values.

This pattern was also observed for tannin concentration for these two sites (Figure 2.1). This suggests that some of the drivers of tannin and anthocyanin biosynthesis are similar. The Griffith (GRI) and Great Southern (GS) sites had the least variation, with differences in anthocyanin concentration no greater than 0.70 mg/g (Figure 2.2)

The Mildura (MIL) site had consistently greater variation across the site, in terms of range of observed anthocyanin concentrations, across all three seasons compared to other sites, with differences of up to 1.85 mg/g berry observed.

Levels of total tannin (Figure 2.1) across most sites correlated positively with observed anthocyanin levels (Figure 2.2). Langhorne creek (LC) and Mildura (MIL) accumulated high concentrations of both anthocyanins and tannins and in the same parts of the vineyard. This further indicates that the same underlying factors drive anthocyanin and tannin accumulation in grape berries. However, this relationship was not observed at Great Southern (GS), where in 2007/08 the tannin concentration was low (1.30 to 1.68 mg/g) and anthocyanins were high (2.41 – 3.10 mg/g berry). This suggests that some factor at that site is having greater impact on anthocyanins. Specific interrogation of environmental and viticultural factors at that site would be required to explain this.

2.1.3 Total soluble solids (TSS) variation

Total soluble solids content (TSS) of berry juice at harvest was determined using a digital refractometer. TSS at the Langhorne Creek (LC) site was relatively uniform in 2005/06 (24.9 to 27.8°Brix) with the majority of the site having differences in TSS no greater than 1.4°Brix (Figure 2.3). In the 2006/07 and 2007/08 seasons however, TSS were considerably more variable with TSS at 20.4 to 24.8 and 24.9 to 29.3°Brix respectively. In 2006/07, the pattern of TSS accumulation resembled the pattern observed for both tannin and anthocyanin accumulation, with TSS higher at the outskirts of the site and lower towards the centre of the site.

In each season, Mildura (MIL) had large differences in TSS across the site, from 17.3 to 26.3°Brix (2005/06) and 20.4 to 26.3°Brix (2006/07 and 2007/08). In all three seasons TSS was higher to the north-west of the site and declined towards the south-east of the site (Figure 2.3). This pattern of accumulation at this site was also observed for both tannins and anthocyanins, particularly for the final two seasons (2006/07 and 2007/08)

The Griffith (GRI) site displayed very little variability in TSS concentrations across the site for all three seasons. In addition, the TSS concentrations at this site were very similar between seasons, 21.9 to 24.8°Brix (2005/06), and 23.4 to 26.3°Brix (2006/07 and 2007/08). With the majority of this site having TSS concentrations between 23.4 to 24.8°Brix for all three seasons (Figure 2.3). This site does not appear to have any one area which accumulated TSS at higher or lower levels across all three seasons.

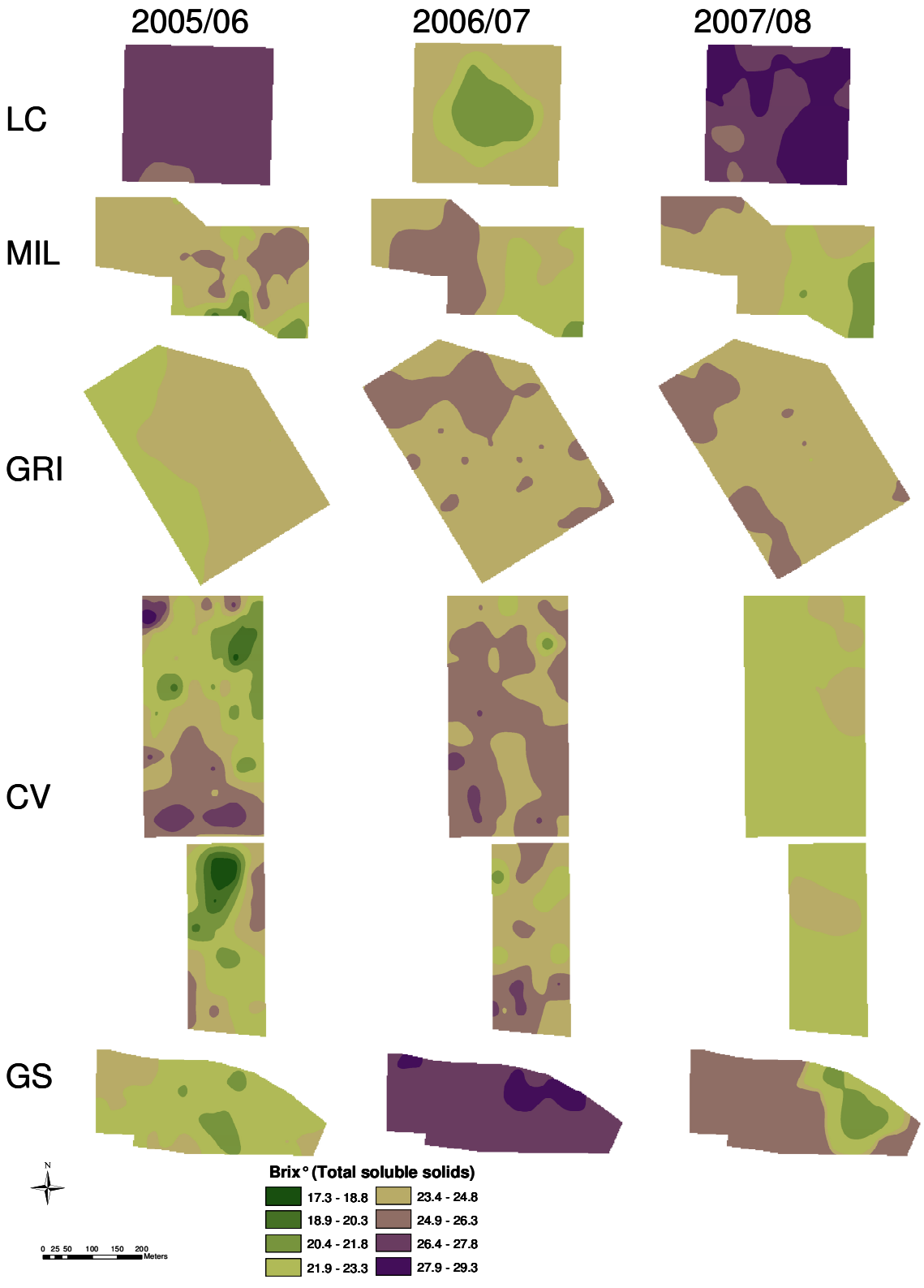


Figure 2.3: Spatial variation maps of total soluble solids (TSS) content of grapes from vines at Langhorne Creek (LC) Griffith (GRI), Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08. Grape samples for TSS analyses were collected from 100 vines and expressed in Brixº before kriging was used to derive the plotted values.

Central Victoria (CV) had very variable TSS across the site particularly for the 2005/06 (17.3-29.3°Brix) and 2006/07 (20.4 to 27.8°Brix) seasons. The 2007/08 seasons however, had less variation (21.9 to 24.8°Brix), with the majority of the site having differences in TSS of no greater than 1.4°Brix. The Great Southern (GS) site had different TSS levels for all three seasons, 20.4 to 23.4°Brix (2005/06), 26.4 to 29.3°Brix (2006/07) and 20.4 to 26.3°Brix (2007/08). Despite having relatively uniform accumulation of tannin and anthocyanins, TSS was more variable with the exception of the 2006/07 season.

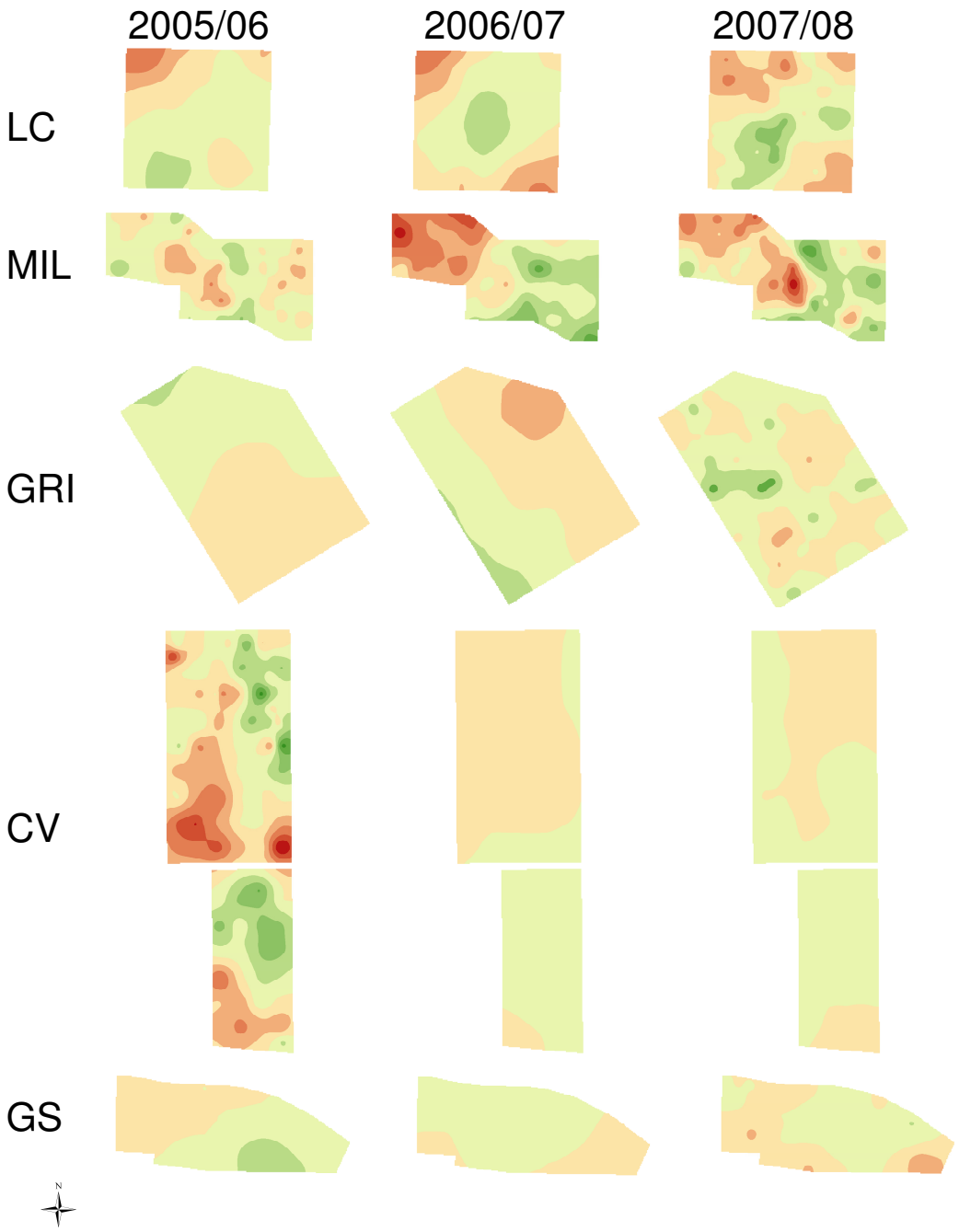
2.2 Between site variations

Normalised data is presented to enable comparison between years for the five different sites without the interactions of the possible drivers of vineyard variability such as seasonal effects. The normalised data was derived from the mean and standard deviation of the variable and removing the data that was either greater than +3 or less then -3, thus removing all data more then three standard deviations from the mean, giving an average measure of variation (Bramley and Hamilton 2004).

2.2.1 Total tannin concentration variation

Average tannin levels in berries varied between sites and seasons (Figure 2.4). Langhorne creek (LC) and Mildura (MIL) fruit had the highest average tannin concentration, with berries accumulating average tannins concentrations of 3.17 mg/g berry and 2.61mg/g berry (2006/07) respectively. Langhorne Creek (LC) fruit was observed to have higher tannin concentrations than fruit from the other four sites for all three seasons with the exception of Mildura (MIL) which had similar overall tannin concentrations for the final two seasons (2006/07 and 2007/08). Overall, the 2006/07 season had the highest average tannin concentrations at all five sites. While the lowest average tannin concentrations occurred in the 2005/06 season, the differences in observed average tannin concentrations between regions changed from season-to-season. In 2005/06, for Griffith (GRI) and Great Southern (GS), tannin averages were observed from 1.35 mg/g berry and 1.64 mg/g berry respectively, with higher tannin concentrations observed in Great Southern (GS). In contrast in 2007/08 there was no difference in the observed tannin concentrations between these two sites with both sites averages to be 1.53 mg/g berry (Figure 2.4).

The greatest variation in tannin occurred in Mildura with tannin averages ranging from 1.53 mg/g fresh weight of berry in the 2005/06 season to 2.61 mg/g fresh weight of berry in the 2006/07 season. The least variation in tannin average was observed in Central Victoria (CV) ranging from 1.92 mg/g fresh weight of berry in 2006/07 to 2.00 mg/g fresh weight of berry in 2007/08. A similar pattern was seen in Great Southern (GS) and Griffith (GRI) in the 2005/06 and 2007/08 seasons (Figure 2.4).



Tannin m/g fresh weight berry						
Site	2005/06		2006/07		2007/08	
	Average	STD	Average	STD	Average	STD
LC	2.15	0.32	3.17	0.58	2.63	0.46
MIL	1.53	0.24	2.61	0.58	2.25	0.43
GRI	1.35	0.19	2.28	0.25	1.53	0.19
CV	1.63	0.37	1.92	0.41	1.99	0.25
GS	1.64	0.42	2.34	0.28	1.53	0.19

STD – Standard deviation

Figure 2.4: Relative spatial variation maps of normalised total tannin concentration at Langhorne Creek (LC) Griffith (GRI), Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08 seasons. Samples of grapes for vine total tannin were collected from 100 vines and expressed in mg/g berry before kriging was used to derive the plotted values.

2.2.2 Anthocyanin Variation

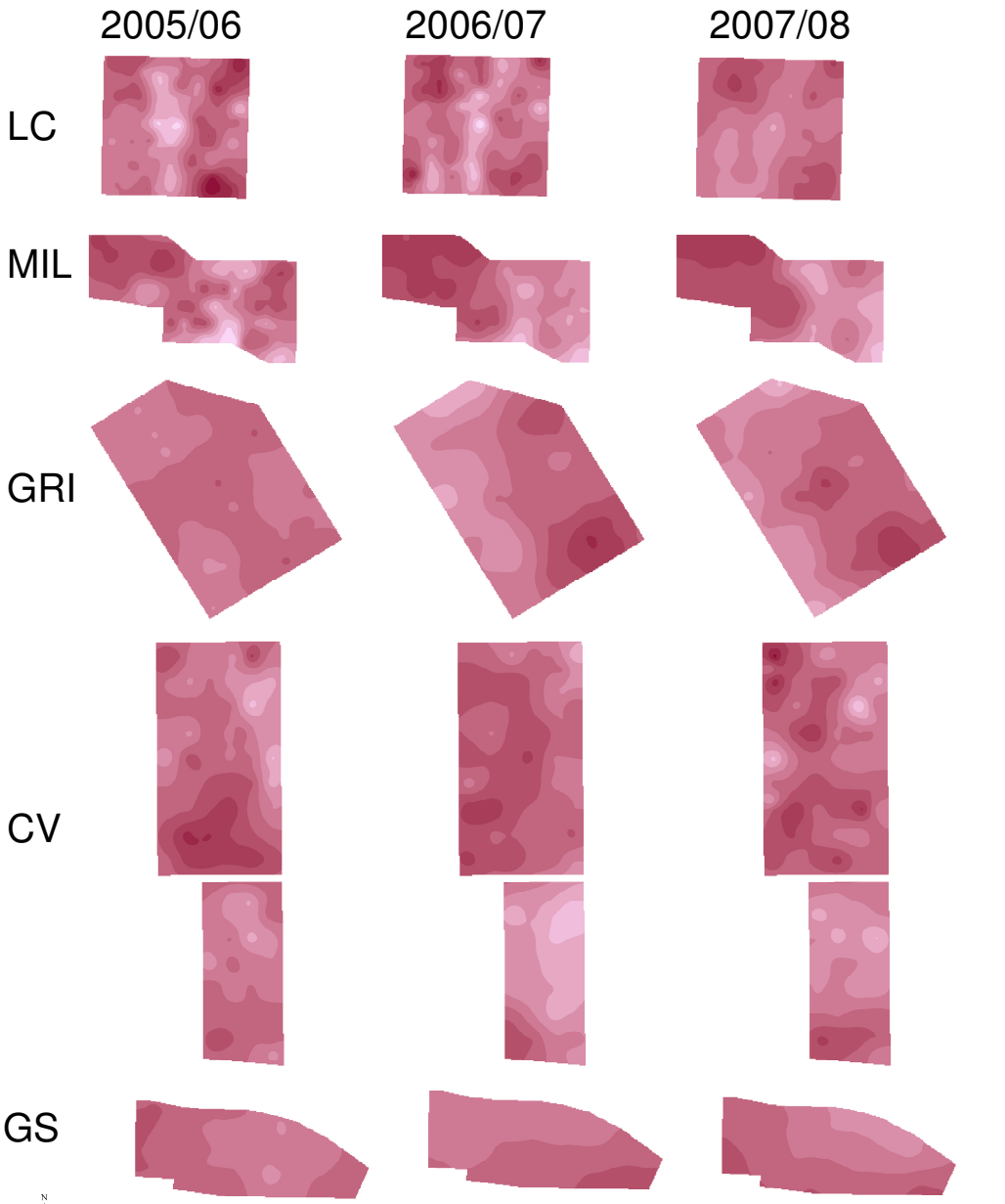
Average anthocyanin levels in grapes varied considerably between sites and seasons and like the observations made for tannin, Langhorne Creek (LC) displayed considerable site variation in anthocyanin concentration for all three seasons (Figure 2.5). Although there were some similarities in the pattern of anthocyanin accumulation within this site, such as areas where anthocyanins tended to be higher, the pattern of accumulation did not remain stable across seasons. Overall, the 2007/08 season had higher average anthocyanins across the site 2.52 mg/g berry. Like tannin accumulation, generally anthocyanins accumulated at greater concentrations towards the outskirts of the site decreasing towards the centre of the site, further supporting the proposition that there are similar environmental drivers for both anthocyanin and tannin. Langhorne Creek (LC) overall showed the highest anthocyanin average of the five sites (Figure 2.5).

Anthocyanin accumulation at Mildura (MIL) was highly variable, with differences across the site of up to 1.85 mg/g berry (2007/08) (Figure 2.2). The range of average anthocyanin concentrations for the Mildura (MIL) site remained similar between seasons, ranging from 1.22 mg/g berry (2005/06), 1.44 mg/g berry (2006/07) and 1.30 mg/g berry (2007/08) (Figure 2.5). Like tannin accumulation, anthocyanin accumulated at higher concentrations to the north-west of the site and declined towards the south-east of the site for all three seasons. This was consistent with more vigorous vines and higher soil water holding capacity towards the east of the site

Anthocyanin averages at Griffith (GRI) was generally low, ranging from 0.91 mg/g berry (2005/2006) and 1.27 to 1.29 mg/g berry (2006/07 and 2007/08). Accumulation of anthocyanins at Griffith (GRI) was also relatively uniform. In the final two seasons anthocyanins tended to accumulate at slightly higher concentrations towards the south-east of the site (Figure 2.2). Griffith (GRI) overall showed the lowest anthocyanin average of the five sites (Figure 2.5).

Like Griffith (GRI), Central Victoria (CV) had low anthocyanin averages across all seasons, ranging from 1.12 mg/g berry in 2005/06, 1.23 mg/g berry in 2006/07 and 1.44 mg/g berry in 2007/08, thus showing no stable accumulation pattern of anthocyanins between seasons (Figure 2.5).

The Great Southern (GS) site had relatively uniform average anthocyanins across the site for all three seasons. Anthocyanin averages ranged from 1.70 mg/g berry in 2005/06 and 1.81 mg/g berry in 2006/07 to 2.01 mg/g berry in 2007/08 (Figure 2.5). This pattern of increasing anthocyanin accumulation throughout the seasons was similar to the pattern of increasing anthocyanins observed in Central Victoria (CV).



Anthocyanin m/g fresh weight berry						
Site	2005/06		2006/07		2007/08	
	Average	STD	Average	STD	Average	STD
LC	2.33	0.24	2.03	0.29	2.52	0.21
MIL	1.29	0.29	1.44	0.40	1.30	0.40
GRI	0.91	0.14	1.27	0.16	1.29	0.14
CV	1.12	0.29	1.23	0.20	1.44	0.19
GS	1.70	0.26	1.81	0.17	2.01	0.28

STD– Standard Deviation

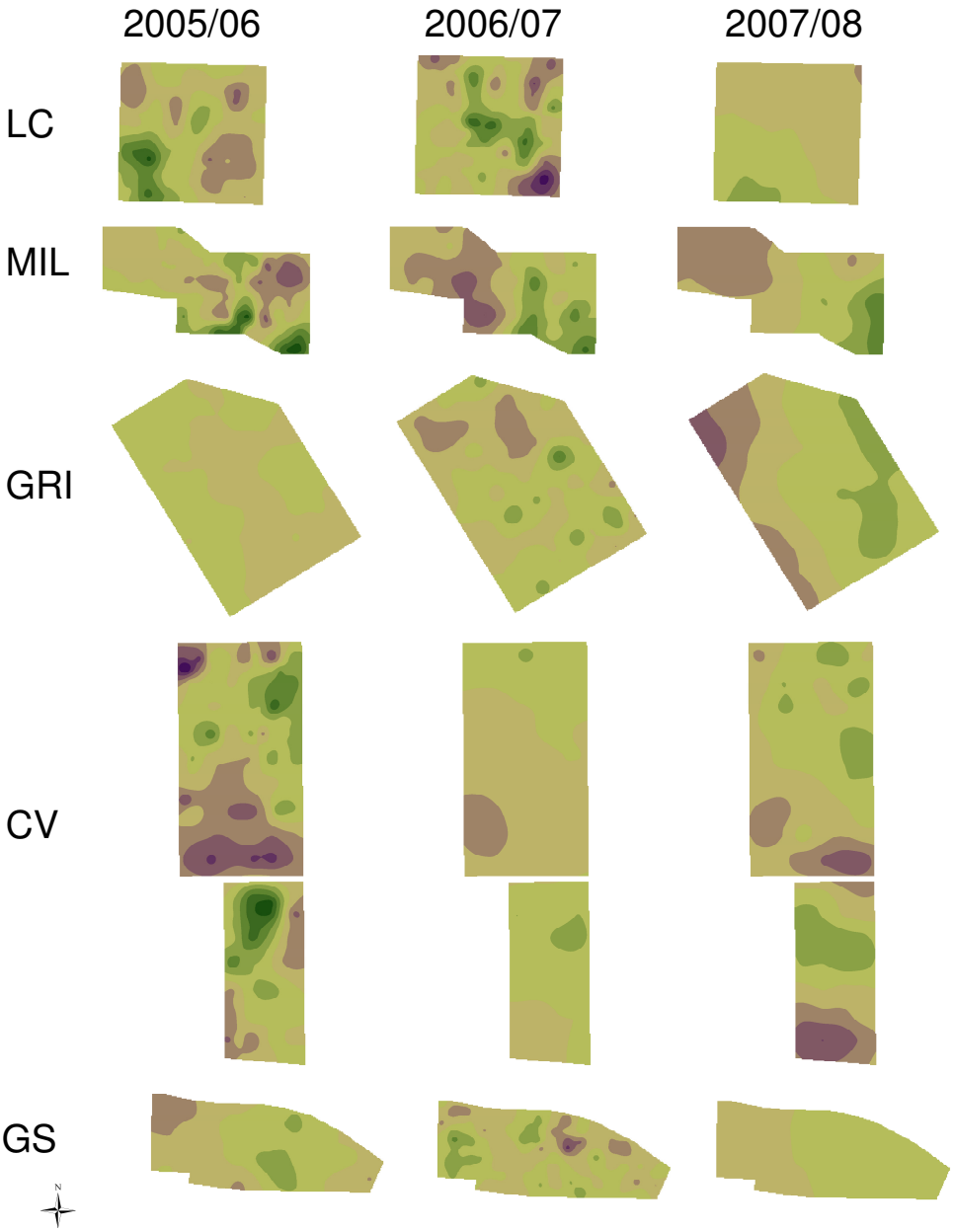
Figure 2.5: Relative spatial variation maps of normalised total anthocyanin concentration at Langhorne Creek (LC) Griffith (GRI), Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08 seasons. Grape samples for vine total anthocyanin were collected from 100 vines and expressed in mg/g berry before kriging was used to derive the plotted values.

Overall the 2007/08 season had the highest average anthocyanin concentrations apart from the Mildura (MIL) site. While the lowest average anthocyanin concentrations occurred in the 2005/06 season, the differences in observed average anthocyanin concentrations between regions changed from season-to-season (Figure 2.5).

2.2.3 Total soluble solids (TSS) variation

Average berry TSS varied considerably between sites and seasons. On average, TSS was the highest at Langhorne Creek (LC), with an average in 2007/08 recorded of 27.7°Brix (Figure 2.6). There was not one particular season which had the highest or lowest TSS for all five sites. However, in general terms, 2007/08 could be considered the season with the highest TSS and 2005/06 the season with the lowest TSS (Figure 2.6). The Mildura (MIL) and Central Victoria (CV) sites both had some of the lowest TSS averages at harvest (approximately 23° Brix) in 2007/08 (Figure 2.6).

For Griffith (GRI) where there was little or no variation in tannin and anthocyanin accumulation (Figure 2.1 and 2.2), there was also little to no variation in TSS no greater than 2.9°Brix for all three seasons (Figure 2.3). In contrast, Great Southern (GS) had very little variation in tannin or anthocyanin accumulation; however there was considerable variability in TSS, particularly for the first and final seasons (2005/06 and 2007/08) Central Victoria (CV) was the site with the greatest variation in TSS. This was most apparent in 2005/06 (17.3 to 29.3°Brix) (Figure 2.3).



Total soluble solids m/g fresh weight berry

Site	2005/06		2006/07		2007/08	
	Average	STD	Average	STD	Average	STD
LC	26.9	0.95	23.4	1.06	27.7	1.18
MIL	24.1	1.43	23.9	1.89	23.4	2.01
GRI	23.4	0.66	24.6	0.79	24.4	1.14
CV	23.1	2.58	24.7	1.40	23.7	1.11
GS	22.8	1.69	27.3	1.17	25.1	1.32

STD– Standard Deviation

Figure 2.6: Relative spatial variation maps of normalised total soluble solids at Langhorne Creek (LC) Griffith (GRI), Central Victoria (CV), Mildura (MIL) and Great Southern (GS) for the 2005/06, 2006/07 and 2007/08. Grape samples for vine total soluble solids were collected from 100 vines and expressed in Brixº before kriging was used to derive the plotted values.

2.3 Summary

Overall, Griffith (GRI) displayed the least variation across the site for tannin, anthocyanin and TSS accumulation (Figure 2.4). The variation in anthocyanin (Figure 2.5) for all five sites was similar to the variation observed for TSS (Figure 2.6). In seasons where TSS was high for a site, anthocyanins were also high. For instance Langhorne creek (LC) had higher TSS levels in 2005/06 and 2007/08, than in the 2006/07 season and anthocyanins were also higher in these two seasons. This relationship was also observed at Great Southern (GS). Like at Central Victoria (CV), Mildura (MIL) also had considerable within site variation for TSS for all three seasons (Figure 2.6) and in areas of the site where TSS was higher so were anthocyanins (Figure 2.5).

The relationship between tannin accumulation and TSS was not as strong as that between anthocyanin and TSS accumulation. On occasions where tannin concentration was high, TSS was low, such as at Langhorne Creek (LC) 2006/07. For this same site in the previous season (2005/06) TSS was high and tannins were low. However, at Mildura in both 2006/07 and 2007/08, tannins were highest where TSS and anthocyanins were highest.

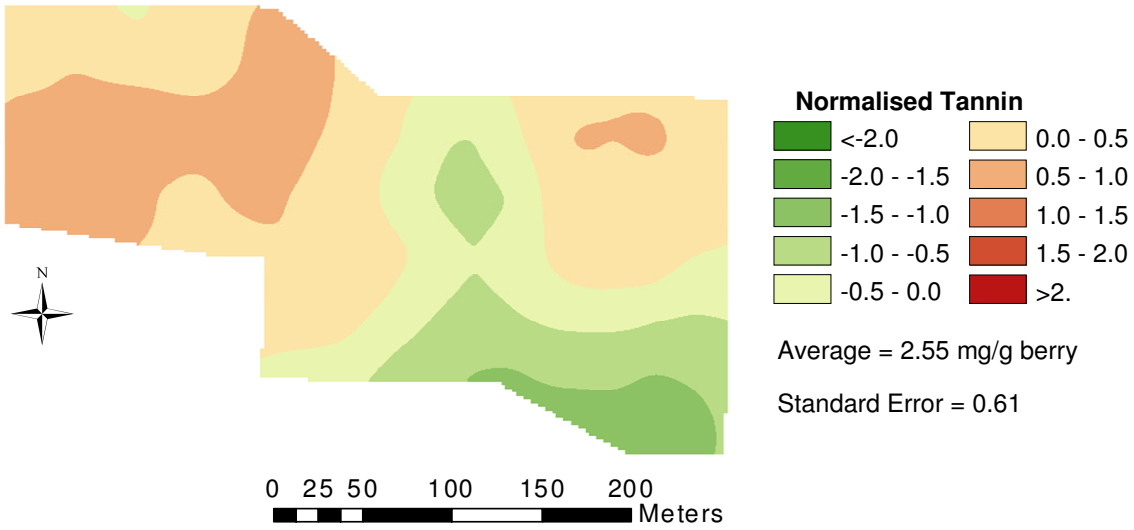


Figure 2.7: Relative spatial variation map of normalised total tannin concentration at Mildura (MIL) for the 2009/2010 season. Samples of grapes for vine total tannin were collected from 100 vines and expressed in mg/g berry before kriging was used to derive the plotted values.

As we know from previous research, there is a good relationship between TSS and anthocyanin but not between TSS and tannin. This is partly because anthocyanin and TSS accumulation are both ripening process as previously discussed by Downey *et al.* . Tannin biosynthesis is not a ripening process. Tannin biosynthesis occurs early in berry development (Downey *et al.* 2003a; Hanlin and Downey 2009); therefore any correlation with sugar is likely to reflect an underlying process that drives both parameters. In looking at all of the data collected for these sites, the major drivers appear to be vine vigour, soil water holding capacity and site topography, which was apparent at the Mildura (MIL) site. Based on this information, a drain was later installed at the western end of the Mildura site to remove excess water and reduce vine vigour. Subsequently, an additional valve was also installed at the western end of the block to enable an alteration to the irrigation scheduling for that part of he block. While the same volume was applied to this portion of the site, it was applied during two irrigation events of shorter duration with additional fertigation. As a consequence of these alterations, which were based on data from these trials, variability within the site was reduced (Figure 2.7).

Objective 3. To determine how changing phenolic composition impacts upon wine colour, wine sensory characteristics and wine score in Australia's major red varieties (e.g. Shiraz and Cabernet Sauvignon)

3.1 Variation in anthocyanin and tannin concentration in Shiraz fruit and the Impact on final wine.

3.1.1 Tannin and anthocyanin concentration of fruit at harvest for winemaking

Low Anthocyanin/Low Tannin (LA/LT) fruit was used to make Wine 1, and High Anthocyanin/Low Tannin (HA/LT) fruit to make wine 2, Fruit used for wines 1 and 2 were measured to have tannin concentrations of 1.56 and 1.54 mg/g fresh weight of whole berry respectively. Low Anthocyanin/High Tannin (LA/HT),fruit was used to make wine 3 and High Anthocyanin/High Tannin (HA/HT) fruit for wine 4. Grapes used to make wines 3 and 4 had tannin concentrations of 2.38 and 2.29 mg/g fresh weight of whole berry respectively (Figure 3.1 A).

The concentration of anthocyanins in fruit with low anthocyanin concentrations used for Wines 1 (LA/LT) and 3 (LA/HT) was 0.89 and 0.94 mg/g fresh weight of whole berry respectively. Whilst levels in the high anthocyanin fruit used for wines 2 (HA/LT) and 4 (HA/HT) were 1.62 and 1.67 mg/g fresh weight of whole berry (Figure 3.1 B).

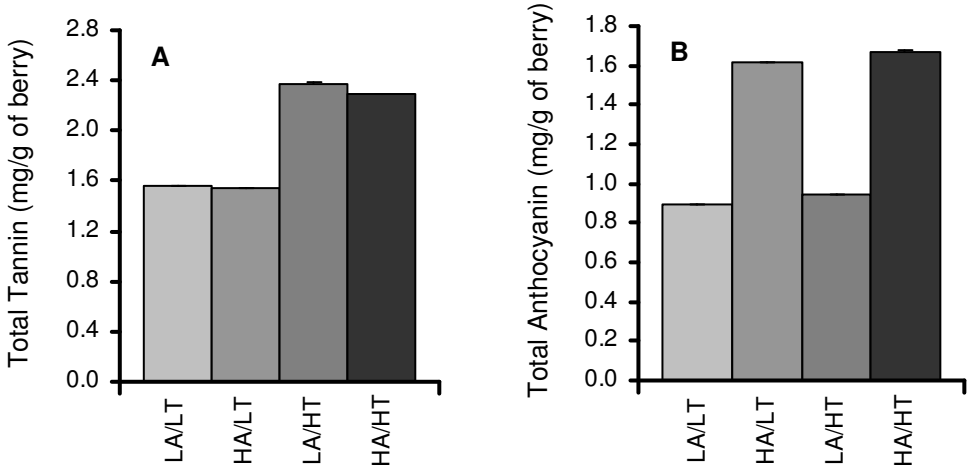


Figure 3.1: Grape phenolic measures at harvest. Shiraz from N.W. Victoria, 2009. **A** Total Tannin mg/g fresh weight of berry expressed as catechin equivalents. **B** Total Anthocyanins mg/g fresh weight of berry expressed as malvidin-3-*O*-glucoside equivalents.

3.1.2 Impact of fruit anthocyanin and tannin concentration on wine colour measures

Wine colour density describes the intensity of the colour of wine. Wines made from fruit containing low concentrations of anthocyanins, Wine 1 (LA/LT) and Wine 3 (LA/HT), had significantly lower wine colour density (WCD) than wines made from fruit containing high anthocyanin concentrations, Wine 2 (HA/LT) and Wine 4 (HA/HT) (Figure 3.2 A). Anthocyanins are the pigments that give red grapes and wine their colour (Ribéreau-Gayon 1982), thus the higher WCD observed in wines made from fruit containing greater anthocyanin concentrations could be reasonably expected, although this is not always the case. Thus, differences in WCD between wines made from fruit containing low anthocyanin (Wines 1 and 3) and high anthocyanin (Wines 2 and 4) concentrations were observed for all time points, although WCD did decrease over time for all wines (Figure 3.2 A). The decrease in WCD observed at bottling is most likely due to sulphur and acid adjustment.

Wine Colour Density is calculated by adding the absorbance of a wine at two wavelengths, 420nm (absorbance of brown pigments) and 520nm (absorbance of anthocyanins). The colour of young wines is due primarily to the anthocyanins in wine and very little due to pigmented polymer. During wine aging anthocyanins react with tannins and other wine components to form pigmented polymers (Somers 1971).

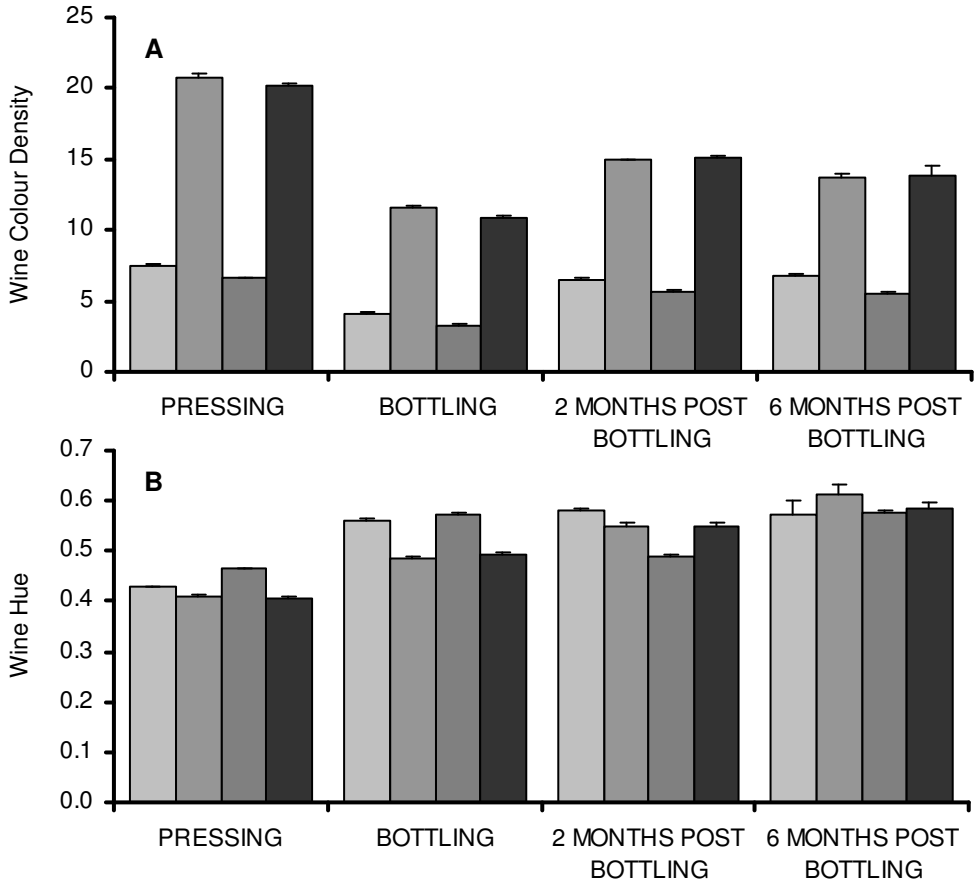


Figure 3.2: Wine colour measures at pressing, bottling, two months post-bottling, and six months post bottling of Shiraz from N.W. Victoria, 2009. **A** Wine Colour Density. **B** Wine Hue for wines: Wine 1 LA/LT □, Wine 2 HA/LT ▒, Wine 3 LA/HT ▒, and Wine 4 HA/HT ■

Wine hue is an indicator of the brownness of wine as opposed to colour intensity, or redness and is calculated by dividing the absorbance of wine at 420 nm by the absorbance at 520nm. As red wines age there is a shift from the bright red and intense colours observed in the young wine to a more red-brown hue in older wines (Liao *et al.* 1992; Somers and Verette 1988). This change in the hue of the wine is partly due to the formation of pigmented polymers (Ribéreau-Gayon 1982; Somers 1971). As the wines age there is a decrease in WCD due to the loss of free (monomeric) anthocyanins and therefore a decrease in the absorbance at 520nm. Free anthocyanins become bound to other compounds forming new pigments. During this process, oxidation of phenolics and other changes in phenolic composition also occur. Phenolic oxidisation produces brown pigments, which increase as the wine ages. These changes result in increased absorbance of wine at 420nm. So as wine ages the decrease in absorbance at 520nm in conjunction with the increase in absorbance at 420nm result in an increase in wine hue (Absorbance 420nm/Absorbance 520nm).

At pressing and bottling wine hue was observed to be significantly higher in wines made from fruit containing low concentrations of anthocyanins (Wines 1 and 3) than the wines made from fruit containing high anthocyanin concentrations (wines 2 and 4) (Figure 3.2 B). At six months post-bottling there was essentially no difference in wine hue between the four wines. The value for wine hue increased over time.

3.1.3 Impact of fruit anthocyanin and tannin concentration on wine tannin and anthocyanin concentration

Tannin concentration in wine decreased over time between pressing and six months post-bottling. Tannins in these wines were detected at the highest levels in wines made from the fruit with the highest levels of anthocyanins (Wine 2 and 4) (Figure 3.3 A). Fruit used to make Wine 2 (HA/LT) had low tannins, however the tannin concentration in this wine was no different to Wine 4 (HA/HT), which was made from fruit containing high concentrations of tannin. The similarity between these two wines being that they were both made from fruit containing a high concentration of anthocyanins. Similarly, wines made from fruit containing low anthocyanins (Wine 1 and 3) contained the lowest concentrations of wine tannin regardless of the fruit tannin concentrations. Unlike Wines 2 and 4, there was not a steady decrease in tannin concentration from pressing through to bottling in Wine 1 and Wine 3. From bottling onwards Wine 3 had no detectable tannins. The difference between these wines was the original tannin content of the fruit for Wine 3 having a higher tannin content.

Similar to the observations for WCD, the anthocyanin concentration was highest in wines made from fruit with high anthocyanin content (Wine 2 and 4) (Figure 3.3 B). Like WCD, anthocyanin concentration was highest for all wines at pressing and declined over time. This was likely due to free anthocyanins binding to other compounds, such as tannin, to form pigmented polymers or converted to other anthocyanin derivatives such as pyranoanthocyanidins (Mateus *et al.* 2006).

The wines made from the high anthocyanin fruit were highest in anthocyanins at pressing and bottling. The high anthocyanin/high tannin wine (Wine 4) had the highest anthocyanin concentration at two and six months post-bottling (Figure 3.3 B)

From this data it is clear that the main driver of wine anthocyanin concentration is fruit anthocyanin concentration, which is consistent with previous reports. While this seems logical, what is counter-intuitive is that fruit (and thus wine) anthocyanin content appears to be the main driver of wine tannin concentration. A series of experiments conducted by Singleton and Trousdale (1992) found that increasing anthocyanin concentration relative to tannin concentration in wine resulted in an increase in the formation of pigmented polymers. The data from this study also suggested that there is an optimum ratio between

anthocyanin and tannin concentration for the formation of pigmented polymers. Other authors have expressed similar ideas (Fulcrand *et al.* 2004) and a similar conclusion is suggested by the data presented here, although there are no data-sets that establish what the optimum ratio is.

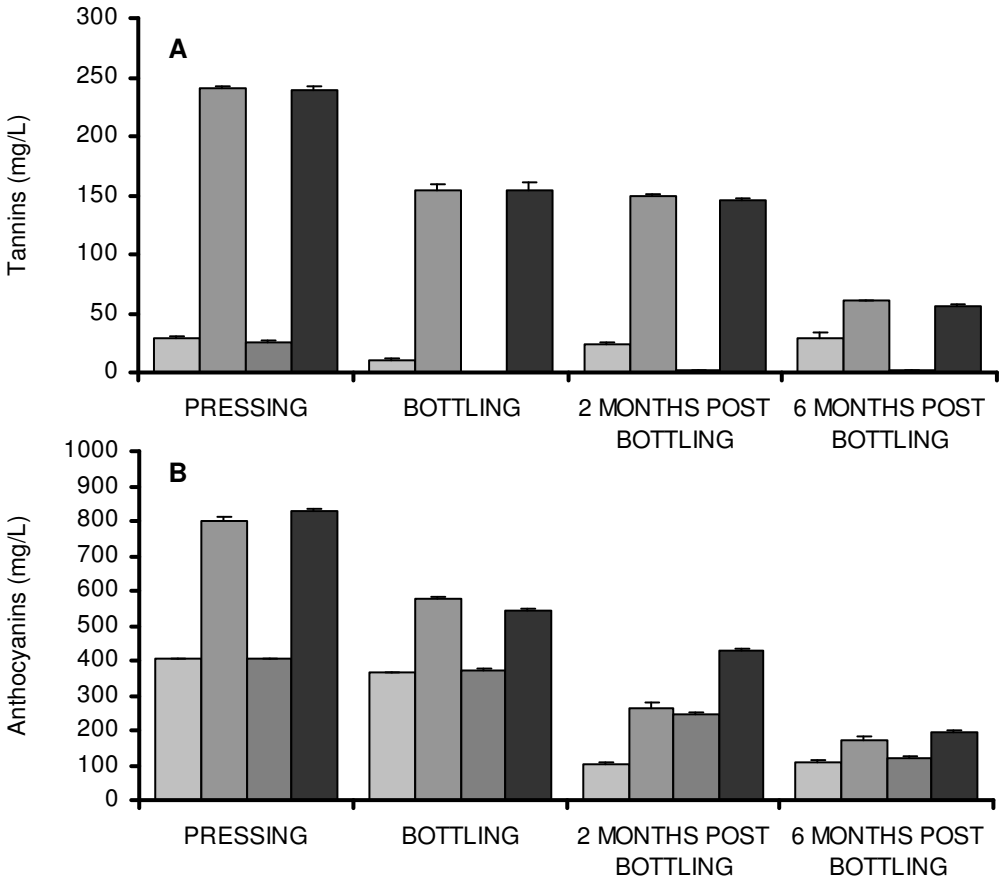


Figure 3.3: Wine phenolic concentrations at pressing, bottling, two months post-bottling and six months post-bottling of Shiraz from N.W. Victoria, 2009. **A** Total Tannin mg/L expressed as catechin equivalents. **B** Total Anthocyanins mg/L expressed as malvidin-3-*O*-glucoside equivalents for wines: Wine 1 LA/LT ☐, Wine 2 HA/LT ■, Wine 3 LA/HT ■, and Wine 4 HA/HT ■ .

3.1.4 Impact of fruit anthocyanin and tannin concentration on wine polymeric pigment measurements.

As red wines age it is the polymeric pigments which are predominantly responsible for the wine colour. As wines reach one year of age, polymeric pigments can be responsible for up to 50% of the wines colour and this can increase up to 90% in wines of 10 years of age (Somers 1971). For all wines made here, polymeric pigment concentration increased over time (Figure 3.4). As anthocyanin concentration decreased (Figure 3.3B) polymeric pigment concentration (Figure 3.4) and wine hue increased (Figure 3.2 B). Polymeric pigments were highest in wines with high anthocyanin concentration (Wine 2 and 4) with no difference between these wines. Wines made from fruit with low anthocyanin content,

and low anthocyanin concentration in the final wine (Wine 1 and 3), had significantly different total polymeric pigment concentrations after bottling. With Wine 3 having the lowest polymeric pigment level. Wine 3 also had a lower tannin concentration than Wine 1

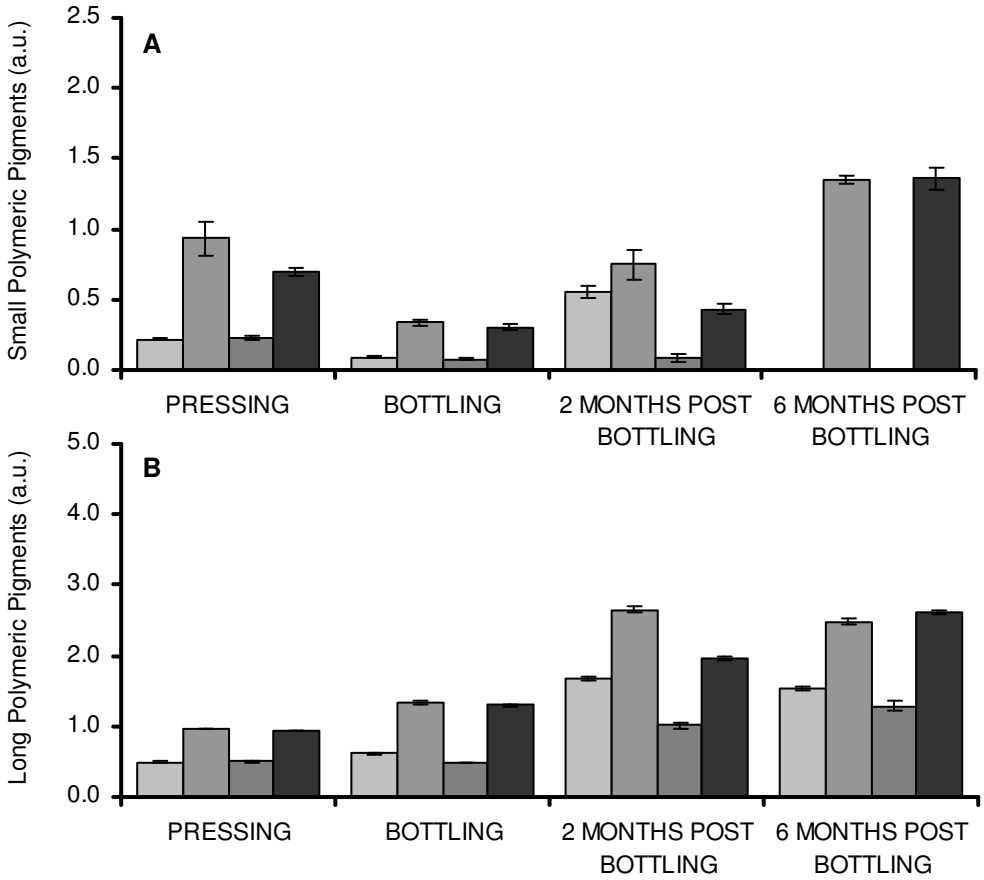


Figure 3.4: Wine polymeric pigment measures at pressing, bottling, two months post-bottling and six months post-bottling of Shiraz from N.W. Victoria, 2009. **A** Short Polymeric Pigments **B** Long Polymeric Pigments for wines: Wine 1 LA/LT , Wine 2 HA/LT , Wine 3 LA/HT , and Wine 4 HA/HT .

Whilst it is obvious that changing phenolic concentration in fruit has an impact on wine colour measures and the phenolic properties of the resultant wines, we are yet to establish the impact on the sensory properties of these wines. Sensory and final wine evaluation is scheduled to be conducted at 12 months post-bottling, which is beyond the life of the current project. The results of this investigation will be reported in the scientific literature.

3.2 Influence of bunch exposure on final wine phenolic and sensory parameters

Wines with different phenolic compositions were also produced from a series of experiments that examined the effect of bunch exposure on grape composition (GWRDC Project No. DNR 02/09) (Krstic and Downey 2005).

3.2.1 Impact of bunch exposure on tannin and anthocyanin concentration

In Cabernet Sauvignon, control fruit had the highest concentrations of skin tannin (5.11 mg/g of skin), followed by the exposed fruit (3.86 of mg/g skin), the shaded fruit (3.65 mg/g skin) and finally, the highly exposed fruit (3.48 mg/g skin) had the lowest concentrations of skin tannin (Figure 3.5 A). In Shiraz, the fruit from the exposed and the shaded treatments had similar concentrations of skin tannin, 5.06 mg/g of skin and 5.31 mg/g skin respectively. Lower concentrations of skin tannin were observed in the control (4.88 mg/g skin) and highly exposed (4.78 mg/g skin) treatments.

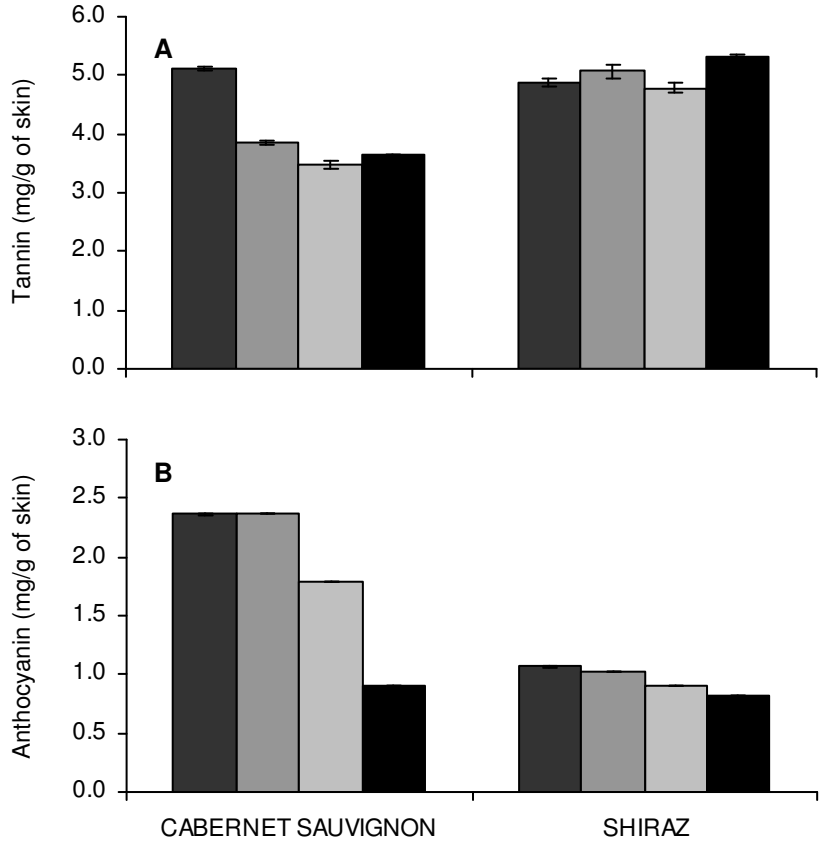


Figure 3.5: Grape phenolic measures at harvest of Cabernet Sauvignon and Shiraz fruit exposed to treatments to alter bunch exposure, Control ■ , Exposed ■ , Highly Exposed ■ , Shaded ■ . **A** Total Tannin mg/g fresh weight of skin expressed as catechin equivalents. **B** Total Anthocyanins mg/g fresh weight of skin expressed as malvidin-3-*O*-glucoside equivalents.

The effect of bunch exposure on anthocyanin accumulation was different for Cabernet Sauvignon and Shiraz. In Cabernet Sauvignon, there was no difference in anthocyanin concentration between the fruit from the control and exposed treatments (2.36 mg/g skin). The highly exposed treatment had significantly lower anthocyanin compared to the control and exposed treatment, with a concentration of 1.79 mg/g of skin. Shading Cabernet Sauvignon fruit caused a significant reduction in total anthocyanins, with a concentration of 0.91 mg/g of skin, a reduction of approximately 60% compared to the control fruit (Figure 3.5 B). Compared to Cabernet Sauvignon, anthocyanin concentration was much lower in Shiraz. Anthocyanin concentration for control and exposed fruit in Shiraz was very similar, 1.07 mg/g of skin and 1.02 mg/g of skin respectively. Slightly lower concentrations of anthocyanins were observed in the highly exposed (0.90 mg/g skin) and shaded fruit (0.81 mg/g skin).

3.2.2 Impact of bunch exposure on wine colour measures

Cabernet Sauvignon wines made with fruit from the exposure treatments had significantly lower Wine Colour Density (WCD) compared to wines made from the control fruit, which had a WCD of 12.75. If WCD is a reflection of the colour intensity of wine and that anthocyanins are responsible for this colour, the concentration of anthocyanins in the fruit should play a significant role in the WCD of the final wine. The fruit from the control and exposed treatment of Cabernet Sauvignon had the same anthocyanin concentration, yet their respective wines had significantly different WCD measurements, with lower values observed for the exposed wines. The wines made from the exposed and highly exposed treatment had similar WCD values at 10.80 and 10.68 respectively (Figure 3.6 A). However, the fruit anthocyanin concentration was significantly lower in the fruit from the highly exposed treatment (Figure 3.5 B). The wine made from the shaded treatment fruit had a significantly lower WCD of 5.43 compared to all other wines.

Shiraz wines made from the shaded fruit had significantly lower WCD in comparison to all other wines, with a wine colour density of 4.56 (Figure 3.6 A). There was no significant difference between wines made from the control, exposed and highly exposed fruit.

In Cabernet Sauvignon and Shiraz wines, Wine Hue was highest in the wine made from the shaded fruit (Figure 3.6 B). In Cabernet Sauvignon, Wine Hue in the wine from the highly exposed treatment was also significantly higher than that of the control wine. Wine Hue of the Shiraz, wine made from the highly exposed fruit was similar to that of the control. However, Shiraz wine made from the exposed fruit had significantly higher wine hue than that made from the control fruit.

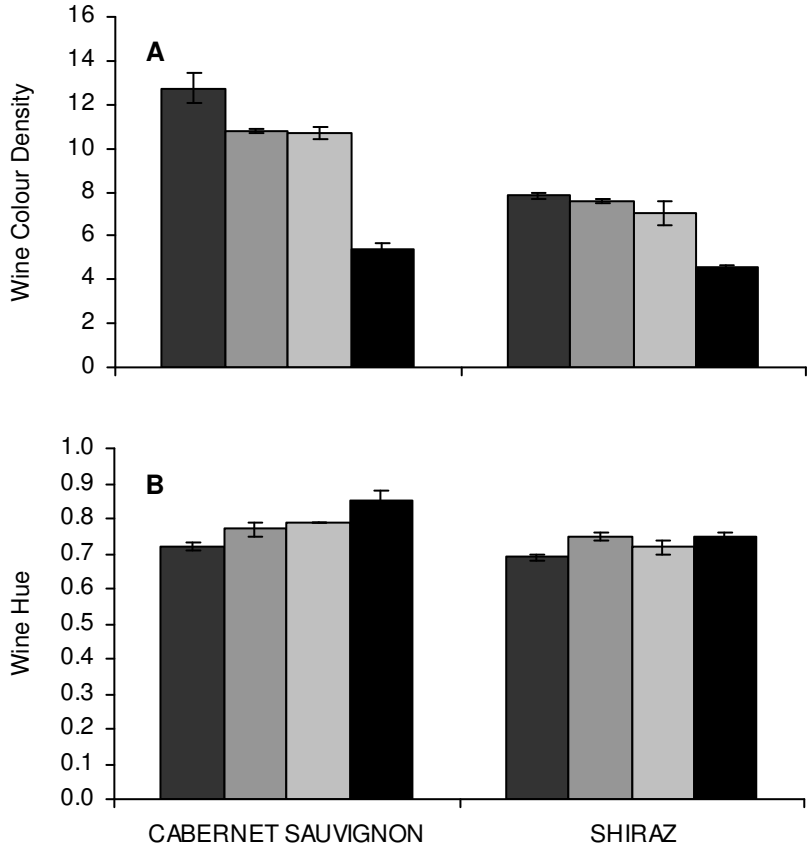


Figure 3.6: Wine colour measures at two years post-bottling of Cabernet Sauvignon and Shiraz wines made from fruit exposed to treatments to alter bunch exposure, Control , Exposed Highly Exposed Shaded **A** Wine Colour Density. **B** Wine Hue.

3.2.3 Impact of bunch exposure on wine tannin and anthocyanin concentration

The lowest concentrations of tannin in Cabernet Sauvignon wines were in the wine made from the shaded fruit (142.9 mg/L) with concentrations of almost half that observed in wine made from the control fruit (274.3 mg/L) (Figure 3.7 A). There was no difference between tannin concentration in wine made from control and highly exposed (246.6 mg/L) fruit. However, concentrations of skin tannins were significantly lower in the highly exposed fruit. Wine made from exposed fruit had tannin concentrations of 214.1 mg/L which was lower than that in the wines made from the control and highly exposed fruit. While little difference was observed in tannin concentration between the fruit from the exposed, highly exposed and shaded treatments, there were differences observed in the wine made from this fruit.

Tannin concentrations in Shiraz wines in response to altered bunch exposure was different to the observations made for Cabernet Sauvignon. In Shiraz wine, there was no significant difference in tannin concentration between wines made from the exposed (268.2 mg/L) and highly exposed (251.8 mg/L) fruit. Wines made from the exposed, highly exposed and shaded (197.1 mg/L) fruit had significantly lower tannin concentrations compared to the wine made from control fruit (339.6 mg/L). The

differences observed in tannin concentration between wines is in contrast to the small differences observed in the fruit. Shiraz wine made from the shaded treatment was 40% lower than that for the control treatment. However, the shaded treatment had higher skin tannin concentration (Figure 3.5 A).

In both the Cabernet Sauvignon and Shiraz wines made from the control, exposed and highly exposed fruit the concentration of anthocyanins (Figure 3.7 B) was similar. For Cabernet Sauvignon, the fruit from the highly exposed treatment had significantly lower fruit anthocyanin concentration compared to both the control and exposed treatments. However, this difference was not observed for wine anthocyanin concentration. For both cultivars, wine made from the fruit of the shaded treatment had significantly lower concentrations of anthocyanins in comparison to all other wines.

While the response of Cabernet Sauvignon and Shiraz to bunch exposure may be different in terms of fruit anthocyanin concentration (Figure 3.5 B) the difference in anthocyanin concentration was not observed in the wines made from the fruit.

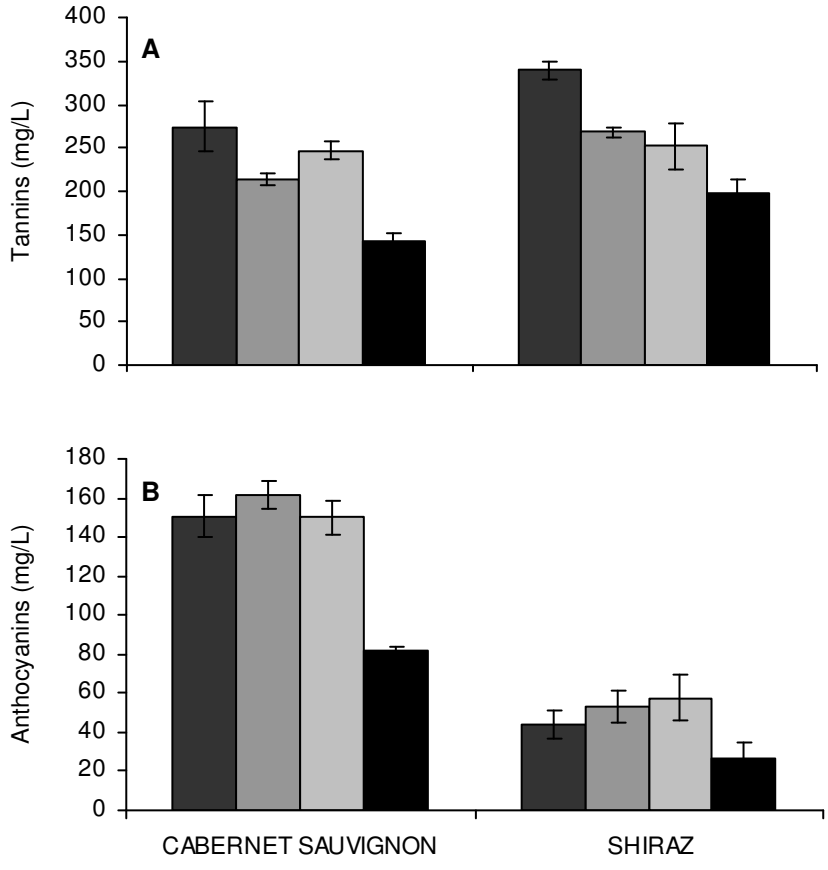


Figure 3.7: Wine phenolics concentrations at two years post-bottling of Cabernet Sauvignon and Shiraz wines made from fruit exposed to treatments to alter bunch exposure, Control ■, Exposed ■, Highly Exposed ■, Shaded ■. **A** Total Tannin mg/L expressed as catechin equivalents. **B** Total Anthocyanins mg/L expressed as malvidin-3-*O*-glucoside.

3.2.4 Impact of bunch exposure on wine polymeric pigments

Unlike the effect of bunch exposure on total anthocyanins and total phenolics, substantial differences in the level of polymeric pigments were observed in the Cabernet Sauvignon and Shiraz wines. The difference in polymeric pigment concentration between bunch exposure treatments resembled the difference between treatments observed for anthocyanin concentration in the fruit (Figure 3.5B). Short polymeric pigments (SPP) and long polymeric pigments (LPP) in Cabernet Sauvignon wine made from the control fruit were significantly higher than in wines made from fruit from the exposure treatments (Figure3.8). There was no significant difference in polymeric pigment levels between the exposed and highly exposed wine. The wine made from shaded treatment fruit had polymeric pigment levels significantly lower than all other wines and more than 50% lower than those observed in wine made from control fruit.

In contrast, altering bunch exposure did not seem to have a major impact on polymeric pigments in wines made from Shiraz fruit. Short polymeric pigments in the wine made from shaded fruit had significantly lower levels than the wines made from the other exposure treatments (Figure 3.8 A). There was no significant difference in long polymeric pigment content between Shiraz wines made from the different exposure treatments (Figure 3.8 B).

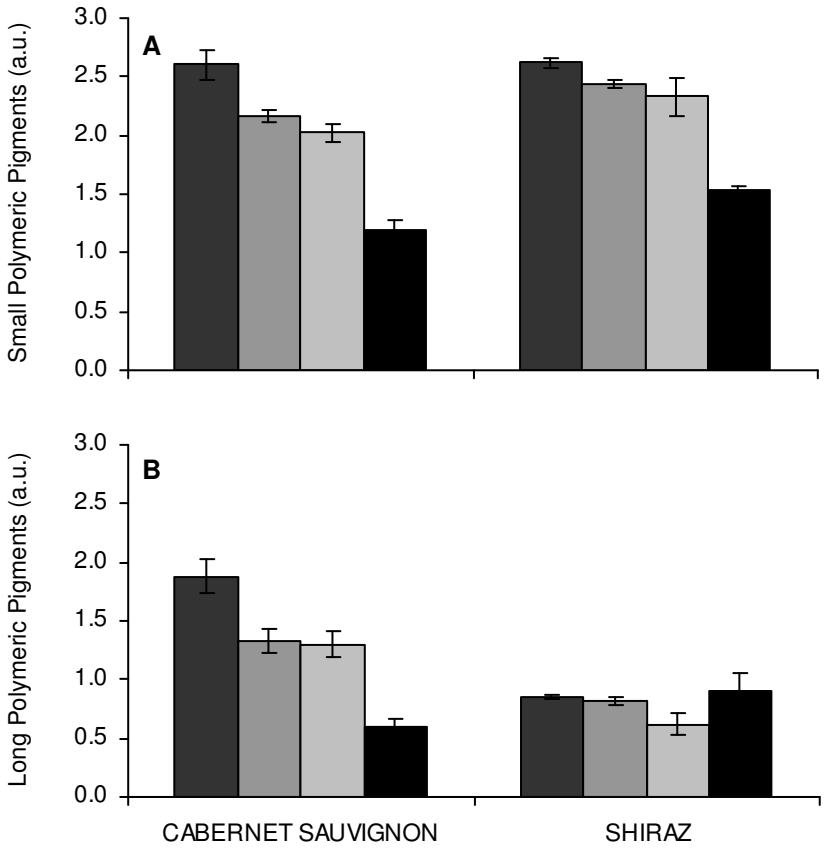


Figure 3.8: Polymeric pigment measures at two years post-bottling of Shiraz and Cabernet Sauvignon wines made from fruit exposed to treatments to alter bunch exposure, Control ■ , Exposed ■, Highly Exposed ■, Shaded ■. **A** Short Polymeric Pigments **B** Long Polymeric Pigments.

3.2.5 Descriptive analysis of wines made from Cabernet Sauvignon and Shiraz fruit with altered bunch exposure.

Descriptive analysis was conducted on these Cabernet Sauvignon and Shiraz wines to determine the sensory impact of altered bunch exposure . Generally, perceived astringency was lower in wines made from shaded fruit although the reverse was generally not observed in wines made from the exposed and highly exposed fruit.

Descriptive analysis by Joscelyne *et al*. (2007), also showed wines from shaded fruit were different from other exposure treatments for a number of flavour and aroma characters. Alcohol was significantly lower in the wines made from the highly exposed and shaded fruit, reflecting lower harvest sugar levels, likely due to extensive leaf removal and therefore reduced photosynthetic capacity (Joscelyne *et al.* 2007).

Cabernet Sauvignon wine made from the shaded treatment fruit was significantly different from wine made from all other bunch exposure treatments for 10 of the 20 attributes that were assessed. These included colour, depth, plum and black cherry aroma, spice and blackberry flavour, bitterness, body, astringency and perceived tannin (Joscelyne *et al.* 2007). Shaded Cabernet Sauvignon wine was more garnet than ruby in hue and had lower colour intensity. The wine was also perceived as lower in tannin, astringency, body, bitterness, blackberry aroma, black cherry aroma, spice flavour and blackberry flavour but higher in plum aroma. Spirity aroma, which is related to alcohol content, was lower in the wine made from shaded fruit. Cabernet Sauvignon wine made from the exposed treatment had a lower level of blackberry aroma than the wine made from the highly exposed fruit (Joscelyne *et al.* 2007).

In Shiraz wines, 7 of the 20 attributes were different in the wines made from shaded fruit compared to those made from fruit from the other exposure treatments. The wines made from shaded fruit were significantly different from all other wines for colour, body, depth, length, dark fruit flavour, astringency and sourness. Wines made from highly exposed fruit were significantly more sour than the control (Joscelyne *et al.* 2007).

Cabernet Sauvignon wines made from shaded fruit were more ruby than violet, had lower colour intensity, lighter body, softer astringency, shorter length and less intense dark fruit flavour, they were also more sour than the control. That there were limited differences between the wines made from control and exposed and highly exposed fruit initially suggests that there is a limited impact of increasing sun exposure on the sensory characters of Shiraz and Cabernet Sauvignon wines (Joscelyne *et al.* 2007).

Certainly for the wines examined here, this appears to be the case However,caution is advised in implicating this data as a general response to bunch exposure with respect to flavour and aroma biosynthesis without considering the location where these trials were undertaken. In the Sunraysia region of northwest Victoria, where these trials were conducted it is unlikely that light was a limiting factor in flavour aroma biosynthesis in the control fruit. It is unsurprising then that there was little difference observed between the wines made from the control and exposed treatment fruit. Slight differences in wine made from the highly exposed treatment fruit would be less likely due to a changed light environment than to leaf removal. Thus, the most important comparison is between the shaded and highly exposed fruit, which both had leaves removed.

Proper sensory evaluation of wines and fruit is a costly and time-consuming process. There are also significant challenges in establishing and maintaining a well-trained sensory panel. In considering these challenges, the project team in discussion with its collaborators, explored a number of alternate approaches. One of these is the application of NMR-based metabolomics to the analysis of the wines and the sensory data described above.

Certain mouth-feel parameters identified from sensory analysis can be strongly correlated to NMR-based metabolomic analysis of wine (Rochfort *et al.* 2010). Metabolomic analysis enabled classification of the wines based on grape variety and bunch exposure treatment in a manner similar to that achieved by sensory analysis. The NMR analysis was rapid, inexpensive and allowed the simultaneous assessment of multiple metabolites contributing to wine quality. Metabolomic analysis of wine may offer a more affordable approach to routine assessment of sensory characters in wine (Rochfort *et al.* 2010). This is an area that warrants further investigation and development of a robust methodology for evaluating a range of important sensory characters for the wine industry.

Objective 4 To determine the major viticultural management practices (crop load, bunch exposure, variety/rootstock/clone, irrigation management and pest and disease pressure) that effect tannin, flavonol and anthocyanin levels in the major Australian winegrape varieties (e.g. Shiraz, Chardonnay, Cabernet Sauvignon and Merlot).

4.1 Influence of bunch exposure on tannin, anthocyanin and flavonol levels

4.1.1 Influence of bunch exposure on tannin content

Changes in tannin synthesis in response to altered bunch exposure varied between cultivars and seasons. For Cabernet Sauvignon, increasing bunch exposure through the

implementation of a lifting wire (exposed) and lifting wire in conjunction with extensive leaf removal (highly exposed) resulted in reduced tannin accumulation for the first two seasons (2002/03 and 2003/04) (Figure 4.1). However, in the final season, 2004/05, the exposed fruit had tannin concentrations greater than the control. The response of Cabernet Sauvignon to the absence of light also varied between seasons where in 2002/03 and 2004/05 an increase in tannin was observed and in 2003/04 there was a significant reduction in tannin concentration due to light exclusion. In Shiraz, altering bunch exposure caused very little or no difference in tannin concentration in comparison to the control with the exception of the highly exposed treatment in the first season of this trial (2002/03); this was consistent with previous reports (Downey *et al.* 2004b). Pinot Noir 114 and Pinot Noir 115 both recorded decreased tannin concentration in response to light exclusion with the reduction in tannin greater in the latter clone. The application of the light exclusion treatment in Merlot (2002/03 and 2003/04) resulted in a decrease in tannin concentration, while in Petit Verdot a significant increase was observed.

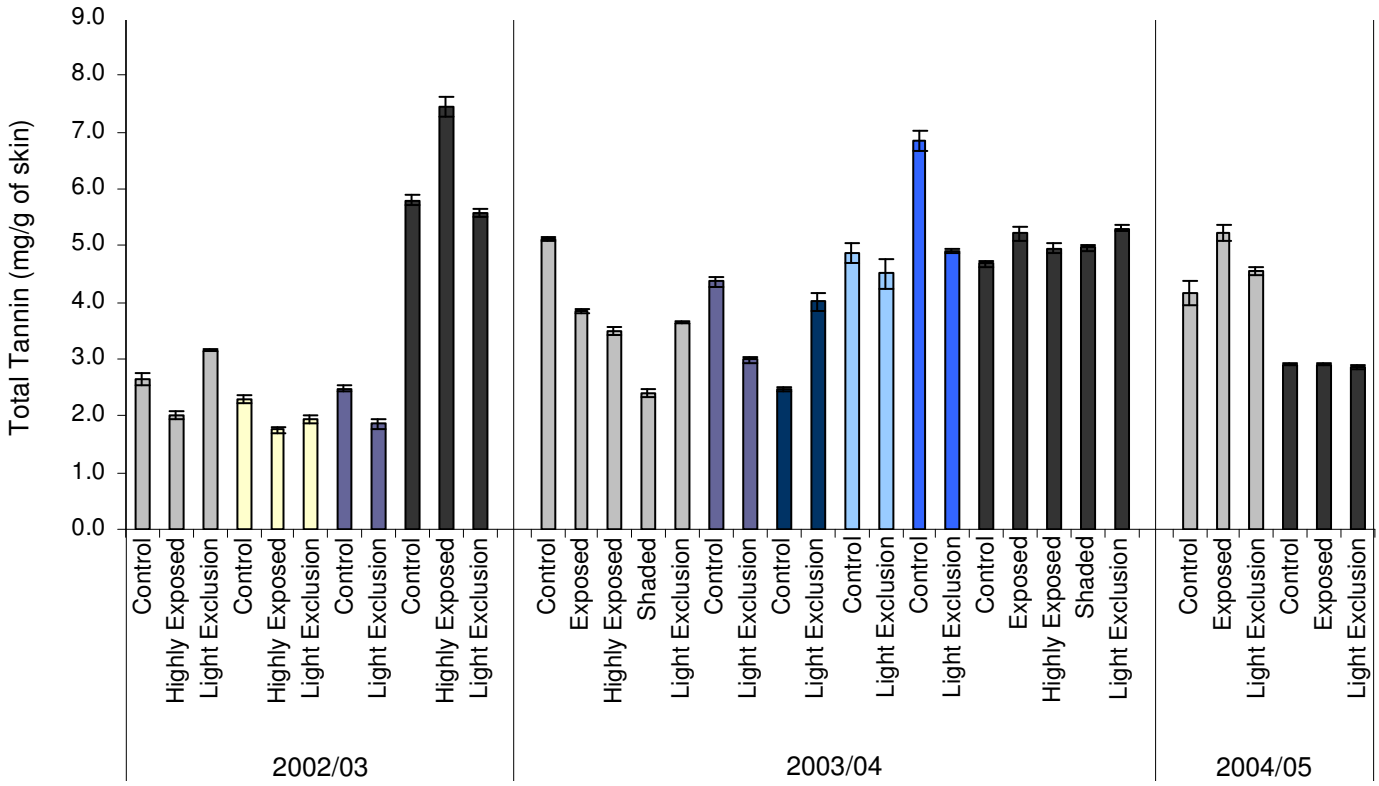


Figure 4.1: Total Tannin mg/g fresh weight of skin expressed as catechin equivalents for seven cultivars (Cabernet Sauvignon , Chardonnay , Merlot , Shiraz , Pinot Noir 114 , Pinot Noir 115 , and Petit Verdot) for standard commercial practice (Control) and in response to altered bunch exposure (Exposed, Highly Exposed, Shaded, and Light Exclusion) over 3 growing seasons (2002/03, 2003/04, and 2004/05).

These results indicate that the effect of altering bunch exposure on tannin concentration varies between cultivars and between seasons for the same cultivar. This suggests that

while bunch exposure my play a role in tannin biosynthesis there are other drivers, such as seasonal effects (climate) that can may have a greater influence than bunch exposure treatments tested here.

4.1.2 Influence of bunch exposure on anthocyanin and flavonol content

Similar to the observations made for tannin accumulation in response to changes in bunch exposure, anthocyanin concentrations varied between cultivars and within the same cultivar between seasons. Generally, light exclusion resulted in a significant decrease in the concentration of anthocyanins with the exception of Shiraz in 2002/03, Pinot Noir 114 in 2003/04 and Cabernet Sauvignon in 2004/05 (Figure 4.2). The highly exposed treatment also resulted in decreased anthocyanin concentration compared to the control with the exception of Shiraz in 2002/03, where anthocyanins were higher. Shading resulted in lower anthocyanins for both Cabernet Sauvignon and Shiraz (2003/04), however the reduction in anthocyanins was greater in Cabernet Sauvignon. For all seasons, Cabernet Sauvignon had greater changes in anthocyanin concentration in response to altered bunch exposure in comparison to Shiraz. The decrease in either tannins or anthocyanins in the Highly Exposed and Shaded treatments may not be a direct effect of light; both of these treatments included extensive leaf removal. Thus, decreases in metabolites could be the result of a decrease in photosynthate accumulation.

For some cultivars the response of tannin and anthocyanin synthesis to altered bunch exposure was similar. In Shiraz, particularly in 2002/03 and 2004/05 the changes in tannin and the anthocyanin concentration in response to changes to bunch exposure were the same, where an increase or decrease in tannin concentration was observed an increase or decrease in anthocyanin concentration was also observed. This would indicate that tannins and anthocyanins share similar drivers for synthesis. Since there isn't a consistent response to light, we would have to conclude that light, or bunch exposure is not the major driver of tannin or anthocyanin biosynthesis. Other authors have concluded that temperature may be a greater driver than light (Cohen *et al.* 2008; Downey *et al.* 2006; Spayd *et al.* 2002) and that would be consistent with changes from season-to-season observed here.

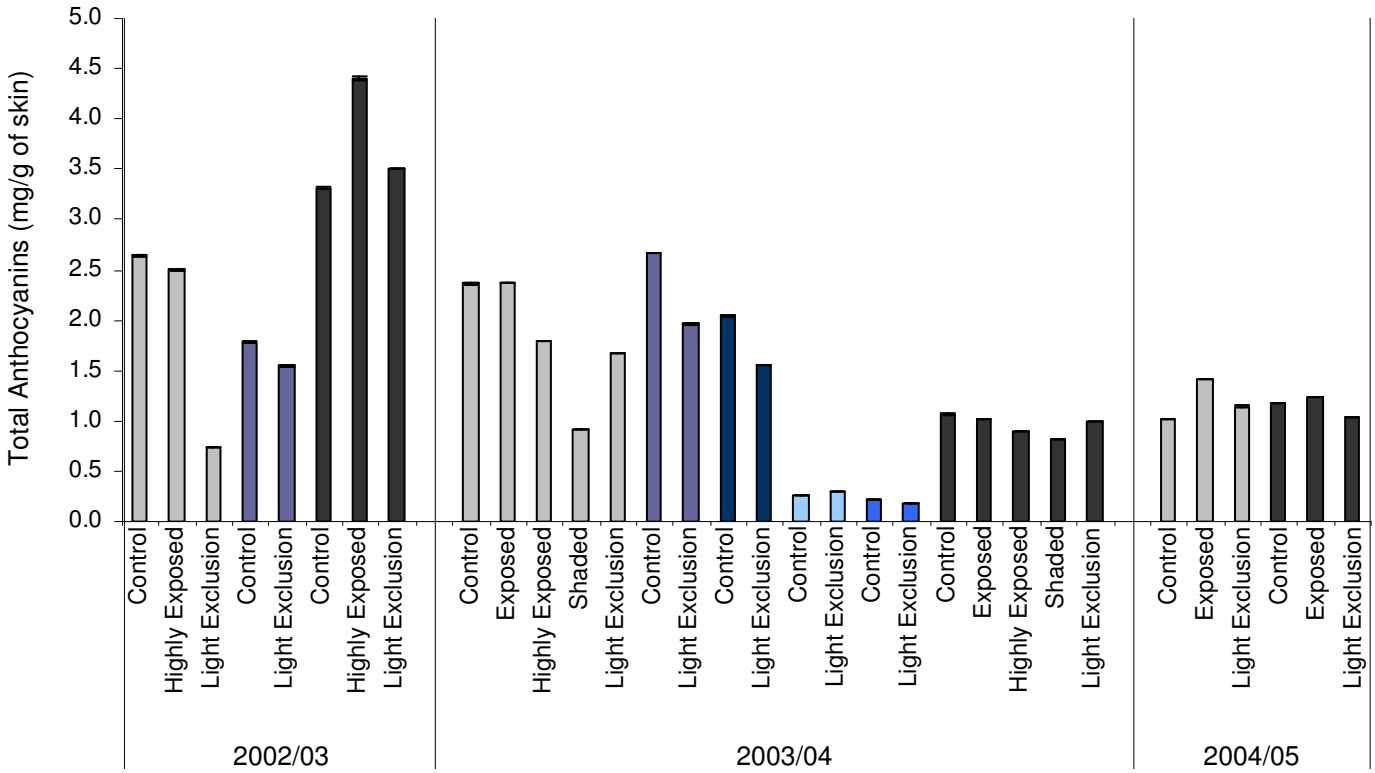


Figure 4.2: Total anthocyanins mg/g fresh weight of skin expressed as malvidin-3-glucoside equivalents for seven cultivars (Cabernet Sauvignon , Merlot , Shiraz , Pinot Noir 114 , Pinot Noir 115 , and Petit Verdot) for standard commercial practice (Control) and in response to altered bunch exposure (Exposed, Highly Exposed, Shaded, and Light Exclusion) over 3 growing seasons (2002/03, 2003/04, and 2004/05).

Unlike anthocyanins and tannins, the response of flavonol accumulation to altered bunch exposures was relatively consistent between treatments for all cultivars, and between seasons. In comparison to the Control, light exclusion resulted in decreased total flavonol concentration in all varieties for all seasons (Figure 4.3). Flavonol concentration varied between seasons, however for all three seasons Shiraz control fruit, had higher flavonol content than Cabernet Sauvignon control fruit. Previous research has indicated that flavonol biosynthesis is light dependent (Downey *et al.* 2003b; 2004c) and this research supports that conclusion. There was a significant reduction in the synthesis of flavonols in the absence of light. That there flavonols present in the light excluded fruit reflects timing of the application of the light excluding boxes. They were applied at fruit-set so the inflorescences and developing berries would have had some light exposure during their development.

In 2002/03 and 2003/04, fruit from the highly exposed treatment had significantly higher total flavonol content than control fruit. When the exposed treatment was applied to Cabernet Sauvignon and Shiraz in 2003/04 and 2004/05 it resulted in increased flavonol concentration in Cabernet Sauvignon and either no significant difference (2003/04) or decreased (2004/05) flavonol concentration in Shiraz. This is a strong indication that increased bunch exposure stimulated the biosynthesis of flavonols and that reducing bunch exposure results in a decrease in the accumulation of flavonols. This result is consistent with previous research findings (Downey *et al.* 2003b; 2004c).

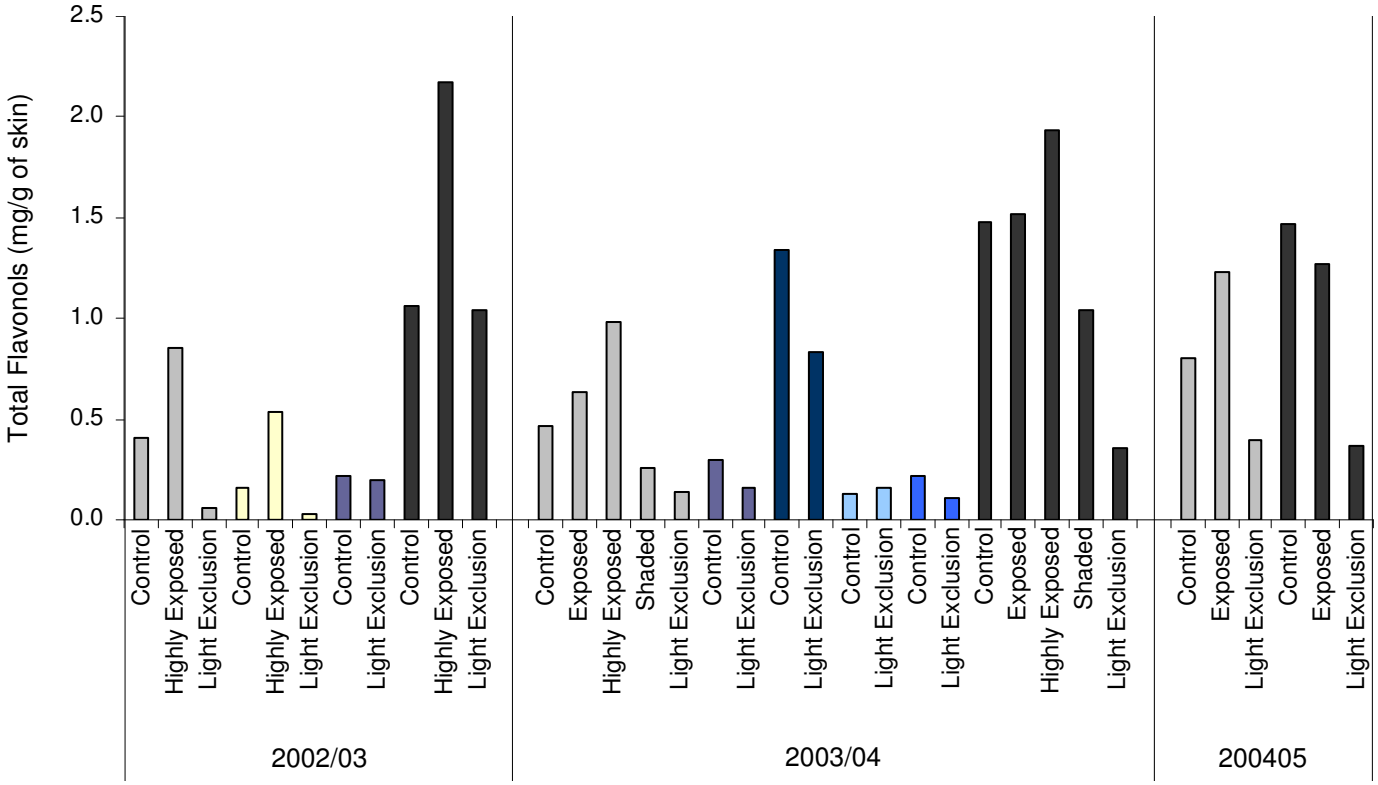


Figure 4.3: Total Flavonols mg/g fresh weight of skin expressed as quercetin-3-glucoside equivalents for seven cultivars (Cabernet Sauvignon , Chardonnay , Merlot , Shiraz , Pinot Noir 114 , Pinot Noir 115 , and Petit Verdot) for standard commercial practice (Control) and in response to altered bunch exposure (Exposed, Highly Exposed, Shaded, and Light Exclusion) over 3 growing seasons (2002/03, 2003/04, and 2004/05).

4.2 Influence of rootstock on tannin, anthocyanin and flavonol levels

4.2.1 Influence of rootstock on tannin content and composition

Tannins were analysed in the skin of Shiraz grapes grown on nine different rootstocks over two seasons. Tannin concentration was significantly higher in 2009 than in the 2008 season for all rootstocks (Figure 4.4). In 2008, total tannin ranged from 3.39 mg/g of skin (Freedom) to 5.90 mg/g skin (MS1010), with a mean of 4.64 mg/g of skin (%CV = 20.8). In 2009, total tannin ranged from 5.73 mg/g skin (1103 Paulsen) to 16.72 mg/g skin (MG5512) with a much higher mean of 9.18 mg/g of skin (%CV = 34.2) compared to the previous season.

There was a strong seasonal effect on tannin concentration in grape skin as we have reported elsewhere in this report. This seasonal effect was generally greater that the effect of rootstock. However, the values are not the same or similar between rootstocks, which indicates some rootstock influence on tannin accumulation. This influence is not consistent suggesting a rootstock by season interaction or the impact of other factors that have not been examined here.

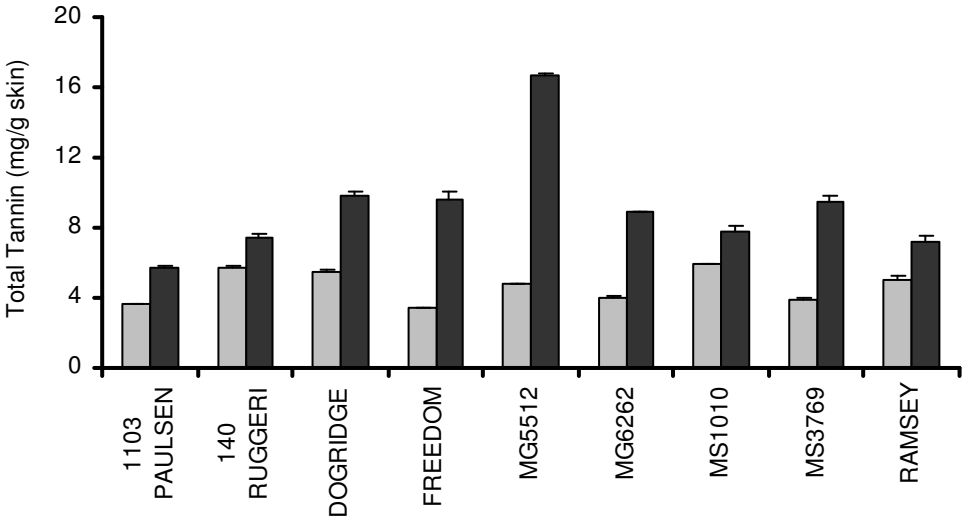


Figure 4.4: Tannin mg/g fresh weight of skin expressed as catechin equivalents for Shiraz on 9 Rootstocks for the 2008 □ and 2009 ■ growing seasons.

Condensed tannin polymer subunit composition was determined by HPLC following acid-catalysed depolymerisation. The most commonly occurring terminal subunit in all nine rootstocks was catechin for both seasons (Figure 4.5 A). In 2008, the percentage of catechin terminal subunits ranged from 51.0% of terminal subunits in MG6262 to 68.8% of terminal subunits in MS3769, with the mean proportion of catechin being 61.2% (%CV = 8.4). In 2009, the percentage of catechin terminal subunits was slightly higher ranging from 59.6% of terminal subunits in Ramsey to 70.2% of terminal subunits in MG5512, with the mean proportion catechin being 63.3% (%CV = 5.6).

The second most common occurring terminal subunit for all nine rootstocks was epicatechin (Figure 4.5 A). In 2008, the percentage of epicatechin terminal subunits ranged from 28.0% of terminal subunits in MS3769 to 45.2% of terminal subunits in MG6262, with the mean proportion of epicatechin being 35.1% (%CV = 13.8). In 2009, the percentage of epicatechin terminal subunits was lower, ranging from 25.9% of terminal subunits in MG5512 to 34.8% of terminal subunits in Ramsey, with the mean proportion epicatechin being 31.3% (%CV = 10.2).

The least common terminal subunit for all rootstocks was epicatechin-gallate (Figure 4.5 A). In 2008, the percentage of epicatechin-gallate terminal subunits in samples where epicatechin-gallate was detected, ranged from 2.5% of terminal subunits in 140 Ruggeri to 5.7% of terminal subunits in MG5512, with the mean proportion of epicatechin-gallate being 3.8% (%CV = 49.1). Epicatechin-gallate as a terminal subunit was not detected in Shiraz on 1103 Paulsen rootstock in 2007/08. In 2008/09, the percentage of epicatechin-gallate terminal subunits was higher, ranging from 3.8% of terminal subunits in Freedom to 5.4% of terminal subunits in MS3769, with the mean proportion epicatechin-gallate being 5.4% (%CV = 23.7).

Of the extension subunits, the most commonly occurring subunit was epicatechin (Figure 4.5 B), with the exception of Freedom in 2009 where epicatechin was the second most prevalent extension subunit. In 2008, the percentage of epicatechin extension subunits ranged from 51.7% of extension subunits in MS1010 to 59.4% of extension subunits in 140 Ruggeri, with the mean proportion of epicatechin being 55.1% (%CV = 4.7). In 2009, the percentage of epicatechin extension subunits was slightly lower ranging from 44.1% of extension subunits in Freedom to 51.4% of extension subunits in Ramsey, with the mean proportion epicatechin being 48.4% (%CV = 5.9).

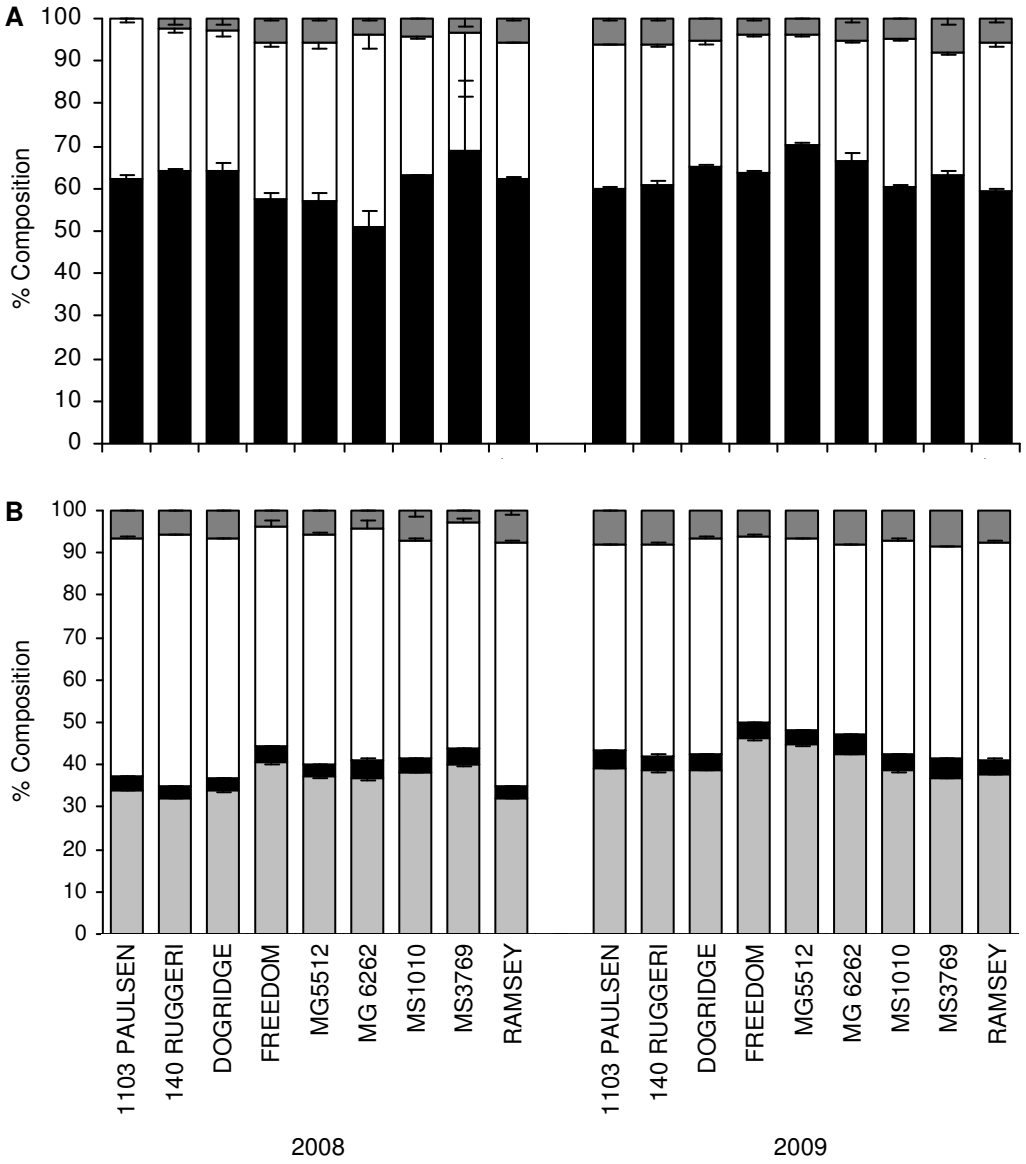


Figure 4.5: Proportional subunit composition of Shiraz grown on nine rootstocks **A** Terminal Subunits **B** Extension Subunits(Epigallocatechin □, Catechin ■, Epicatechin □, Epicatechin-gallate ■) for the 2008 and 2009 growing seasons.

The second most commonly occurring extension subunit was epigallocatechin (Figure 4.5 B), with the exception of Freedom in 2009 where epigallocatechin was the most common extension subunit. In 2008, the percentage of epigallocatechin extension subunits ranged from 32.2% of extension subunits in Ramsey to 40.6% of extension subunits in Freedom, with the mean proportion of epigallocatechin being 36.1% (%CV = 8.8). In 2009, the percentage of epigallocatechin extension subunits was higher, ranging from 37.0% of extension subunits in MS3769 to 46.1% of extension subunits in Freedom, with the mean proportion epigallocatechin being 40.3% (%CV = 8.1).

The next most common extension subunit was epicatechin-gallate (Figure 4.5 B), with the exception of MG6262 and MS3769 in 2008 where epicatechin-gallate was the least common extension subunit. In 2008, the percentage of epicatechin-gallate extension subunits ranged from 2.6% of terminal subunits in MS3769 to 7.5% of extension subunits in Ramsey, with the mean proportion of epicatechin-gallate being 5.5% (%CV = 29.8). In 2009, the percentage of epicatechin-gallate extension subunits was higher, ranging from 6.0% of extension subunits in Freedom to 8.7% of extension subunits in MS3769, with the mean proportion epicatechin-gallate being 7.4% (%CV = 12.5).

The least common extension subunit was catechin, with the exception of MG6262 and MS3769 in 2008 where the least common extension subunit was epicatechin-gallate (Figure 4.5 B). In 2008, the percentage of catechin extension subunits ranged from 2.7% of extension subunits in MG5512 to 4.5% of terminal subunits in MG6262, with the mean proportion of catechin being 3.3% (%CV = 18.1). In 2009, the percentage of catechin extension subunits ranged from 3.6% of extension subunits in Ramsey, 140 Ruggeri and MG5512 to 8.7% of extension subunits in MS3769, with the mean proportion catechin being 3.9% (%CV = 9.9).

These observations for rootstocks are consistent with our observations for tannins generally. While catechin is usually the most common terminal subunit and epicatechin the next most common terminal subunit, this is not always the case. Here we observed that in Freedom in 2008/09 epigallocatechin was the most common extension subunit not epicatechin. Consistent with other work, there were also examples of some subunits not being detected. But this was also not consistent from season-to-season and while absent as a terminal subunit, for example epicatechin-gallate in Shiraz grown on 1103 Paulsen in 2007/08, was present as an extension subunit in the same sample.

4.2.2 Influence of rootstock on anthocyanin content and composition

Unlike observations for tannin concentration there was not one season that had overall higher or lower anthocyanins for the nine rootstocks investigated (Figure 4.6). In 2008, total anthocyanins ranged from 2.03 mg/g of skin (140 Ruggeri) to 7.72 mg/g skin

(MS3769), with a mean of 5.06 mg/g of skin (%CV = 37.7). In 2008/09 total anthocyanin concentration ranged from 2.21 mg/g skin (1103 Paulsen) to 7.12 mg/g skin (MG5512) with a mean of 4.67 mg/g of skin (%CV = 28.9) Coincidentally, the rootstocks with the highest anthocyanin content in the 2009 season also had the highest tannin content and the rootstock with the lowest anthocyanin concentration also had the lowest tannin concentration suggesting a seasonal impact on overall flavonoid biosynthesis.

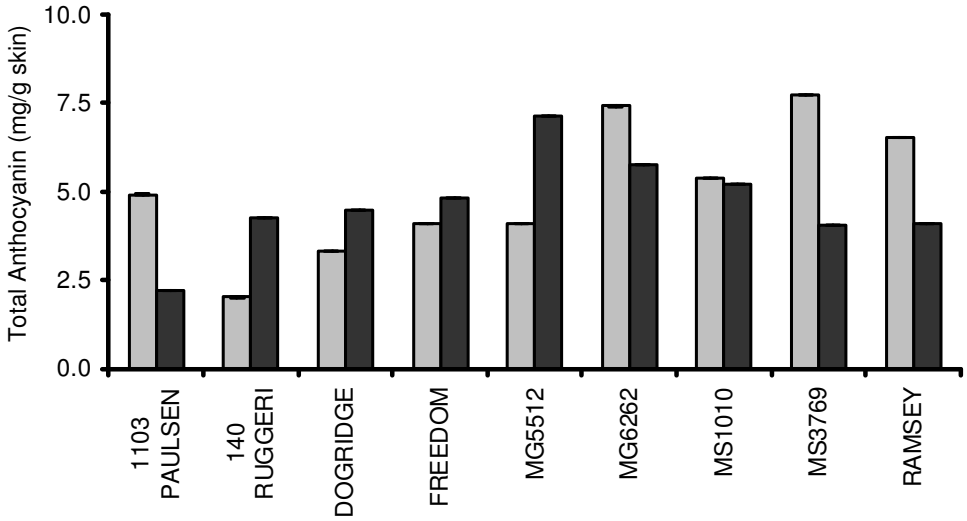


Figure 4.6: Anthocyanin concentration (mg/g) fresh weight of skin expressed as malvidin-3-glucoside equivalents for Shiraz on nine rootstocks for the 2008 and 2009 growing seasons.

Anthocyanin composition in grape skin was determined by HPLC and showed that the least common anthocyanins for all rootstocks were those derived from cyanidin (Figure 4.7). In 2008, the percentage of cyanidin derived anthocyanins ranged from 0.3% in 140 Ruggeri, MG6262 and MS3960, to 0.9% in 1103 Paulsen, with a mean of 0.5% (%CV = 38.0). In 2009, the proportion of cyanidin derived anthocyanins was similar ranging from 0.1% in Ramsey to 0.9% in 140 Ruggeri, with a mean of 0.4% (%CV = 61.2).

Malvidin derived anthocyanins were the most commonly occurring anthocyanins for all rootstocks accounting for more than half of the anthocyanins present for all rootstocks in both seasons. In 2007/08, the proportion of malvidin derived anthocyanins ranged from 66.6% in 1103 Paulsen to 83.8% in MG6262, with a mean proportion of 76.4% (%CV = 7.0). In 2009, the proportion of malvidin derived anthocyanins ranged from 67.8% in 140 Ruggeri to 84.3% in Ramsey, with a mean of 77.1% (%CV = 6.9).

In 2008 the proportion of delphinidin derived anthocyanins ranged from 1.7% in MG6262 to 4.3% in Paulsen, with a mean proportion of 2.6% (%CV = 33.5). In 2009, the proportion of delphinidin derived anthocyanins ranged from 1.3% in Ramsey to 4.9% in MG6262, with a mean of 3.2% (%CV = 40.2).

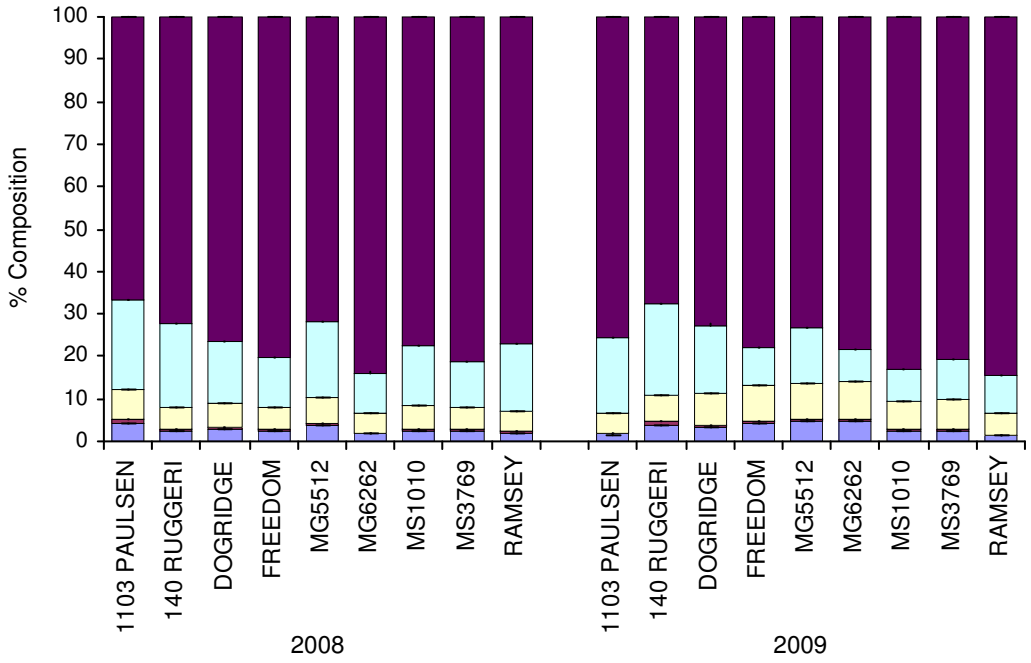


Figure 4.7: Proportional composition of anthocyanins grouped according to parent anthocyanidin; Delphinidin , Cyandin , Petundin , Peondin and Malvidin for Shiraz on nineRootstocks for the 2007-08 and 2008-09.

In 2007/08, petunidin derived anthocyanins ranged from 6.9% in Paulsen to 4.7% in MG5512, with a mean proportion of 5.6% (%CV = 12.1) and in 2008-09, from 4.5% in Paulsen to 8.8% in MG6262, with a mean of 6.9% (%CV = 20.7). Peonidin derived anthocyanins ranged from 9.4% in MG6262 to 21.3% in Paulsen with an average proportion of 15.0% (%CV = 27.0) in 2007-08. In the following season (2009), peonidin derived anthocyanins ranged from 7.6% in MG6262 and 21.3% in 140 Ruggeri, with a mean of 12.4 (%CV = 40.6).

The proportional composition of anthocyanins in Shiraz has been reported by Downey *et al.* (2004).This data are consistent with previous reports in that malvidin derived anthocyanins are the major anthocyanin present in Shiraz grape skin. Both seasonal and rootstock influences were apparent in the data but these were not consistent suggesting a rootstock by season interaction and/or the presence of other factors that were not evaluated as part of this trial. For example, differences in soil structure or chemistry, plant available water or possible pest and disease pressure. A long term study of all of these parameters as well as of the content and composition of quality parameters would be needed to tease elucidate these relationships. The existing trial that these samples were

collected from could be modified for this purpose, or appropriate analyses added to the existing experiment. This would add substantially to the value of the current work.

4.2.3 Influence of rootstock on flavonol content

Like anthocyanins, the concentration of flavonols in grapes grown on the nine rootstocks studied did not vary significantly between seasons (Figure 4.8). In 2008, flavonol content ranged from 0.32 mg/g of skin in fruit grown on 140 Ruggeri to 3.43 mg/g in fruit grown on MG6262, with an average of 1.35 mg/g of skin (%CV = 67.3). In 2009, flavonol concentration ranged from 0.31 mg/g of skin in fruit from vines on 1103 Paulsen rootstock to 2.29 mg/g skin in fruit grown on MG6262 with an average of 1.09 mg/g skin (%CV = 55.8). In both seasons, fruit grown on MG6262 had the highest concentrations of flavonols whilst those grown on 1103 Paulsen, 140 Ruggeri, and Dogridge rootstocks tended to have lower concentrations in both seasons.

Individual flavonol composition was determined by HPLC simultaneously with anthocyanin composition after Downey and Rochfort (2008).. Differences were observed between rootstocks and season (data not shown) but no obvious trend was observed. These data, together with the anthocyanin and tannin data for these nine rootstocks will be presented independently in peer-reviewed publication (Ebrahim *et al.* in prep.) which is in preparation.

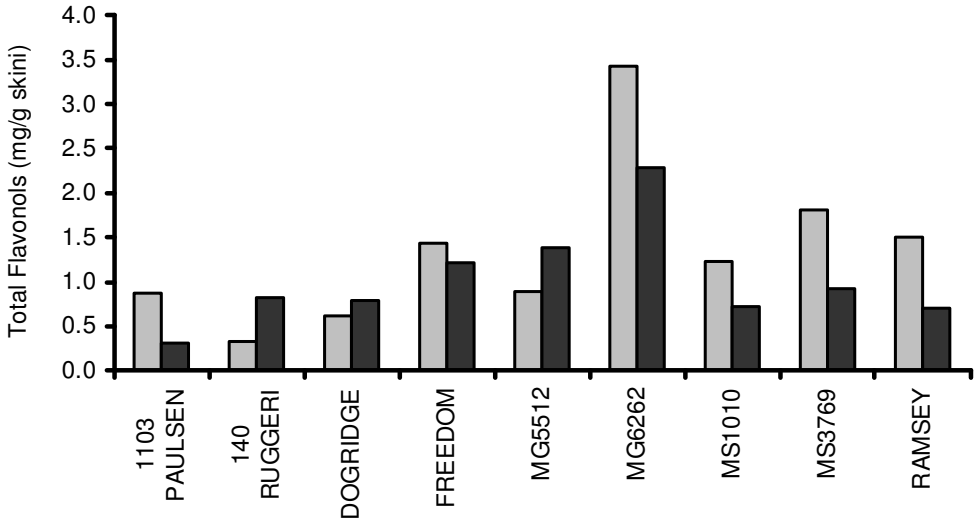


Figure 4.8: Flavonol concentration (mg/g fresh weight of skin) expressed as Quercetin-3-glucoside equivalents for Shiraz on nine rootstocks for the 2008 □ and 2009 ■ growing seasons.

4.2.4 Summary of rootstock influence on flavonoids

Considered together, the tannin, anthocyanin and flavonol data for Shiraz grape skin from vines grown on nine different rootstocks indicated two clear outcomes. While there were differences between rootstocks a strong seasonal effect appears to override rootstock differences and the factors influencing tannin biosynthesis and those affecting anthocyanin and flavonol biosynthesis are different and even the seasonal impact varies between sub-classes of flavonoids. To some extent this is not unexpected as tannin biosynthesis in Shiraz occurs very early in berry development (Downey *et* al. 2003a; Hanlin and Downey 2009) while anthocyanin biosynthesis occurs later in development during berry ripening (Somers 1976). Consequently early season factors that impact on tannin biosynthesis may not affect anthocyanin and flavonol biosynthesis.

Other research on the effect of rootstock on tannin accumulation in fruit has also shown little difference in the tannin content and composition between rootstocks (Sampaio 2007). While there is clearly a rootstock effect as evidenced by differences in levels of tannins, anthocyanins and flavonols between each rootstock, there is not sufficient data from either these or other studies to indicate rootstocks consistently conferring high or low tannin, anthocyanin or flavonol levels in the fruit. Such rootstocks may exist but a more detailed study of a large number of rootstocks over a period of multiple seasons would be required to identify these performance indicators. While a suitable site exists (where these samples were collected) this detailed analysis is not a component of the current project (GWRDC CSP 99/2).

4.3 Influence of sustained deficit irrigation on tannin and anthocyanin levels

4.3.1 Influence of sustained deficit irrigation on tannin content

Condensed tannins were determined by protein precipitation assay on fruit collected from a deficit irrigation trial conducted on Shiraz and Cabernet Sauvignon grapevines (Chalmers 2007). Harvest samples from multiple deficit irrigation treatments were collected over four successive seasons (2003/04, 2004/05, 2005/06 and 2006/07). There was little or no difference in the concentration of tannins in whole berries between any of the treatments for both Cabernet Sauvignon and Shiraz (Figure 4.9).

In Cabernet Sauvignon, tannin concentration ranged from 2.93 to 3.62 mg/g of whole berry (%CV = 10.5), 2.28 to 2.86 mg/g of whole berry (%CV = 9.5), 1.05 to 1.14 mg/g of whole berry (%CV = 3.3) and 5.67 to 6.72 mg/g of whole berry (%CV = 7.2) for the four consecutive seasons (2003/04, 2004/05, 2005/06 and 2006/07).

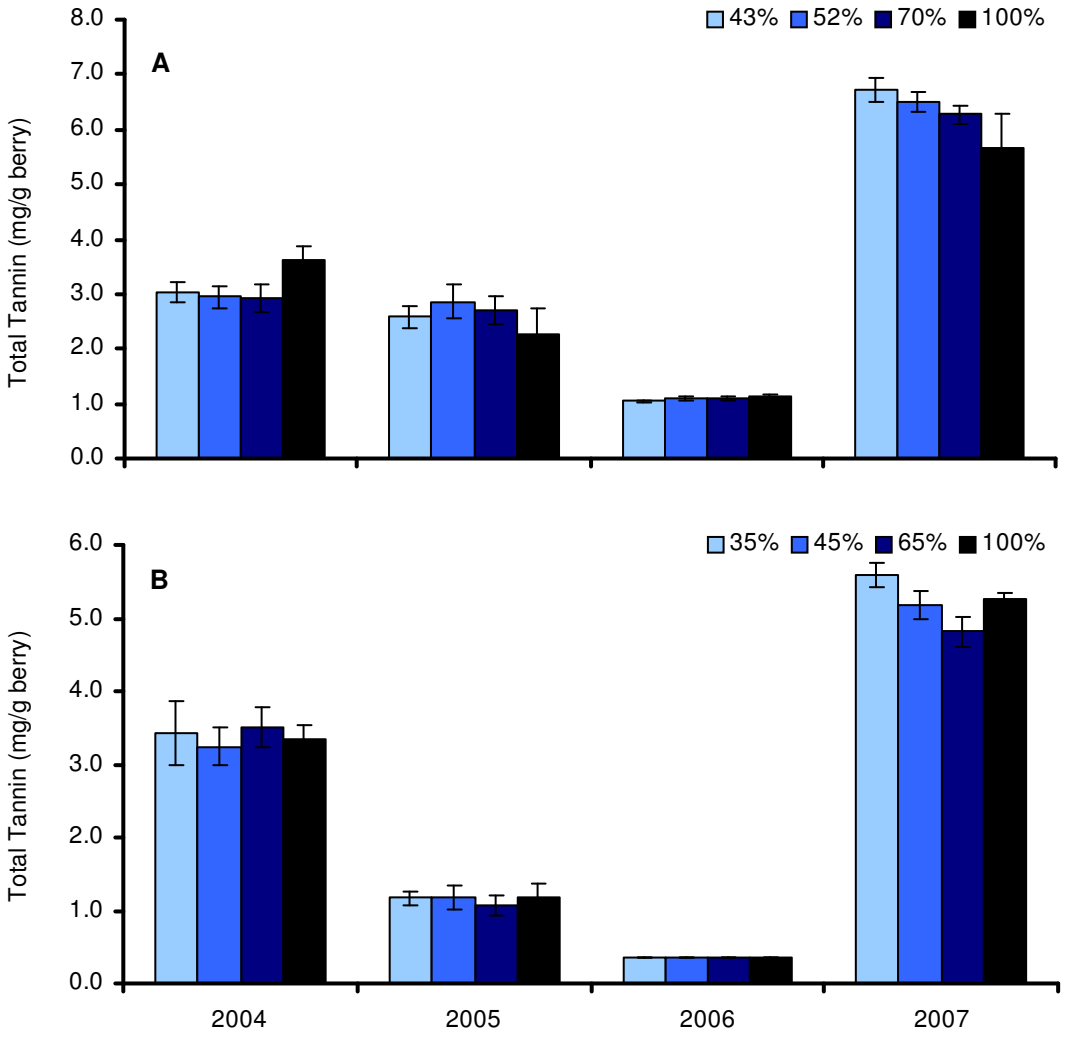


Figure 4.9: Total tannin (mg/g fresh weight of berry expressed as catechin equivalents) of **A** Cabernet Sauvignon and **B** Shiraz of Control fruit, and fruit with SDI treatments imposed

In Shiraz, tannin concentration ranged from 3.24 to 3.51 mg/g of whole berry (%CV = 3.4), 1.07 to 1.18 mg/g of whole berry (%CV = 4.5), 0.36 to 0.37 mg/g of whole berry (%CV = 0.6) and 4.81 to 5.59 mg/g of whole berry (%CV = 6.2) for the four consecutive seasons (2003/04, 2004/05, and 2005/06 2006/07). Tannin concentrations varied between seasons for both cultivars with 2005/06 having low tannin concentration across all treatments for both Cabernet Sauvignon and Shiraz, and 2006/07 having high tannin concentrations.

These observations are consistent with previous reports where deficit irrigation treatments were applied to Cabernet Sauvignon and no difference in tannin levels was observed (Kennedy *et al.* 2002).

4.3.2 Influence of sustained deficit irrigation on anthocyanin content and composition

Anthocyanin data was collected for three seasons of the deficit irrigation trial, 2003/04, 21004/05 and 2005/06 for both Shiraz and Cabernet Sauvignon vines (Chalmers 2007).

From this data it is immediately apparent that the2004/05 season was a low anthocyanin year for both varieties and this was consistent across all treatments (Figure 4.10). A cursory assessment of the data also suggests that there is an increasing trend in anthocyanin concentration with increasing water deficit; however close examination of the data is less convincing.

For Cabernet Sauvignon, the lowest irrigation treatment (43%) in 2003/04 had the highest concentration of anthocyanins, significantly higher than the other SDI treatments (52% and 70%) but there was no difference compared to the 100% treatment (Figure 4.10). Anthocyanin concentrations in Cabernet Sauvignon in 2003/04 ranged from 3.43mg/g of skin for the 52% and 70% SDI treatments to 4.23 mg/g of skin in the 43% SDI treatment. In 2004/05 anthocyanin concentration ranged from 1.07mg/g of skin in the 100% treatment 1.25mg/g in the 43% treatment. In 2005/06, anthocyanin concentration in Cabernet Sauvignon ranged from 2.7mg/g in the 52% irrigation treatment to 3.6mg/g in the43% treatment; concentration in the 100% treatment was not significantly different from the 52% treatment at 2.8mg/g.

In Shiraz in 2003/04, there was no difference between each of the SDI treatments, 35%, 45% and 65%, however all SDI treatments had a significantly higher anthocyanin concentration than the 100% treatment (Figure 4.10). In 2003/04, anthocyanin concentration in Shiraz ranged from 2.66 mg/g of skin in the 100% treatment to 4.23 mg/g of skin in the 65% SDI treatment. In 2004/05, the anthocyanin concentration was lowest in the 100% treatment at 1.77mg/g fresh weight of skin and highest in the 45% irrigation treatment at 2.25mg/g. There was no significant difference between the 45% and 65% irrigation treatments in 2004/05, nor was there any difference in anthocyanin between the 100% and 35% irrigation treatments. In 2005/06, the anthocyanin concentration in Shiraz grape skins ranged from 3.37mg/g of anthocyanins in the 65% irrigation treatment to 4.14mg/g in the 35% irrigation treatment. There was no difference between the 45%and 65% irrigation treatment or between the 35% and 100% irrigation treatments.

While there was a very weak general trend between irrigation levels and anthocyanin concentration, one could not conclude that deficit irrigation had an effect on anthocyanin concentration in the fruit. Although such an observation has been previously reported (Kennedy *et al.* 2002). There are several possible explanations for this, one of which is that there is no relationship between irrigation level and anthocyanin concentration; however widespread use of deficit irrigation to manage fruit quality, particularly colour tends to argue against that. The other and more likely explanation is that the 100% treatment, which was the standard vineyard practice at the site, was probably substantially greater than the plant water requirement, thus the applied deficits had not significantly decreased plant available water in the rootzone and little or no effect was observed. This highlights one of the risks in sourcing sample material from other projects

where there is little control over the project management and by inference the applied treatments and therefore the quality of the samples collected and the data generated.

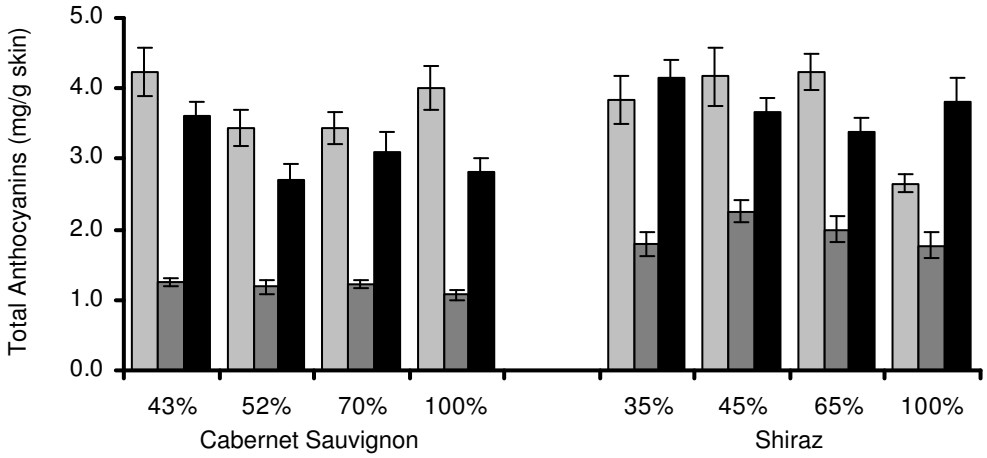


Figure 4.10: Total anthocyanins (mg/g fresh weight of skin) expressed as malvidin-3-glucoside equivalents of Cabernet Sauvignon and Shiraz of Control fruit, and fruit with SDI treatments imposed for 3 growing seasons 2003/04 □ , 2004/05 ■ , and 2005/06 ■.

While some minor differences in total anthocyanin concentration were observed between the irrigation treatments there was essentially no difference in composition based of anthocyanins derived from the parent anthocyanidin (Figure 4.11). In 2003/04, Malvidin derived anthocyanins were present in the highest concentrations for all SDI treatments for both cultivars ranging from 61.1% to 63.1% in Cabernet Sauvignon and 69.6% to 74.8% in Shiraz. Delphinidin derived anthocyanins contributed to 15.5% to 16.8% and 6.4% to 9.9% of anthocyanins in Cabernet Sauvignon and Shiraz respectively. Petunidin derived anthocyanins were present in higher proportions in Cabernet Sauvignon compared to Shiraz for all treatments, ranging from 10.8% to 11.4% and 6.2% to 7.8% respectively. Peonidin derived anthocyanins formed a larger portion of total anthocyanin concentration in Shiraz compared to Cabernet Sauvignon, ranging from 10.9% to 13.6% and 7.7% to 8.1% respectively. Cyanidin derived anthocyanins contributed the least to total anthocyanin concentration ranging from 1.2% to 1.5% in Cabernet Sauvignon and 0.3% to 0.4% in Shiraz.

It is clearly apparent that there were some compositional differences between the cultivars Cabernet Sauvignon and Shiraz, with Shiraz, for example, having the higher proportion of Malvidin derived anthocyanins. It is also clear from this data that deficit irrigation had a minimal effect on the proportional composition of anthocyanins, based on parent anthocyanidin. A similar compositional pattern was observed in the 2004/05 and 20005/06 seasons (data not shown) (Chalmers 2007).

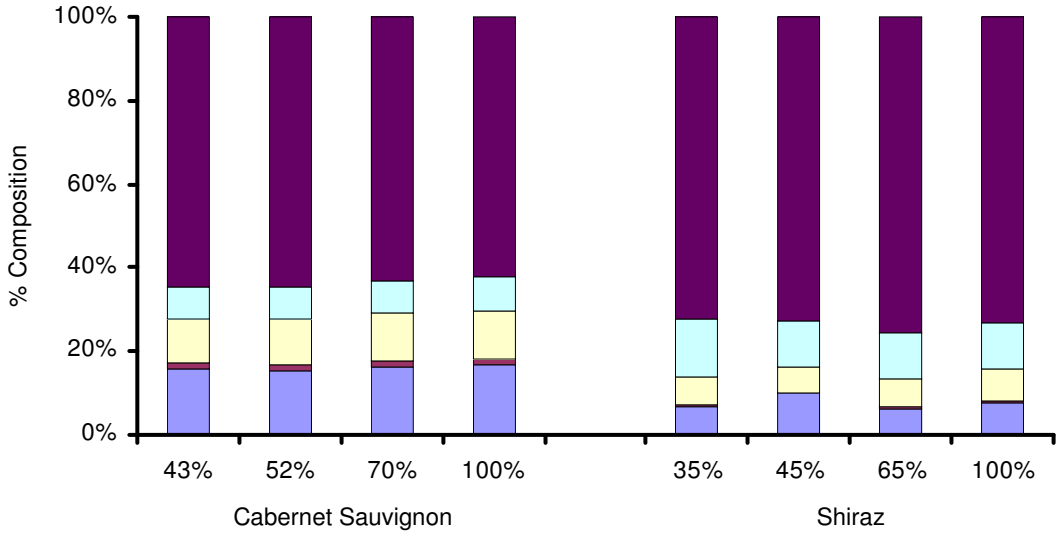


Figure 4.11: Percent composition of anthocyanins grouped according to those derived from Delphinidin , Cyandin , Petundin , Peondin and Malvidin

4.4 Varietal differences in tannin and anthocyanin levels for Californian winegrapes.

4.4.1 Varietal differences in tannin content

Total tannin was determined in whole berry homogenates for a range of varieties collected from across California (USA) over three seasons. Samples were collected at random from harvest samples from individual vineyard blocks brought to a central grape assessment laboratory. The variety and number of samples reflected the vineyards being harvested at the time of sample collection. In 2005, samples from 16 varieties were analysed, 13 varieties were analysed in 2006 and 18 in 2007. For the varieties such as Cabernet Sauvignon, Merlot and Rubired more than 50 samples were analysed for all three seasons. For a number of varieties very few samples were collected, being close to either the beginning or end of harvest for those varieties at the time of sample collection. Overall, a total of 1720 samples were collected from a potential pool of around 8,000 vineyard blocks, covering 22 varieties over three seasons

Tannin concentration varied considerably between varieties and within the same variety. For Cabernet Sauvignon, tannin concentration was observed to range from 0.20-1.76 mg/g of whole berry in 2005, 0.39-4.57 mg/g whole berry in 2006, and 0.36-5.81 mg/g of whole berry in 2007. For Merlot tannin concentration ranged from 0.08-1.92 mg/g of whole berry in 2005, 0.83-4.22 mg/g of whole berry in 2006, and 0.75-5.20 mg/g of whole berry in 2007. The concentration of tannin observed for Rubired ranged from 0.09-0.99 mg/g of whole berry (2005), 0.32-3.87 mg/g of whole berry (2006) and 0.44-3.23 mg/g of whole berry (2007).

For each season the highest observed tannin concentration was found in Merlot with a concentration of 1.92 mg/g of whole berry (2005), Cabernet Sauvignon with 4.57 mg/g of whole berry (2006) and 5.81 mg/g of whole berry (2007).

The lowest recorded tannin concentration in the 2005 samples was found in Cabernet Sauvignon with a concentration of 0.02 mg/g of whole berry. In 2006,. Barbera had 0.29 mg/g of whole berry and in 2007, Barbera had a tannin concentration of 0.31 mg/g of whole berry.

The range of in tannin concentrations for samples within the same cultivar varied between seasons. The range of tannin concentrations observed for all cultivars was greater in 2006 and 2007 compared to the range observed in 2005. It is uncertain whether this relates to the sampling or reflects a seasonal impact, because there is not a comprehensive collection of the total soluble solids data for all of the samples collected. However, based on other research observations it is likely that this represents a seasonal effect as there is not a strong relationship between tannin concentration and ripeness (Hanlin and Downey 2009; Seddon and Downey 2008).

The data from 2006 and 2007 show quite large ranges in tannin concentration for some varieties, for example in Cabernet Sauvignon, the tannin concentration in whole berry homogenates ranged from 0.36 to 5.81 mg/g in 2007 a 16 fold difference, while in Merlot the range for the same season was 0.75 to 5.20 mg/g almost a 7-fold range (Figure 4.14).

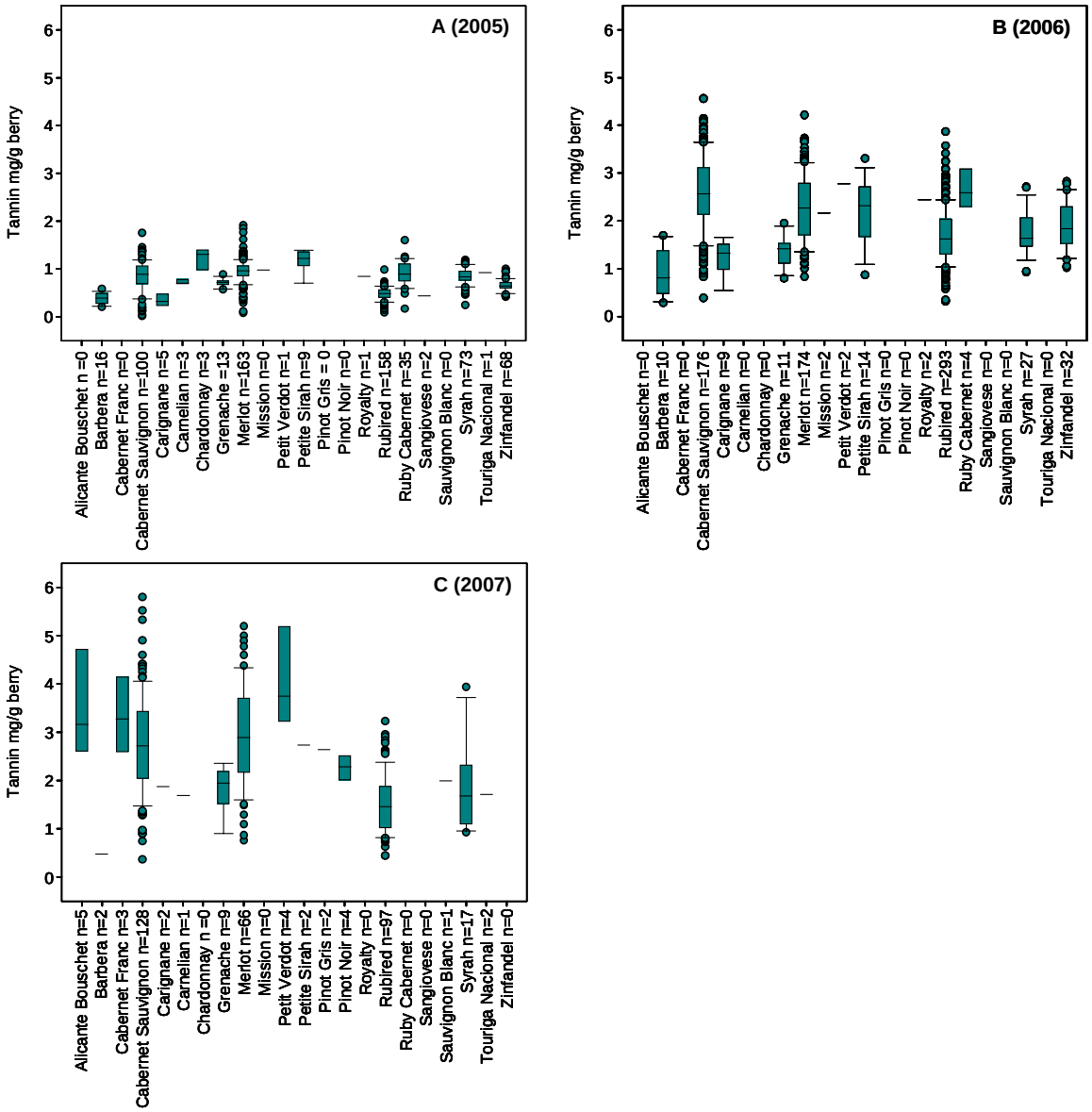


Figure 4.14 Total tannin concentration, mg/g fresh weight of berry for whole berry homogenates collected in California during the **A** 2005, **B** 2006, and **C** 2007 growing seasons.

4.4.2 Varietal differences in anthocyanin content

In the second and third seasons of data collection anthocyanins were also analysed in the whole berry homogenates collected from California. Like observations for tannin, anthocyanin concentration varied considerably between varieties and within the same variety. In Cabernet Sauvignon, anthocyanin concentration ranged from 0.14 - 2.96 mg /g of whole berry (2006), and 0.23 - 4.32 mg/g skin (2007). Variation in total anthocyanin concentration was greater in Merlot and Rubired. Merlot in 2006 was observed to have a concentration in total anthocyanin ranging from 0.19 - 4.38 mg/g of whole berry. The extent of variation observed for Merlot was greater in the following season (2007), with anthocyanin concentration ranging from 0.27 - 5.55 mg/g of whole berry, a twenty-fold difference. Some of the highest observations of anthocyanin concentration were observed in Rubired for both seasons. In 2006, anthocyanin concentration ranged from 0.20 - 5.86 mg/g of whole berry, whilst in 2007 anthocyanin concentration in Rubired ranged from 0.31 - 6.95 mg/g of whole berry. Within the same variety and season, these ranges for anthocyanin concentration could be partly attributed to a seasonal effect, but because anthocyanin accumulation is a ripening process, riper fruit would tend to have higher anthocyanin levels.

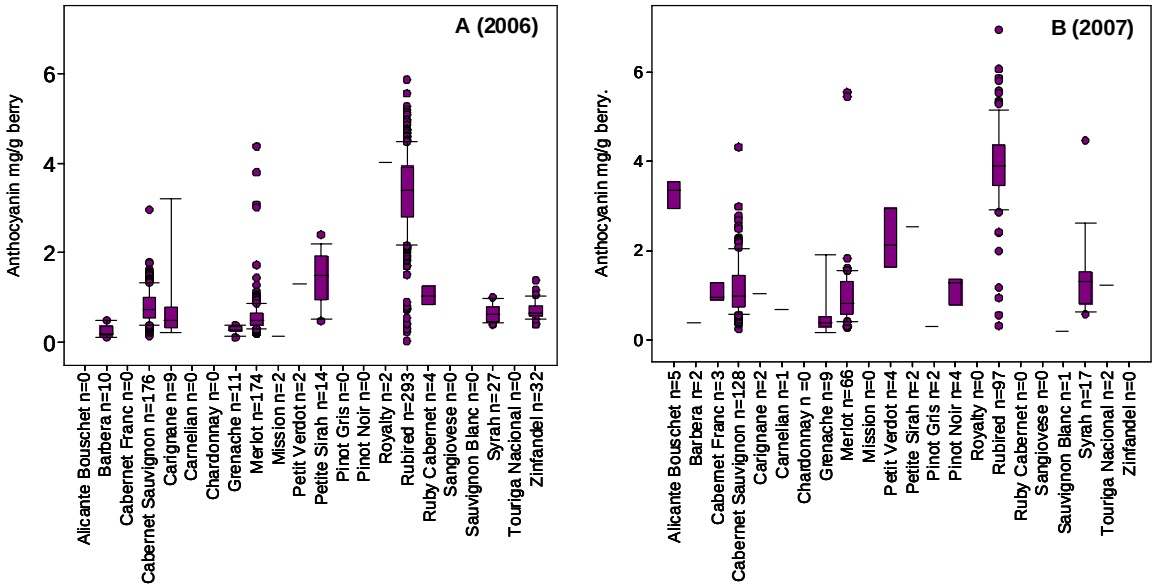


Figure 4.15 Total anthocyanin concentration, mg/g fresh weight of berry for whole berry homogenates collected in California during the **A** 2006 , and **B** 2007 growing seasons.

What is interesting to observe between the tannin and anthocyanin data-sets is that in some varieties, for example, Cabernet Sauvignon, there is a greater range in the tannin data than in the anthocyanin data, which suggests that tannin biosynthesis is more highly variable and that tannin biosynthesis is more sensitive to environmental or viticultural drivers. What is also possible is that early in the season when tannin biosynthesis occurs

the environmental conditions are more variable than later in the season when anthocyanin biosynthesis occurs. However, the data presented here is not sufficiently comprehensive in term of environmental and viticultural management data to demonstrate this. An attempt at dissecting these relationships is discussed in more detail under Objective 3.

These results indicate considerable variation in tannin concentration not only between different cultivars but also within the same cultivar. Differences in tannin concentration for samples of the same cultivar were as high as 5.44 mg/g of whole berry (Cabernet Sauvignon, 2007). While differences in anthocyanin concentration was observed to be as great as 6.64 mg/g of whole berry (Rubired, 2007). There were also considerable seasonal differences in tannin and anthocyanin concentration, there was an overall increase in tannin concentration in 2006 and 2007 compared to 2005. In addition to variation within varieties and between varieties, there was a seasonal difference in anthocyanin concentration. There was an overall increase in tannin concentration for the bulk of the samples analysed for these varieties in 2007 in comparison to 2006. In addition to this increase in tannin concentration there was also an increase in variation of tannin concentration within the same cultivar between seasons, with the bulk of the samples analysed in the 2005 season having concentrations within a smaller range compared to the subsequent seasons.

4.5 Yield influence on tannin and anthocyanin content

At harvest yield for each vine was determined through a series of measurements including bunch number and weight for a given area of the canopy (50cm transect) and also row spacing. Yield information for 100 vines for three seasons was collated in addition to the tannin and anthocyanin concentration for each of the 100 targeted vines.

There was a negative correlation between tannin concentration and yield ($r^2 = 0.2771$) and between anthocyanin concentration and yield ($r^2 = 0.572$) (Figure 4.14). Previous researchers have reported similar trends in anthocyanin content with respect to crop load (Kliewer and Weaver 1971; Petrie and Clingeleffer 2006; Walker *et al.* 2005; Wolf *et al.* 2003), here we have demonstrated a similar trend with tannin concentration, although the relationship is weaker, which indicates that crop load, or yield has less of an impact on tannin concentration that it does on anthocyanin accumulation (Figure 4.14). This may be a reflection of the difference in timing between anthocyanin and tannin biosynthesis during berry development. It also suggests that there are factors that contribute more to final tannin concentration at harvest than yield.

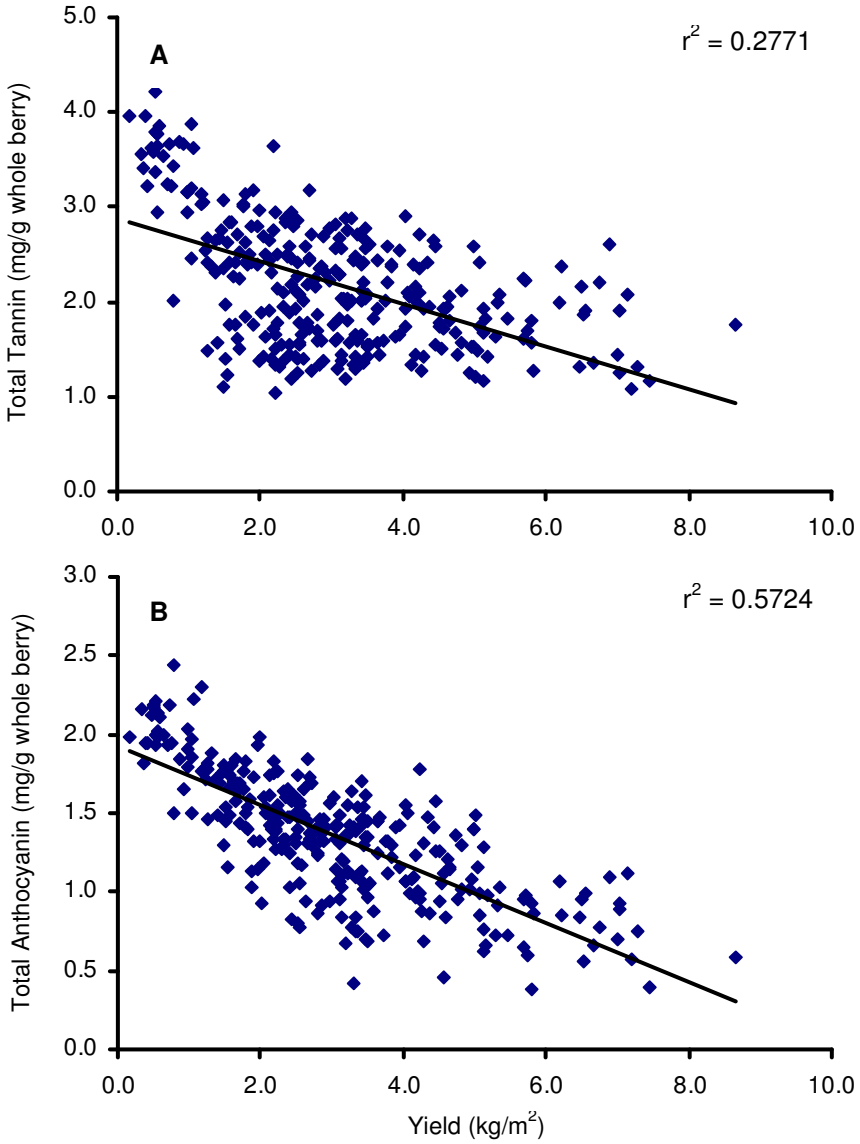


Figure 4.14 Comparison of yield for Shiraz grapes collected from 100 vines, for three seasons (2005, 2006, and 2007) to **A** Total tannin concentration (mg/g fresh weight of berry) and **B** Total anthocyanin concentration (mg/g fresh weight of berry) and yield (kg/m^2)

Yield, crop load per vine, vine balance and fruit quality are intimately linked. The viticultural parameters can be managed using a range of techniques, although the most common approach in modern Australian viticulture is the use of deficit irrigation. Reducing irrigation inputs can decrease vegetative growth, effectively reducing vine vigour. As will be discussed later in this report, vigour appears to be a major driver of tannin accumulation in winegrapes. Reduced irrigation inputs can also decrease berry size, effectively concentrating quality components like anthocyanins. Observations to date have not reported such a strong relationship with grape tannins. Again this may be due to the different timings of synthesis between anthocyanins and tannins but may also indicate

that other factors have a greater influence over tannin biosynthesis in the grape berry than plant water status.

Objective 5. Development of standardised industry methods for the sampling and analysis of grape samples for the determination of total tannin (spectrophotometer/NIR/FTIR), tannin composition (HPLC) and anthocyanin composition (HPLC).

5.1 Vineyard sampling of winegrapes

Sampling protocols have been previously established and are in widespread use in the Australian industry and also internationally, including with a number of collaborators on this project. The protocols were developed as part of a previous DPI Vic. GWRDC-funded project (GWRDC Project No. CRV 99/6). These protocols have been adapted for this project; for vineyards two bunches were taken from 20 vines, for treatments, two bunches were taken from each target vine within the treated panels. The number of treated panel varied between trials (see Methods Sections 1.1 and 3.1.1).

A large number of samples analysed as part of this project were collected as part of the GWRDC-funded Soil and Water Initiative Five Sites Project (GWRDC Project No. DPI 04/04). Collection of samples for this project followed protocols established and outlined in the project report(Goodwin 2009). Briefly, target vines were selected based on NDVI and EM38 data (see Methods Section 2.1). At harvest, bunches from a transect of each target vine were collected.

5.2 Grape Tissue Extraction for Analysis

Methods for extraction of various classes of phenolics, for example anthocyanins, flavonols and condensed tannins, have been published previously; however some issues had been raised regarding their effectiveness. These methods were investigated and refined here to address perceived shortcomings.

In previous work it had been observed that the values for individual anthocyanins obtained by high-performance liquid chromatography (HPLC) appeared to be time sensitive. This had not previously been identified as an issue, largely because much of the published research on colour in numerous systems, not only grapes and wine, utilised measures for determining total colour or total anthocyanins. While HPLC analysis had been employed it was usually only to determine the anthocyanin profile, not as a routine analytical tool. In using HPLC as a routine analytical tool it became apparent that the anthocyanin profile was changing over time, with individual anthocyanins being degraded. Over a period of around six hours virtually all malvidin-3-*O*-coumaroly glucoside lost the coumaroyl moiety and while this drastically altered the profile of individual anthocyanins,

total anthocyanins remained relatively constant. Thus, in a total colour analysis this was not an issue,

To overcome this limitation to routine HPLC analysis of the anthocyanin profile of grape skin we examined the extraction solvent and mobile phases in the HPLC separation. It was identified that acid in the extraction solvent was the cause of the hydrolysis of the coumaroyl moiety and a number of alternate extraction solvents were trialed. Of these, 50% methanol-water (v/v) was shown to be the most effective when used in combination with 10% formic acid in the HPLC mobile phase. The outcomes of this work were published in the peer-reviewed literature (Downey *et al.* 2007; Downey and Rochfort 2008).

During the course of the research undertaken as part of this project, discrepancies were identified between several analytical methods for the determination of condensed tannins. One of the possible sources of difference was the choice of extraction solvent. In phenolic research there are two solvents in widespread use, 70% acetone-water (v/v) and 50% ethanol-water (v/v). Various publications have compared different solvents and different mixtures of solvents, but there was not a published comprehensive study of different solvents and the previous work had not looked at HPLC analysis as an end-use of the extract, only total tannin or total phenolic assays.

To address this we compared total tannin and tannin subunit composition in mixtures of both ethanol and acetone with water from zero to 100% solvent (Downey and Hanlin, submitted). This work categorically demonstrates the differences between the two solvent systems, but also highlights similarities and occasions where the two solvent systems might be used interchangeably. The most effective extraction solvent was 70% acetone-water (v/v); 50% ethanol-water (v/v) was the most effective of the ethanol-water mixtures. More tannin and longer polymers were extracted in acetone than in ethanol, but the composition of all polymers was similar. Thus, subunit composition determined in a 70% acetone extract would be comparable to that in 50% ethanol or less, for example 10-15% ethanol as might occur in wine. This has benefits and applications to food grade analysis of tannins as well as being a tool to fractionate tannins for further structural and functional examination.

5.3 Total Tannin Analysis of Whole Berry Homogenate by Protein Precipitation

At the time this project commenced there were a number of published methods for condensed tannin analysis. These fell into two main groups, assays for total tannins and HPLC separation of monomeric material or of subunits following acid-catalysed depolymerisation (Bate-Smith 1973; Hagerman and Butler 1978; Kennedy and Jones 2001; Peng *et al.* 2001).

For analysis of total tannins, HPLC analysis was too costly and too time consuming to be used for routine analysis of large sample numbers, thus attention was directed to the many published total tannin assay methods.

At the outset of this project in July 2005, only one published method had been adapted for use in grape and wine analysis (Harbertson *et al.* 2004; Harbertson *et al.* 2002; Harbertson *et al.* 2003). This method was employed as the primary investigative tool for total tannin analysis for grape samples in this project.

In 2006, an alternate method was published that used methylcellulose as a precipitating agent (Sarneckis et al. 2006) rather than protein (bovine serum albumin; BSA) as used by Harbertson *et al.* (2003). With the publication of this method by the Australian Wine Research Institute, an opportunity was identified to compare the two methods for effectiveness in terms of throughput. Interestingly, there were significant differences between the results generated by the two methods and between these methods and the standard HPLC method of quantification following acid-catalysed depolymerisation (Seddon and Downey 2008). This work prompted further investigations into the nature of the difference between these methods. Understanding these differences was identified as a potential tool for generating different data on tannin extracts and for differential separation, given that both precipitants could be resuspended and further analysed (Harbertson and Downey 2009). As described above, part of this work prompted investigations of the solvent systems (Hanlin and Downey, submitted) as well as of the mechanism of precipitation and quantification (Harbertson and Downey 2009). This work is ongoing.

To increase sample throughput, the original method of Harbertson et al. (2003) was adapted to a 96-well plate format (Heredia *et al.* 2006). This method was revised to further increase throughput and this revision will be published with data in relation to the development of a rapid high-throughput analytical method based on UV-visible spectra.

5.4 Rapid determination of phenolics components in red wines from UV-visible spectra

The major barriers to adoption of routine analytical methods for analysis of grape and wine quality parameters are the time required to undertake the analysis, the cost per sample and the need for highly trained technical staff to undertake the analyses. In other fields, the application of rapid analytical tools such has near-infrared (NIR) spectroscopy, has been fundamental to the development of quality based payment schemes and high-throughput screening of products for processing (Osborne 2000)

In the grape and wine industry, NIR analysis has also been employed to evaluate fruit and wine quality parameters (Kupina and Shrikhande 2003). In recent years attempts have been made to apply this technology to the analysis of grape and wine phenolics

(Cozzolino et al. 2004). While some success has been reported in the analysis of grape and wine colour, robust predictions of the phenolic and tannin content were less successful.

Discussions on the limitations of an NIR based approach to the determination of tannins and low molecular weight phenolics with Prof. Roger Boulton, a colleague and collaborator at the University of California, Davis (UC Davis), identified inclusion of both the UV-visible and NIR spectra as a possible solution to this problem. A project was developed to collect the UV-visible and NIR spectra, and tannin and phenolic data on wine samples collected in both California and Australia as part of the project reported here.

Partial Least Squares (PLS) based chemometric analysis of the complete dataset was not able to generate a sufficiently robust model; however analysis of just the UV-visible spectra produced a strong predictive model (Skogerson *et al.* 2007). Currently, development of this approach is being undertaken by researchers and wineries in California, USA to produce a tool suitable for widespread adoption.

During the course of analysing the grape samples for Objectives 2 and 4 of this project, UV-visible spectra were also collected on whole berry extracts. The data for phenolic parameters determined by the Harbertson-Adams assay and the UV-visible spectra have been incorporated in a PLS model for the prediction of these parameters in whole berry homogenates. This work is ongoing with collaborators from Constellation Wines US and the Californian wine industry Gold Standards Technical Group. The outcomes of this collaborative work will be reported to the Australian industry and research community through peer-reviewed and industry publications as it becomes available.

Objective 6. Develop and implement a targeted extension and evaluation strategy to facilitate the adoption of research findings throughout the Australian Wine Industry, using various change agents, ie Grapecheque, Viticare, industry grower groups, industry trade journals (ie Australian Viticulture) and scientific publications (ie Australian Journal of Grape and Wine Research).

When this project was conceived there was a widespread belief in the Australian wine industry that reward for quality based payment schemes would underpin all future winery-grower contracts and that while there may be some shortcomings in the some of the measures used at the time, efforts to develop rapid assessment tools for various parameters, including tannin, would overcome those limitations.

As time has shown us, this has not been the case to date and there has been something of a shift away from reward for quality based payment schemes, particularly where these

were based on one or two fruit metrics such as sugar or colour. The trouble being that while sugar was a good indicator of overall ripeness and alcohol level in the final wine it was not well correlated with the price point or quality of the wine. Colour was a much better indicator of quality, but spectacularly fallible.

However, rather than abandon the concept altogether, it was envisaged that a suite of analyses including sugar, anthocyanins and tannins, would be the solution and that growers would need to learn to mange tannin in the vineyard the same way that they have learnt to manage colour.

Since then, payment for tannin has become increasingly less likely as the industry has realised how little they actually know about tannin and managing tannin in the vineyard has not turned out to be as straightforward as say managing colour.

Thus, the initial assumption that the end-user of innovations developed in this project would be winegrowers and the main benefit would be an increase in the farm gate value of fruit was flawed.

The major flaw in the logic process was the assumption that the wineries understood tannin and that if they had a metric they would able to use it to better stream fruit, manage ferments and tailor wine styles to meet consumer demand.

As it turned out, while winemakers knew what tannin was more or less, what it tasted like and what tannin sensations they preferred, they had no mechanism for relating that back to a numerical value for tannin.

In fact, while winemakers may have had a very good understanding of subtle mouthfeel differences that were largely attributed to "tannin" they knew as little about it as anyone else. This was borne out in study of the industry commissioned by GWRDC and undertaken by Megan Hill and Jeff Kaine, (DPIV) that looked at the market for tannin innovations in the Australian wine industry (Hill and Kaine 2007).

At this point it became apparent that the there would be very little impact on individual growers or the value of fruit until there was sufficient winery and winemaker understanding of tannins to be able to establish tannin specifications for fruit at the weighbridge. Thus, while this project had started out intending to show growers how to increase or alter tannin levels in their fruit, it was forced to re-focus when it became apparent that without the economic incentive to produce fruit to a specification only a small number of grower-winemakers would be likely to take up and use the knowledge.

Thus, while the project continued to focus on the viticultural and environmental drivers of tannin, it was identified that considerable resources would need to be devoted to educating all segments of the industry about the nature of not just tannins, but phenolics generally.

It was also identified, that significant gaps in knowledge existed across a range of areas that had not been considered in during project development, including wine analytical methods, impacts of exogenous tannins, fundamental tannin structure and factors affecting the extraction of tannins into wine.

To address the matter of educating the industry a large number of communication activities were undertaken that ranged from peer-reviewed publication through industry articles, posters, workshops and other extension media as detailed below (Section 6.2 Communication Activities). To address some of the research areas highlighted by Hill and Kaine (2007) that were not incorporated into the original project, a number of collaborations were developed both nationally and internationally (Section 6.1).

6.1 Collaborations

From the outset of this project, collaboration was identified as one of the keys to delivering aspects of the project. For example, the number of samples that could readily be collected and analysed by the project team would be fairly limited if the project team had to undertake all of the sample collection and analysis.

In order to increase the number of samples analysed, the project initially sought two key collaborations. The first of these was with the GWRDC-funded Soil and Water Initiative 5 Sites Project (GWRDC PROJECT No. DPI 04/04). This project comprised five Shiraz vineyards with100 GPS tagged vines at each site, in addition to a large amount of physical and physiological data, harvest samples of fruit were collected from each vine at each site. These samples were transported to DPI Mildura where they were analysed for anthocyanins and tannins and the information shared between the two projects. This resulted in some 1,500 samples being processed during the course of that project, which added substantially to our understanding of tannin variation within a single site and from year-to-year across a site (see Results Discussion Section 2).

The second of these collaborations was between DPI Victoria and E&J Gallo Winery (California, USA). This collaboration capitalised on E&J Gallo Winery's harvest assessment process, which collects a harvest sample (see Results/Discussion Section 5.1) from every vineyard block. These samples were homogenised for their own analysis and a sub-sample set aside, frozen and shipped to DPI Mildura for analysis of tannins and anthocyanins. This collaboration netted a further 1,750 grape samples across 22 varieties over three seasons (see Results/Discussion Section 4.4).

In addition to these collaborations, opportunities to develop collaborative research activities were identified throughout the project. The first of these was between DPI Mildura and researchers at UC Davis, Department of Viticulture and Enology, which lead to several joint projects with Professor Roger Boulton and later with Professor Doug Adams and Professor Hildegarde Heymann. With Prof. Boulton, a small piece of work

was undertaken on temperature gradients in fermentation vessels. This work was assisted by a student, who later undertook a Master's project jointly between DPI Mildura and UC Davis. This project worked on the development of a predictive model for phenolics in wine described above (Results\Discussion Section 5.4). Collaborative work was also undertaken with Prof. Adams in tannin analytical methods, which lead to further collaborations with Prof. Heymann and with Dr James Harbertson (Washington State University). This work has resulted in several research papers either published or in preparation. These collaborations are ongoing and will contribute to future research in this area by the DPI Mildura tannin research team.

During the course of collaborations with UC Davis, a collaborative link was established with Dr James Harbertson at Washington Sate University. This link has resulted in several exchanges between DPI Mildura and Washington State (IAREC; Irrigated Agricultural Research and Extension Centre, Prosser WA). These exchanges have focussed on tannin analytical methods, comparing exiting methods and looking at ways to use methods that generate different data to understand more about tannin structure and tannin interactions.

During the life of this project a number of collaborations have been developed with the University of Adelaide (Waite Campus). Some of these arose from previous work on bunch exposure with Prof. Patrick Iland and Renata Ristic. This later expanded to include sensory analyses in collaboration with Dr Sue Bastian and graduate students within the program at the School of Agriculture, Food and Wine. This work subsequently contributed to further collaborations with Dr Bastian and Dr Simone Rochfort (DPI Werribee). Collaborations with the University of Adelaide expanded to include Prof. Graham Jones and Dr Kerry Wilkinson through the joint supervision of a PhD student, Rachel Hanlin. This work now also includes Dr Maria Hrmova (Australian Centre for Plant Functional Genomics) and Dr Tony Bacic (University of Melbourne).

Throughout the life of this project there has been a close relationship with CSIRO Plant Industry (Horticulture Unit, Adelaide), specifically Dr Simon Robinson and Dr Mandy Walker. This relationship has seen joint contribution to papers as well as joint supervision of students.

During the course of this project, the project supervisor was invited to present a summary of DPI research to the research and development team at Constellation Wines US (Madera, CA). Following this presentation, Mark Downey was invited to spend time with the research team discussing Constellation's current phenolic research interests and met Dr Mark Kelm, the senior chemist. This meeting led to an ongoing collaboration between the two groups and a number of exchanges between the two groups. The primary area of interest has been tannin extraction and characterisation. This work is ongoing and there are publications currently in preparation for publication arising from this work.

6.2 Communication Activities

Communication activities undertaken as part of the current project have primarily consisted of presentations at various forums and publications within various industry and scientific journals. A considerable effort in the early life of the project was devoted to easily digested industry articles (Section 6.2.4). Another major area of activity has been poster presentations at various meetings, conferences and other events (Section 6.2.3), as well as presentations and display at field days, workshops and seminars (Section 6.2.2).

6.2.1 Field Days

1. Stand at the 56th MILDURA HORTICULTURAL FIELD DAYS showcasing tannin research (2007).
2. Stand at the 57th MILDURA HORTICULTURAL FIELD DAYS showcasing tannin research (2008).
3. Winegrape Tannin Project and Soil & Water Initiative farm walk in conjunction with the Riverland Viticulture Technical Group. The farm walk was conducted on the Mildura Soil & Water Initiative site (Wingara Wines, Nangiloc) 8 April 2008. 40 people attended.
4. Stand at the 58th MILDURA HORTICULTURAL FIELD DAYS showcasing tannin research (2009).
5. Stand at the 59th MILDURA HORTICULTURAL FIELD DAYS showcasing tannin research (2010).

6.2.2 Workshops, Seminars, Conferences and other Media

6. Workshop: Downey, M.O., Mazza, M. and Baker, N.B. Spatial variation in tannin and anthocyanin composition in Shiraz vineyards. 14th Australian Wine Industry Technical Conference, Adelaide, South Australia; 3-8 July 2010.
7. Conference presentation: Downey, M.O. Exogenous tannin contributions to wine phenolic profiles and sensory character. 61st ASEV Meeting, Seattle Washington; 24-25 June 2010.
8. Conference presentation: Downey, M.O. The Myth of Fingerprints; 7th International Cool Climate Symposium, Seattle Washington; 20-23 June, 2010.
9. Workshop: Areas of current research and future research directions; Murray Valley Winegrowers Association; November 2009, Mildura.

10. Workshop: Areas of current research and future research directions; Murray Valley Winegrowers Association; May 2009, Mildura.

11. Seminar: Recent advances in tannin research - In collaboration with Washington State University and Constellation Wines US; August 2008, Mildura.

12. Seminar: Recent advances in tannin research - In collaboration with CSIRO, E&J Gallo Winery, AWRI and University of Adelaide; July 2008, Barossa Valley.

13. Seminar: Impacts of light and bunch exposure on anthocyanins and tannins in winegrapes; Queensland Winegrowers and Winemakers; June 2009, Stanthorpe.

14. Conference presentation: Hanlin, R.L. Tannin accumulation and composition during grape berry development. American Society for Enology and Viticulture, Portland, Oregon, USA, 17th – 20th June, 2008.

15. Conference presentation: Downey, D.O. Variation in the skin tannin content, composition, and polymer length distribution of 36 different grape cultivars. American Society for Enology and Viticulture, Portland, Oregon, USA, 17th – 20th June, 2008.

16. Presentation: Hanlin, R. L. Changes in tannin content and composition during berry development. School of Agriculture Food and Wine, The Univeristy of Adelaide, 17 October 2007

17. Presentation: Hanlin, R. L. From grape to wine - tannin interactions with cell walls. School of Agriculture Food and Wine, The Univeristy of Adelaide, 22 May 2008.

18. Presentation: Hanlin, R. L. From grape to wine - Isolation and characterisation of winegrape tannin by diol phase separation and phloroglucinol analysis. School of Agriculture Food and Wine, The Univeristy of Adelaide, 22 September 2009.

19. Workshop: Krstic, M.P. and Downey, M.O. 13th Australian Wine Industry Technical Conference Measures of key indicators of wine quality. 13th Australian Wine Industry Technical Conference, Adelaide, South Australia, 29th July - 2nd August, 2007.

20. Media: Farmview, Episode 6 - The Wine Grape Tannin Research Project
The Wine Grape Tannin Research Project aims to develop robust methods of measuring tannins pre harvest and to investigate the viticultural, environmental and varietal factors that affect tannin content and composition in premium wine grape varieties, (2009).

    http://new.dpi.vic.gov.au/agriculture/horticulture/farmview-videos/farmview-season-1-videos

6.2.3 Posters

1. Tardaguila, J., Downey, M.O., Njegovan, M., Dini, S. and Krstic, M.P. (2005) The evaluation of different *Vitis vinifera* L. var. Sangiovese Clones in a warm Australian Climate. *ISHS International Workshop on Advances in Grapevine and Wine Research*, Venosa, Italy, 15-17 September 2005.

2. Chalmers, Y.M., Dry, P.R., Downey, M.O., Loveys, B.R. and Krstic, M.P. (2005) Impact of sustained deficit irrigation on berry size and composition of warm climate winegrapes. *ISHS International Workshop on Advances in Grapevine and Wine Research*, Venosa, Italy, 15-17 September 2005.

3. Downey, M.O., Tardaguila, J., Njegovan, M. and Krstic, M.P. (2005) The effect of bunch shading on composition of Sangiovese, Tempranillo and Grenache grapes. *ISHS International Workshop on Advances in Grapevine and Wine Research*, Venosa, Italy, 15-17 September 2005.

4. Tardaguila, J., Njegovan, M., Downey, M.O. and Krstic, M.P. (2005) Determining the Effect of Bunch Thinning on Sangiovese and Grenache Composition. *ISHS International Workshop on Advances in Grapevine and Wine Research*, Venosa, Italy, 15-17 September 2005.

5. Downey, M.O., Hannah, R., Mazza, M. and Krstic, M.P. (2006) Effects of Shading on Anthocyanin and Flavonol Accumulation in Grape Berries. *4th International Workshop on Anthocyanins*, Rotorua, New Zealand, 14-17 February, 2006.

6. Downey, M.O., Mazza, M., Hannah, R. and Krstic, M.P. (2006) Anthocyanin accumulation in Shiraz. *4th International Workshop on Anthocyanins*, Rotorua, New Zealand, 14-17 February, 2006.

7. Mazza, M. and Downey, M.O. (2006) Measuring Total Tannin in Grape Skins and Homogenates. *Mildura Horticultural Field Days*, Mildura, Australia, 30-31 May 2006.

8. Seddon, T.J., Mazza, M., Hannah, R., Millikan, M. and Downey, M.O. (2006) Variation in Tannins and Anthocyanins across Grape Cultivars. *Mildura Horticultural Field Days*, Mildura, Australia, 30-31 May 2006.

9. Skogerson, K.J., Mazza, M., Downey, M.O. and Boulton, R.B. (2006) Rapid Determination of Phenolic Compounds in Red Wine Fermentations Using UV-vis Spectroscopy. *57th Annual Meeting of the American Society for Enology & Viticulture*, Sacramento, USA. 28-30 June 2006.

10. Hogg, A.S., Bogs, J., Gendall, A.R., Robinson, S.P. and Downey, M.O. (2006) Flavonoid Biosynthesis in Skins of Cabernet Sauvignon and Chardonnay. *8th International Congress of Plant Molecular Biology*, Adelaide, Australia, 20-24 August 2006.

11. Seddon, T.J., Mazza, M., Hannah, R., Millikan, M., Downey, M.O. (2006) Variation in Tannins and Anthocyanins across Grape Cultivars. *11th Annual Romeo Bragato Conference*, Queenstown, New Zealand, 24-27 August 2006.

12. Downey, M.O. Mazza, M. and Krstic, M.P. (2008) Extraction of grape skin anthocyanins and flavonols for HPLC analysis. *13th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 29th July - 2nd August, 2007.

13. Hogg, A.S., Bogs, J., Gendall, A.R., Robinson, S.P. and Downey, M.O. (2008) Flavonoid Biosynthesis in Skins of Chardonnay and Cabernet Sauvignon. *13th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 29th July - 2nd August, 2007.

14. Seddon, T.J., Mazza, M., Millikan, M. and Downey, M.O. (2008) Variation in Tannins and Anthocyanins across Grape Cultivars. *13th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 29th July - 2nd August, 2007.

15. Seddon, T.J. and Downey, M.O. (2008) Comparison of analytical methods for determining total tannin in grape skin. *13th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 29th July - 2nd August, 2007.

16. Skogerson, K.J., Mazza, M., Downey, M.O. and Boulton, R.B. (2008) Comparison of UV-visible and visible-NIR spectroscopies in the rapid prediction of phenolic compounds in red wine fermentations. *13th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 29th July - 2nd August, 2007.

17. Seddon, T.J., Downey, M.O. (2008) Comparison of methods for analysing tannin in grape skin. 59th Annual meeting, American Society for Enology and Viticulture, Portland, Oregon, USA, 17th – 20th June, 2008.

18. Hogg, A.J, Bogs, J, Gendall, A, G., Robinson, S.P, Downey, M.O. (2008) Tannin Biosynthesis in Chardonnay and Cabernet Sauvignon grape skin, American Society for Enology and Viticulture, Portland, Oregon, USA, 17th – 20th June, 2008.

19. Baker. N.K., Mazza, M., Downey, M.O. (2008) Variation in winegrape tannin, anthocyanin and total phenolic levels and their seasonal differences between varieties. *8th International symposium on Grapevine Physiology and Biotechnology*, Adelaide, South Australia, 23rd-28th November, 2008.

20. Kelm, M.A., Hanlin, R.L., Downey, M.O. (2008) Isolation and characterisation of winegrape tannin by diol phase separation and phloroglucinol analysis. *8th International symposium on Grapevine Physiology and Biotechnology*, Adelaide, South Australia, 23rd-28th November, 2008.

21. Hanlin, R.L., Downey, M.O. (2008) Extraction of condensed tannin from grape skin into acetone and ethanol mixtures. *8th International symposium on Grapevine Physiology and Biotechnology*, Adelaide, South Australia, 23rd-28th November, 2008.

22. Chalmers, Y.M., Loveys, B.R., Downey, M.O., Krstic, M.P., Dry, P.R. Physiological response of red wine grape varieties to sustained deficit irrigation. *8th International symposium on Grapevine Physiology and Biotechnology*, Adelaide, South Australia, 23rd-28th November, 2008.

23. Baker, N.K., Mazza, M., Downey, M.O. (2010) Spatial and temporal variability of grape quality characteristics. 59th MILDURA HORTICULTURAL FIELD DAYS. Mildura Victoria, 26th - 27th May, 2010

24. Mazza, M., Baker, N.K., M., Downey, M.O. (2010). The impact of anthocyanin and tannin concentration on wine quality parameters. 59th MILDURA HORTICULTURAL FIELD DAYS. Mildura Victoria, 26th - 27th May, 2010

25. Baker, N.K., Mazza, M., Downey, M.O. (2010) Variation in winegrape tannin levels and their seasonal differences between varieties. *14th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 3rd–8th August, 2010.

26. Hanlin, R.L., Downey, M.O. (2010) The use of 100-berry micro-ferments to analyse phenolic content and composition during winegrape fermentation, *14th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 3rd–8th August, 2010.

27. Baker, N.K. Mazza, M., Downey, M.O. (2010) Spatial and temporal variability of grape quality characteristics. *14th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 3rd–8th August, 2010.

28. Mazza, M., Downey, M.O. (2010) Influence of bunch exposure on flavonols and anthocyanins in major Australian winegrape varieties. *14th Australian Wine Industry Technical Conference*, Adelaide, South Australia, 3rd–8th August, 2010.

6.2.4 Industry Articles

1. Downey, M.O. and Krstic, M.P. (2005) Winegrape tannins at a glance. *Australian Viticulture*, January-February, 71.

2. Downey, M.O. and Krstic, M.P. (2005) Shedding light on shaded grapes. *Australian Viticulture*, March-April, 67-68.

3. Downey, M.O. and Adams, D.O. (2005) Practical use of a total tannin assay for red winegrapes. *Australian Viticulture*, May-June, 68-71.

4. Downey, M.O., Hannah, R. and Krstic, M.P. (2005) Managing and manipulating winegrape tannins. *Australian Viticulture*, September-October, 77.

5. Crothers, N.J. and Downey, M.O. (2006) Effect of Sun Exposure on Chardonnay Grape and Wine. *Australian Viticulture*, January-February, 19-20.

6. Downey, M.O. and Harbertson, J. (2006) Tannin Research in Washington, USA. *Australian Viticulture*, March-April, 68-69.

7. Downey, M.O. and Skogerson, K.J. (2006) Continuous Temperature Monitoring during Red Wine Fermentation. *Australian and New Zealand Wine Industry Journal*, May-June, **21**:26-30.

8. Downey, M.O. and Mazza, M. (2006) Changes in Grape Anthocyanins and Tannins During Berry Development, *Australian Viticulture*, July/August, 88-89.

9. Seddon, T. Mazza, M. and Downey, M. (2006) Which grape cultivar has the highest tannin concentration. *Australian Viticulture*, September/October, 89-91.

10. Harbertson, J.F. and Downey, M.O. (2006) Know Your Phenolics. *Australian Viticulture*, November/December, 98-100.

11. Mazza, M., Baker, N.K., Downey, M.O. and Krstic, M.P. (2007) Tannin levels in popular winegrape varieties from California. *Australian Viticulture*, May/June, 53-54.

12. Downey, M.O., Mazza, M. and Krstic, M.P. (2007) Recent Advances in Grape Tannin Research. *Australian and New Zealand Grapegrower and Winemaker*, July, 55-56.

13. Downey, M.O. and Hogg, A.S. (2007) Extending our understanding of tannin biosynthesis in grapevines. *Australian Viticulture,* July/August, 82-85.

14. Downey, M.O. and Mazza, M. (2007) Rapid determination of phenolics in red wines and musts. *Australian and New Zealand Wine Industry Journal,* September/October, 22(4):34-35.

15. Downey, M.O. and Seddon, T.J. (2007) Choose you method: Comparison of tannin levels in grape skin by different analytical methods. *Australian and New Zealand Grapegrower and Winemaker*, August, 54-56.

16. Hanlin, R. and Downey, M. (2008) Tannin accumulation during grape berry development. *Australian Viticulture*, March/April 70-71.

17. Hanlin, R. and Downey, M. (2008) Tannin composition of grape berry skin during development of Shiraz and Cabernet Sauvignon fruit. *Australian Viticulture*, July/August, 41-42.

18. Jones, L. US scientists in Mildura to continue tannin research collaboration. *Australian Viticulture*, May/June 56-57.

6.2.5 Peer Reviewed Papers

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

2. Ristic, R., Downey, M.O., Iland, P.G., Bindon, K., Francis, I.L., Herderich, M. and Robinson, S.P. (2007) Artificial bunch shading of Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research*, 13:53-65.

3. Skogerson, K.J., Downey, M.O., Mazza, M. and Boulton, R.B. (2007) Rapid determination of phenolic components in red wines from UV-visible spectra and the method of partial least squares. *American Journal of Enology & Viticulture*, 58:318-325.

4. Downey, M.O., Mazza, M. and Krstic, M.P. (2007) Development of a stable extract of anthocyanins and flavonols from grape skin. *American Journal of Enology and Viticulture*, 58:358-364.

5. Joscelyne, V.L., Downey, M.O., Mazza, M. and Bastian, S.E.P. (2007) Partial shading of Cabernet Sauvignon and Shiraz vines altered wine color and mouthfeel attributes, while increased exposure had little impact. *Journal of Agricultural and Food Chemistry,* 55:10888-10896.

6. Seddon, T.J. and Downey, M.O. (2008) Comparison of analytical methods for determining total tannin grape skin. *Australian Journal of Grape and Wine Research* 14:54.61.

7. Downey, M.O. and Rochfort, S.J. (2008) Simultaneous separation of anthocyanins and flavonols in grapes and wine by reversed-phase HPLC. *Journal of Chromatography A,* 1201:43-47.

8. Hanlin, R.L. and Downey, M.O. (2009) Condensed tannin accumulation in the skin of Cabernet Sauvignon and Shiraz grapes during berry development. *American Journal of Enology and Viticulture,* 60:13-23.

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

10. Hanlin, R.L., Hrmova, M., Harbertson, J.F. and Downey, M.O. (2010) Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine – a review. *Australian Journal of Grape and Wine Research*, 16:154-172.

11. Rochfort S, Ezernieks V, Bastian SEP, Downey MO (2010) Sensory attributes of wine influenced by variety and berry shading discriminated by NMR metabolomics. *Food Chemistry* 121, 1296 -1304.

12. Downey, M.O. and Hanlin, R.L. (submitted) A comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin. *South African Journal of Enology and Viticulture*.

13. Downey, M.O. Seddon T.J., Mazza, M., Rochfort, S. and Millikan, M. (submitted) Condensed Tannin Content, Composition and Polymer Length Distribution across 36 Grape Cultivars. *American Journal of Enology and Viticulture,*

6.2.6 Papers in prep.

1. Hanlin, R.L., Kelm, M., Willkinson, K., and Downey, M.O. (in prep.) Characterising condensed tannin distribution in Shiraz and Cabernet Sauvignon winegrapes by semi-preparative diol phase separation. *Journal of Agricultural and Food Chemistry*

2. Hanlin, R.L., Hrmova, M. and Downey, M.O. (in prep.) Isolation and characterisation of grape berry cell wall polysaccharide composition in Shiraz and Cabernet Sauvignon. *Plant Physiology*

3. Mazza, M., Downey, M.O. (in prep.) Influence of malvidin-3-O-glucoside on the solubility of condensed tannin in water, ethanol and model wine. *Journal of the Science of Food and Agriculture*

4. Baker, .N.K., Mazza, M., Goodwin, I., McClymont, L., Downey, M.O. (in prep.) Within vineyard variation in Shiraz grape berry composition. *Australian Journal of Grape and Wine Research*

5. Ebrahim, A. Millikan, M. and Downey, M.O. (in prep.) Influence of rootstock selection on Shiraz grape berry tannin, anthocyanins and phenolic content. *South African Journal of Enology and Viticulture*

6. Hogg, A.S., Mazza, M. Gendall, A., Robinson, S.P. and Downey, M.O. (in prep.) Expression of genes involved in flavonoid biosynthesis and accumulation of flavonoids in the skin of Chardonnay and Cabernet Sauvignon grapes. *Australian Journal of Grape and Wine Research*,

7. Harbertson, J.F., Parpinello, G.P., Heymann, H.G. and Downey, M.O. (in prep.) Impact of exogenous tannin additions on wine chemistry and wine sensory character. *American Journal of Enology and Viticulture,*

8. Harbertson, J.F., Hanlin, R.L., Kelm, M. and Downey, M.O. (in prep.) Characterisation of the interaction of condensed tannin with bovine serum albumin, salivary proteins and carboxy-methylcellulose. *Journal of Agricultural and Food Chemistry,*

6.2.7 Conference Proceedings

1. Downey, M.O. Mazza, M. and Krstic, M.P. (2008) Extraction of grape skin anthocyanins and flavonols for HPLC analysis. In: Proceedings of the 13th Australian Wine Industry Technical Conference, 29th July - 2nd August, 2007, Adelaide, South Australia. (Blair, R.J., Williams, P.J. and Pretorius, I.S. Eds.) pp 378. Australian Wine Industry Technical Conference Inc. Adelaide.

2. Hogg, A.S., Bogs, J., Gendall, A.R., Robinson, S.P. and Downey, M.O. (2008) Flavonoid Biosynthesis in Skins of Chardonnay and Cabernet Sauvignon. In: Proceedings of the 13th Australian Wine Industry Technical Conference, 29th July - 2nd August, 2007, Adelaide, South Australia. (Blair, R.J., Williams, P.J. and Pretorius, I.S. Eds.) pp 377-378. Australian Wine Industry Technical Conference Inc. Adelaide.

3. Seddon, T.J., Mazza, M., Millikan, M. and Downey, M.O. (2008) Variation in Tannins and Anthocyanins across Grape Cultivars. In: Proceedings of the 13th Australian Wine Industry Technical Conference, 29th July - 2nd August, 2007, Adelaide, South Australia. (Blair, R.J., Williams, P.J. and Pretorius, I.S. Eds.) pp 378-379. Australian Wine Industry Technical Conference Inc. Adelaide.

4. Seddon, T.J. and Downey, M.O. (2008) Comparison of analytical methods for determining total tannin in grape skin. In: Proceedings of the 13th Australian Wine Industry Technical Conference, 29th July - 2nd August, 2007, Adelaide, South Australia. (Blair, R.J., Williams, P.J. and Pretorius, I.S. Eds.) pp 379-380. Australian Wine Industry Technical Conference Inc. Adelaide.

5. Skogerson, K.J., Mazza, M., Downey, M.O. and Boulton, R.B. (2008) Comparison of UV-visible and visible-NIR spectroscopies in the rapid prediction of phenolic

compounds in red wine fermentations. In: Proceedings of the 13th Australian Wine Industry Technical Conference, 29th July - 2nd August, 2007, Adelaide, South Australia. (Blair, R.J., Williams, P.J. and Pretorius, I.S. Eds.) pp 335-336. Australian Wine Industry Technical Conference Inc. Adelaide.

6. Chalmers, Y.M., Krstic, M.P., Downey, M., Loveys, B.R. and Dry, P.R. (2008) Impacts of sustained deficit irrigation on quality attributes and flavonoid composition of Shiraz grapes and wine. In: 5th International Symposium on Irrigation of Horticultural Crops. Mildura, Australia, 28 August – 2 September, 2006. Acta Horticulturae, 792:163-169.

6.2.8 Student Theses

1. Hogg, A.S. (2006) Tannin biosynthesis in Cabernet Sauvignon and Chardonnay grape berries. Honours Thesis, School of Botany, La Trobe University, Melbourne.

2. Seddon, T.J. (2006) Exploring the extent of variability in tannin content and composition found in the skins of grape cultivars. Honours Thesis, School of Molecular Sciences, Victoria University, Melbourne.

3. Skogerson, K.J. (2006) Rapid determination of phenolics compounds in red wine fermentations using predictive spectral methods. Masters Thesis, School of Viticulture and Enology, University of California, Davis.

4. Hanlin, R. (2007) Seasonal changes in tannin content and composition during berry development in major commercial winegrape cultivars. Honours Thesis, School of Agriculture, Food and Wine, University of Adelaide, Adelaide.

5. Ebrahim, A. (2009) Rootstock effect on winegrape tannin content and composition. Honours Thesis, School of Molecular Sciences, Victoria University, Melbourne.

6. Hanlin R. (current) Characterisation of tannins and cell walls in winegrapes and their interactions during extraction and winemaking. PhD Thesis, School of Agriculture, Food and Wine, University of Adelaide, Adelaide.

Outcome/Conclusion

This project has expanded our knowledge of condensed tannins in grape berries. At the beginning of this project tannins had been examined in perhaps half-a-dozen varieties, with the bulk of the published research on Shiraz. This project has examined around 40 varieties and greatly increases our overall knowledge of grape tannin.

For all varieties total tannins were often measured across multiple sites and seasons, which has shown that there is considerable variation in tannin content between varieties, sites and seasons. All of the varieties examined contained tannins. For a large number of samples, the polymer composition was examined as was the polymer length distribution and the bulk of this work has been published, submitted for publication or is currently in preparation for publication.

While considerable data on tannin content and composition was generated, shortcomings in many of the analytical methods were identified. Some methods were refined and alternate methods trialled. A range of methods that generate different data for the same samples appears to be a useful approach to better understanding the detail of tannin composition and structure, and this work is ongoing. Preliminary work on rapid high-throughput methods was conducted and further development of these methods would be a valuable tool for widespread routine tannin analysis by the Australian wine industry

That tannins are variable means that theoretically the levels can be manipulated when the drivers of this variability are understood and when target levels have been established. Establishing these targets requires a deeper understanding of the sensory contribution of individual tannins to wine, the chemical characterisation of desirable wine tannin profiles and a clear understanding of the relationship between grape tannins and wine tannins.

While examining the sensory character of individual tannins was beyond the scope of this project, investigations of the viticultural and environmental drivers of tannin variability was one of the aims and this was largely addressed through a collaboration with the Soil and Water Initiative Five Sites Project. Samples from some 1,500 vines over three seasons showed clear patterns in accumulation that were stable from season-to-season and indicated underlying causes of variation within sites. This knowledge has been used at one of the sites to alter management of vine vigour and to reduce within vineyard variability. However, the observed general pattern at each site was not always apparent which demonstrated that in some years seasonal effects were as important as other influences. Indications are that the biggest seasonal driver is temperature and it is likely that specific viticultural management practices adapted both for high and low temperature years is required. Examination of other drivers such as deficit irrigation and rootstocks showed they have important effects at times but they were inconclusive as the studies were not detailed enough to examine the interactions between all drivers of berry and

wine quality. Clarifying our understanding of these factors will require further investigative effort.

While this project generated a lot of data on tannins in various grape varieties, and has expanded our general knowledge of grape tannins, there are a few major areas that were not covered by the project. Of these we have mentioned the sensory nature of tannins and tannin structure, but another area is the biosynthesis of the tannin polymer. Large variation in polymer length was observed here, but this area needs to be revisited with a view to discerning the mechanism of polymerisation, this would need to include a search for tannin mutants in wild or non-vinifera populations or production of mutants through molecular techniques.

In this project, winemaking was a relatively small component and largely focussed on wines from the spatial trials. While the spatial variation was interesting in its own right, some of the more interesting wine outcomes resulted from the opportunity provided by markedly different grape compositions from the same site being made into comparable wines. Although the sensory evaluation of these wines has not been completed, a significant observation has been the apparent affect of anthocyanin concentration on the extraction or solubility of condensed tannins in wine. This is an area that warrants closer examination and may provide an important to key to understanding the relationship between grape composition and final wine.

Exploration of this relationship commenced during this project with a thorough review of the interactions of condensed tannins with other cellular components. This review concluded that the interaction of tannins with cell wall material was likely to be the major factor influencing tannin extraction and may be the main driver of the decreasing tannin extractability during berry development reported by numerous investigators. Research in this area is ongoing as part of a PhD project undertaken during the life of this project, but which will not conclude until early 2011. Outcomes of both the review of tannin interactions and the PhD research project are expected to direct future research on tannin interactions in wine, particularly during winemaking and aging.

In summary, this project sought to rapidly capture a snapshot of the extent of variation in tannin content and composition in grape berries, to examine whatever trials were to hand to gain an insight into the possible drivers of that variation and to evaluate possible methodological approaches to routine tannin measurement such that at the end of the project, both researchers and industry would have identified some clear opportunities for future research; and perhaps have eliminated some of the less fruitful lines of investigation. Certainly the project has generated a large volume of data that allows us to contextualise tannin values as they are reported in the literature from our own investigations. What is beyond the realms of this research is a way to convert much of this information into economic opportunities.

Recommendations

This research has identified as many questions as answers with respect to managing and measuring tannins in grapes and wine and how this reflects grape and wine quality. Even so, some recommendations for the management of tannin variation in the vineyard and winery and the likely drivers for the observed variation are proposed:

- Variation within vineyards must be assessed in terms of both anthocyanin and tannin levels.
- Look at the underlying drivers of in-vineyard variation based on local knowledge of the site and other studies as appropriate (eg. soil surveys, NDVI).
- Apply management strategies, such as irrigation to control vigour, to eliminate variation where possible.
- Investigate opportunities for routine tannin and anthocyanin assessment across vineyards, rather than treating vineyard blocks as single entities.
- Generally, more is better, given that too much tannin or anthocyanin is not a widespread problem (yet).

From a research perspective there are a range of additional recommendations that identify areas for further investigation:

- The direct effect of irrigation and deficit irrigation on tannin accumulation needs to be properly and thoroughly investigated. This needs to be investigated in a background of actual plant water requirement and managing vine vigour and this in turn needs to be applied in response to actual environmental conditions, particularly temperature.
- The mechanism of polymer formation is still unknown. An effort needs to be made to screen a wider range of varieties, as well as other non-vinifera species and wild relatives to find polymer mutants, for example Calzin, a mutant that makes epicatechin homo-polymers in the flesh. Screening needs to be supported by molecular studies, part of which might also include the production of grapevine genetically modified tannin mutants.
- The relationship between grape tannin content and tannin levels in the final wine is unclear but it is not always a direct relationship. The factors that influence tannin

extractability and solubility need to be further investigated as do the winemaking strategies that can be used to manipulate tannin extraction, for example enzymatic treatments.

- Sensory characteristics of individual tannins are not well understood. If the Australian wine industry is to develop specification s for tannin that will enable greater control over product quality, a thorough understanding of the sensory character of individual tannins is required. Different tannins need to be studied in combination, tannins in combination with other wine matrix components and an understanding of the interactions between tannins and other grape and wine matrix elements, such as other tannins, anthocyanins, protein and polysaccharides (cell wall material) needs to be undertaken. The impact that enzymatic treatments have on cell walls and thus sensory character would be a useful corollary to this study.
- Sensory studies can be costly and time-consuming, particularly if undertaken on a large scale or on a routine basis. Development of alternate approaches such as the NMR-based metabolomic approach reported here should be further developed as a tool for both research and industry application.
- Rapid, high-throughput analytical methods that do not require a high level of sophistication in either the winery or the technical staff, need to be developed to facilitate routine analysis of tannins by the industry, whether this be in a grape assessment lab, at the weighbridge, press room or barrel cellar.

Appendix 1: Communication

- 11 Peer-reviewed papers published.
- 2 Peer-reviewed papers submitted for publication.
- 8 Peer-reviewed papers in preparation for publication.
- 18 Industry journal articles published.
- 4 international conference presentations
- 4 AWITC workshops.
- 2 Recent Advances in Tannin Research workshops convened.
- 28 Conference and field day posters.
- 6 Conference abstracts.

For full details refer to Results/Discussion Section 6.

Appendix 2: Intellectual Property

Nil.

Appendix 3: References

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Appendix 4: Staff

Name	Position/Role	Dates
Dr Mark Krstic	Project Supervisor	July 2005 – Nov. 2007
Dr Mark Downey	Chief Investigator	July 2005 – Jan. 2008
	Project Supervisor	Jan. 2008 – June 2010
Marica Mazza	Research Scientist	July 2005 – Jan. 2008
	Chief Investigator	Jan. 2008 – June 2010
Nardia Baker	Technical Officer	Nov. 2005 – July 2008
	Research Officer	July 2008 – June 2010
Kate Willcock	Summer Student/Casual Technical Assistant	Dec. 2009 – Feb. 2010
Jo Behncke	Casual Technical Assistant	June 2008 – June 2010
Tara Seddon	Honours Student (Victoria University)	2006
Allison Hogg	Honours Student (La Trobe University)	2006
Rachel Hanlin	Honours Student (University of Adelaide)	2008
Abdu Ebrahim	Honours (Victoria University)	2009
Kirsten Skogerson	Masters (UC Davis)	2005-2006
Rachel Hanlin	PhD Student (University of Adelaide)	2009-2011

Appendix 5: Budget Reconciliation

The End of Project Financial Statement will be forwarded at a later date, once the end of year financial year processing is completed.

END OF PROJECT FINANCIAL STATEMENT

Statement of Receipts and Expenditure

At the conclusion of each Project, the GWRDC requires a Statement of Receipts and Expenditure of GWRDC funds received in relation to the Project.

Project Title:	Viticultural management of grape tannin and anthocyanin levels to achieve desired wine quality specifications

GWRDC Project Number	Project Start Date	Project End Date
DPI 05/01	**01 July 2005**	**30 June 2010**

Financial Year (e.g. 2006/07)	**(a)** ***Funds brought forward from previous year***	**(b)** **#Approved GWRDC Budget (full year)**	**(c)** **Total budget available** *(a)+(b)*	**(d)** **Actual expenditure ($)**	**(e)** **Difference ($)** *(c)-(d)*
Year 1: 2005/2006					
Year 2: 2006/2007					
Year 3: 2007/2008					
Year 4: 2008/2009					
Year 5: 2009/2010					
TOTAL					

* "Funds brought forward" refers to any unspent GWRDC funds from the previous year and approved by the GWRDC to be spent the following year (i.e. column *(e)* from previous year).
# "Approved GWRDC Budget" is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes ☒ No ☐

*If **NO**, please provide reasons:*

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes ☒ No ☐

*If **NO,** please provide reasons:*

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative)
……

…… ……..
……
Name: *Title:*
Date: