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GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Vines to wine – linking fruit quality to wine flavour and aroma

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSP 05/04**

Principal Investigator: **Dr Paul K. Boss**

Research Organisation: **CSIRO Food Futures Flagship**

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Vines to wine – linking fruit quality to wine flavour and aroma (CSP 05/04)

Author details:

Dr. Paul K. Boss

Project Leader
CSIRO Food Futures Flagship
PO Box 350
Glen Osmond
SA 5064
Australia

Tel: 61 (0)8 8303 8614
Fax: 61 (0)8 8303 8601
Email: paul.boss@csiro.au

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Abstract

The aims of this project were to determine the links between grape composition and the volatile compounds and sensory attributes of the resultant wine. Correlative studies and microfermentation allowed grape precursors to wine volatiles and sensory predictors to be identified. It was also shown that viticultural treatments can be used to manipulate wine sensory attributes. Both pre and postveraison stages of development were found to be important for flavour development in fruit. The knowledge generated in this project will form the basis for the future development of measures of grape flavour potential and strategies for producing fruit fit for purpose.

Executive Summary

A research stream of the CSIRO Food Futures Flagship was established to study the influence of the grape on wine flavour and aroma and represented a considerable CSIRO investment in grapevine research. The aims of this project were to determine the links between grape composition and the volatile compounds and sensory attributes of the resultant wine, and to understand how to optimise grape composition and communicate this information to grape growers so that it is incorporated into their viticultural practices. Initial experiments were based on a correlative approach where grape and wine samples were obtained from matched commercial vineyards and analysed for sensory characteristics and chemical composition. Following co-investment in the project by the GWRDC, the scope was expanded to include sampling from viticultural trials for chemical and sensory analyses. This enabled the further investigation of grape-wine chemistry links and the analyses of flavour and aroma compounds in wines from several viticultural studies conducted by CSIRO researchers.

The identification of grapevine precursors contributing to wine flavour and aroma was a key outcome of the project. Several approaches were taken to meet this challenge. First, correlative studies were undertaken to identify links between grape composition and the chemical and sensory attributes of the resultant wine. These were conducted over three vintages across multiple sites in South Australia and Western Victoria. The data generated were mined for correlative relationships between sensory descriptors and both grape and wine chemical composition. Strong relationships were observed for some sensory attributes and these grape compounds are potentially measures of flavour potential in fruit. Techniques utilising model musts and controlled fermentations were also applied to identify grape-dependent wine volatiles. This approach was developed to confirm the links between grape and wine chemistry identified in the correlative study and as a means to identify wine components whose production is dependent on the composition of grape must. Significantly, these experiments have revealed that the concentration of many wine esters, previously thought to be entirely yeast-derived, can be affected by berry composition. In another approach, methods for the fractionation of organic compounds from grapes using a variety of chromatographic techniques were established. The resulting fractions were hydrolysed and the resulting volatile compounds analysed by GCMS to highlight those that contain grape-derived non-volatiles that act as precursors to wine volatiles. Those that do release volatiles have been further fractionated in an iterative process for isolation and identification of non-volatile grape components that may contribute to wine aroma. Our research has shown that this is a valid technique for the potential identification of such compounds and has confirmed the importance of grape composition in the production of yeast-derived wine flavour compounds. Eventual identification of these precursors or modulators of yeast activity will allow them to be measured in grape samples to predict the volatile composition and sensory attributes of the resulting wine.

An understanding of the timing of the synthesis of grape target compounds in the berries and the variables that affect their production will enable the development of strategies to alter their levels in berries through vineyard management, or assist in the generation of novel cultivars. This section of the research in this project was based on field trials designed to elucidate the timing of the production of grape flavour and aroma compounds. It also tested the impact of environmental or viticultural

management practices on wine flavour and aroma. The work was focussed on some of the industry's most pressing concerns, namely effects of irrigation regimes, yield and harvest timing on wine composition.

In collaboration with the precision viticulture team at CSIRO Ecosystem Sciences, we have continued to show the potential benefits that could be gained by vineyard mapping and selective harvesting of parcels of grapes, and this work has now been put in the context of wine sensory outcomes. Robust sensory effects, that is, differences that were stable across vintages, were seen in one vineyard situation where vigour and yield was mapped. However, in another vineyard where topographical differences were the main driver of vineyard variation, parcels of grapes produced wines with different sensory properties, but the nature of these differences changed from year to year.

It was shown that the timing of grape flavour compound accumulation is different depending on the class of compound and this has implications for harvest timing. Furthermore, some flavour and aroma components, such as methoxypyrazines, are synthesised preveraison. Therefore, preveraison metabolite levels may act as indicators of flavour potential in grapes and enable intervention in the field to alter berry composition postveraison. An examination of changes in wine chemical composition and sensory attributes during late ripening identified correlations which will allow the development of flavour ripeness markers in the future. Many of the sensory and chemical changes were robust across vintages, although it needs to be tested if they are also robust across regions and varieties.

Studies demonstrated that irrigation strategies and yield manipulation have the potential to alter the sensory properties of wines produced from grapes within a single vineyard block. Therefore, there is much potential for changing grape composition through vineyard management to produce grapes fit for purpose. Knowledge gained through an understanding of the biochemistry behind the changes in grape metabolism that underpins these different wine outcomes will allow defined viticultural strategies to alter wine flavour to be developed. The information gained about the changes in wine chemistry that accompany the wine sensory differences provides the starting point for future studies that will provide guidelines for growing grapes to winery flavour specifications.

The range of techniques and different intellectual approaches from analytical and separations chemists, plant physiologists, biochemists and sensory scientists gave this project a genuinely multidisciplinary approach. The research team's insights into grape chemistry have produced some novel and exciting results and have established a foundation of basic research that will eventually lead to changes in the way grapes are assessed and, eventually, grown.

Background

The future competitiveness of the Australian wine industry will depend on its ability to reliably export a high quality product at reasonable cost and to produce new wine styles suited to changing market needs. Expansion of the industry into new markets will require the generation of a product suited to the new consumers. A major determinant of consumer preference is the flavour and aroma of the wine. However, little is understood of how compounds in the grape berries contribute to the final flavour and aroma characteristics of the wine and how the biophysical environment, within-vineyard variability and management influences flavour development. There is no technology for the objective measurement of grape flavour attributes that growers and wineries can easily use to assess their product. In order to develop technologies to measure compounds contributing to consumer appeal we need to identify berry-derived compounds contributing to wine flavour and aroma.

Wine flavour and aroma is determined by a complex mixture of compounds that are derived from multiple sources during vinification (Ebeler and Thorngate 2009). Major contributions to the sensory attributes of wine come from compounds originating from grapes, from yeast and bacterial metabolism during vinification and, if used, oak wood. The complexity of the system is increased by the fact that biological transformation of compounds originating from grapes may occur due to microbial activity during fermentation, and that chemical transformations may occur in the acidic conditions found in wine. Wine style is determined by the relative concentration of compounds from each of these sources. Variables introduced during winemaking can influence wine style (Swiegers et al. 2005). For example, the use of different yeast strains has been shown to alter sensory properties of the resulting wine (e.g. Loscos et al. 2007, Torrens et al. 2008), as has the use of malolactic bacteria (Bartowsky 2005). However, grapes also have a significant impact on wine flavour attributes. At a coarse level this is evident in the ability of different grape varieties to produce wines of distinct sensory characteristics, and this will be largely due to genetic differences that lead to different chemical profiles in the berries (Dunlevy et al. 2009). At a subtle level, effects of vintage, region and vineyard management can alter wine flavour and aroma made from a specific variety (e.g. Heymann & Noble 1987, Kliewer & Dokoozlian 2005, Fang & Qian 2006, Lund et al. 2009). This suggests that environmental factors can alter berry composition, presumably through changes in gene expression, enzyme activity or the rate of chemical reactions in the berries. There is also evidence (sometimes anecdotal) that viticultural practices such as irrigation management, pruning, modification of bunch exposure and nutrition can influence grape quality and the style and value of the derived wine but virtually nothing is known about the influence of these practices on specific flavour-active compounds or their non-volatile precursors. Equally important in the determination of optimal harvest time is the question of the apparent temporal difference between the accumulation of sugars and flavour compounds which has led to a gradual increase in the alcohol content of Australian red wines as attempts are made to improve the balance of flavour components.

The complexity of wine chemistry and how this relates to the sensory properties of wine restricts studies into the impact of vineyard variables on wine style. Therefore, the determination of a compound or group of compounds that contribute to a certain flavour or aroma character is a goal for scientists trying to develop a better

understanding of how wine style is constructed chemically. Some compounds that contribute to varietal sensory characteristics have been identified such as the methoxypyrazines, which contribute vegetal or earthy aroma to certain varieties (e.g. Cabernet Sauvignon, and Sauvignon Blanc) and volatile thiols, which are important contributors to Sauvignon Blanc flavour and aroma (for review see Dunlevy et al. 2009). However, these compounds are not solely responsible for all the sensory attributes of wines made from these varieties and changes in the total volatile profile will alter consumer preference. Furthermore, the wine volatile composition depends much on the composition of the grapes used to produce that wine. A means of predicting wine style from grape composition and practical measures to alter grape composition during the growing season will help to guide the production of grapes with a chemical profile that can be used to make wines of a specified flavour profile. Therefore the identification of grapevine precursors contributing to wine flavour and aroma is a key research outcome that is essential for growing grapes fit for purpose. Determining the relationship between grape compounds and the volatiles in wine will provide chemical targets for researchers studying viticultural effects on wine composition to enable more focus to their work. It will also provide targets for the development of grape quality measures and will relate grape composition to sensory attributes defined as being important for consumer preference.

As well as developing an understanding of how grape composition affects wine outcomes, this project also sought to generate knowledge that would allow a more definitive measure of optimal harvest time based on flavour rather than sugar. Integration of such knowledge with information on vineyard variability, the effects of irrigation and yield, and relationships between the variable biophysical environment of the vineyard (soils, slope, aspect, etc) offers the potential for final wine style and quality (i.e. flavour and aroma) to be driven, at least in part, by decisions taken in the vineyard, whether during the season or at vintage. Evidence available from recent research, suggests that such a capability has the potential to offer significant commercial benefits (e.g. Bramley and Hamilton, 2005).

In summary, this project aimed to provide a breakthrough in the identification of molecules that contribute to or may act as indicators of certain flavour and aroma characteristics of wine. This would enable the development of technologies to measure the abundance of these molecules in grapes and monitor the product for its ability to match certain consumer appeal. Adoption of such technologies could aid the grape and wine industry in streaming fruit for batches of wine to be marketed to specific groups of consumers. There are also no scientific validated methods of flavour management in the vineyard that provide producers the ability to better manage the flavour potential of their grapes. Relationships between location, viticultural practices, environmental variables and grape quality are largely unknown and these studies need to include investigating the sensory characteristics of the wine produced from these grapes. Such research will also lead to better advice to industry on how to manage vineyard variability to optimise returns and eventually a more directed approach to grape-growing to achieve certain flavours as our understanding of the viticultural and environmental effects on the production of these compounds improves

Project aims and performance targets

The original project objectives were:

- Develop technologies to measure the abundance of flavour compounds and precursors in grapes, providing more objective measures of fruit quality linked to wine flavour and aroma.
- Understand the impact of viticultural management on fruit composition leading to an improved ability to grow grapes to meet desired wine specifications.
- Understand changes in fruit composition during ripening to optimise harvest timing for specific flavour levels.

These are the planned outputs as described in the original grant application:

Outputs and Performance Targets 2005-06

Outputs	Performance Targets
1. Capital equipment to advance current grape & wine flavour research purchased	Capital equipment ordered
2. Suitable sites in the GWRDC Soil & Water initiative and other GWRDC sites identified for flavour research	Sites identified and linkages established between projects.
3. Study initiated and completed into the feasibility of applying statistical and computerised methods to rapidly analyse GCMS data	Study completed and decision taken on the usefulness of the method for additional data analyses compared to existing conventional analyses.
4. Study into database development and analyses of diverse datasets	Activities initiated for determining suitable database structure and requirements for holding and analysing diverse datasets. Framework study completed. Experimental treatments, metabolite data, management and environmental information entered into database for analyses including entry of data from linkages to other projects in the Food Futures Quality Biosensors theme.
5. Berry samples collected from viticulture management treatments	Berry samples collected and processed. Some berry samples also sent to other Flagship 4B & 4A projects for additional analyses including sensory analysis using a trained descriptive panel.
6. Wine samples produced	Wine making completed. Some wine samples also sent to other Flagship 4B & 4A projects for additional analyses including sensory analysis using a trained descriptive panel
7. Sample purification methods using MLCCC initiated	At least 2 compounds purified to a point suitable for further analysis
8. Measurement of compounds	Measurement of carotenoids, volatile and non-volatile flavour and aroma compounds in grape berries, leaves and wine

Outputs and Performance Targets 2006-07

Outputs	Performance Targets
1. Berry developmental samples collected	Berry samples collected and processed. Some berry samples also sent to other Flagship 4B & 4A projects for additional analyses.
2. Berry samples collected	Berry samples collected and processed. Some berry samples also sent

from viticulture management treatments	to other Flagship 4B & 4A projects for additional analyses
3. Wine samples produced	Wine making completed. Some wine samples also sent to other Flagship 4B & 4A projects for additional analyses
4. Measurement of compounds	Measurement of volatile and non-volatile flavour and aroma compounds in grape berries, leaves and wine
5. Non-volatile precursor list extended	Compounds functionally characterised following MLCCC, HPLC-MS, and hydrolytic analysis
6. Volatile list extended	Compounds functionally characterised following GCMS
7. Sensory analysis obtained	Sensory analyses obtained for linking to chemical data and experimental treatments.
8. Database development and analyses of diverse datasets	Experimental treatments, metabolite data, management and environmental information entered into database for analyses including entry of data from linkages to other projects in the Food Futures Quality Biosensors theme.
9. Extension and communication	Presentation to industry forum and paper submitted to industry journal

NB: There were some delays in the completion of 06/07 project outputs 4, 5 and 6 due to the late appointment of a postdoctoral Fellow and a Technical Officer. The resignation of "in kind" staff also caused some delays while those positions were being refilled. The extension and communication output (9) was be fulfilled at the 13th AWITC in July 2007.

Outputs and Performance Targets 2007-08

Outputs	Performance Targets
1. Berry developmental samples collected	Berry samples collected and processed. Some berry samples also sent to other Flagship 4B & 4A projects for additional analyses.
2. Berry developmental series -analytical	Berry samples collected and characterised according to standard berry descriptors and content of key flavour compounds
3. Berry development series – mini-ferments	Berries of advancing physiological age adjusted to uniform sugar/acid and vinified
4. Berry samples collected from viticulture management treatments	Berry samples collected and processed. Some berry samples also sent to other Flagship 4B & 4A projects for additional analyses.
5. Wine samples produced	Wine making completed. Some wine samples also sent to other Flagship 4B & 4A projects for additional analyses
6. Measurement of compounds	Measurement of volatile and non-volatile flavour and aroma compounds in grape berries, leaves and wine
7. Non-volatile precursor list extended	Compounds functionally characterised following MLCCC, HPLC-MS, and hydrolytic analysis
8. Volatile list extended	Compounds functionally characterised following GCMS
9. Sensory analysis obtained	Sensory analyses obtained for linking to chemical data and experimental treatments.
10. Database development and data analyses	Experimental treatments, metabolite data, management and environmental information entered into database for analyses including data from linkages to other projects in the Flagship Quality Biosensors theme.
11. GCO used to assist in identification of key flavour compounds	GCO data obtained to link flavour character as perceived by an individual to a specific volatile compound separated by GCMS

NB: Outputs 7 and 11 were delayed due to the late start of a new postdoctoral fellow, Rob Keyzers. He was appointed in late January 2008 and made significant progress towards the separation and identification of non-volatile grape components that may

contribute to wine flavour and aroma (output 7). This output actually is a continuing milestone that extends through next year's outputs as well, and so achievement of the performance target is on track. GCO (output 11) was conducted in collaboration with Food Science Australia (now CSIRO Food and Nutritional Sciences).

Outputs and Performance Targets 2008-09

Outputs	Performance Targets
1. Berry developmental samples collected	Berry samples collected and processed. Some berry samples also sent to other Flagship 4B & 4A projects for additional analyses.
2. Berry developmental series -analytical	Berry samples collected and characterised according to standard berry descriptors and content of key flavour compounds
3. Berry development series – mini-ferments	Berries of advancing physiological age adjusted to uniform sugar/acid and vinified
5. Wine samples produced	Wine making completed. Some wine samples also sent to other Flagship 4B & 4A projects for additional analyses
6. Measurement of compounds	Measurement of volatile and non-volatile flavour and aroma compounds in grape berries, leaves and wine
7. Non-volatile precursor list extended	Compounds functionally characterised following MLCCC, HPLC-MS, and hydrolytic analysis
8. Volatile list extended	Compounds functionally characterised following GCMS
9. Sensory analysis obtained	Sensory analyses obtained for linking to chemical data and experimental treatments.
10. Database development and data analyses	Experimental treatments, metabolite data, management and environmental information entered into database for analyses including data from linkages to other projects in the Flagship Quality Biosensors theme.
11. GCO used to assist in identification of key flavour compounds	GCO data obtained to link flavour character as perceived by an individual to a specific volatile compound separated by GCMS
12. Flavour re-creation	Flavour recreation to validate identification of key flavour compounds using chosen clusters of volatile compounds from the berries. Base matrix for experiments defined and at least 2 target compounds defined.
13. Extension and communication	Presentation to industry forum and paper submitted to industry journal
14. Industry applicability of measurement technologies	Targeted measurements made in a field situation and applied to problems relevant to industry

NB: Output target 11 "flavour re-creation" was not achieved and was flagged as such in the appropriate annual report. When the grant was written it was predicted that this would be an important demonstration of the link between the sensory attributes of berries and their chemical composition. However, it became obvious during the project that berry sensory data is not a good predictor of wine sensory attributes, and so we have focused more on developing methods to investigate how grape-derived compounds are transformed during wine-making.

Outputs and Performance Targets 2009-10

Outputs	Performance Targets
1. Industry applicability of measurement technologies	Targeted measurements made in a field situation and applied to problems relevant to industry
2.Flavour findings across vintages assessed	This will validate the work over the life of the project and will highlight the key areas to allow an extension of the findings beyond the project life
3. Models of wine flavour from	Combine viticultural, analytical and sensory information to determine

precursors to consumption developed	interrelationships
4. Final report produced and recommendations to industry finalised	All data analysed and key linkages between grapes and wine flavour determined and summarised and communicated to industry in a Final GWRDC Report.

NB: In May 2010 an extension of project until June 2011 was agreed to with a re-scheduling of the target dates for the 09/10 outputs until June 10/11.

Output 3 was delayed. While we had gathered the appropriate data from field studies involving yield, vigour and irrigation, we had only produced wine from the third vintage of a study into harvest timing in early 2010, and this was an integral part of this output. As the analyses of the wines involved subsequent sensory and chemical profiling, these experiments were not completed until early 2011. The revised target date for this output is June 2011.

Method

Introduction

The methods used in this project are outlined below. Some have been used in several of the subprojects that are described in this report. While much of the field-based work utilised already established skills and methodologies one of the initial tasks of the project was to develop analytical chemistry methods to compliment the capabilities in viticulture, plant physiology and biochemistry already present in the CSIRO teams. As the initial target compounds were volatile, gas chromatography mass spectrometry (GCMS) techniques were established in the laboratory. In general, these have involved the use of solid phase microextraction (SPME) fibres to extract the volatile compounds from the headspace of grapes and wine which are then separated and quantified using GCMS. The development of these methods was the subject of a peer reviewed manuscript (Kalua and Boss, 2008). We have also used the stirbar-sorptive microextraction (SBME) technique to analyse volatiles and semi-volatiles in grapes, and stable isotope dilution assays (SIDA) to quantify methoxypyrazines in grapes and wine. The research group now has a suite of analytical methods to use for the analysis of volatile compounds grapes and wine and an extensive collection of standards for compound identification and quantification.

Experiments designed to identify and/or confirm links between grape metabolites and wine volatile compounds have also required the establishment of new techniques in the Adelaide laboratory. For the natural products chemistry/separations chemistry approach to the identification of grape precursors, chromatographic techniques, including both normal- and reversed-phase stationary phases, in low, medium and high pressure systems (LP-,MP-, HPLC and MLCCC) have been developed for fractionation of grape extracts. We have also established the use of model must microfermentation techniques for the identification of fractions containing volatile precursors. This technique has also proven useful for studying the impact of grape composition on wine composition by analysing dilutions of grape juices or different berries taken throughout development.

Our collaborators at CSIRO Food and Nutritional Sciences established trained panel sensory techniques for grapes and wine, as well as utilising a winemaker panel chaired by Louisa Rose from Yalumba to analyse the same samples. In order to provide materials for this work, a method for the production and storage of grape homogenates was developed by CSIRO Plant Industry in Adelaide. Suitable statistical treatments for these comparisons have been generated by Emlyn Williams, a statistician at the Australian National University. Winemaking and other sensory tests were conducted by the service provider Provisor, and their methods are included here for reference.

Plant Material

Each subsection of work described in the next chapter utilised vines and fruit from various sources and these are listed below. While the assistance of the many wine companies who most generously allowed us to sample fruit from their vineyards is greatly appreciated, their names are not included in case they do not wish their vineyards, fruit and wine to be identified by third parties.

Subsection A1: Cabernet Sauvignon grapes were sourced from commercial vineyards from viticultural regions in South Australia and Victoria namely; Barossa Valley (BV), Coonawarra (CO), Eden Valley (EVA and EVB), Langhorne Creek (LCA & LCB), Mildura (WH & WL) and the Riverland (RL). The LCA and LCB samples were obtained from two blocks from the same vineyard located within 100 metres of each other, but which had historically been graded at different quality levels. The two samples obtained from Mildura were from high (WH) and low (WL) vigour regions of a single vineyard that were classified by remote sensing (Bramley and Hamilton, 2007). The EVA and EVB samples were obtained from different vineyards in Eden Valley located less than two kilometres from each other. Samples were obtained from these sites across three vintages: 2003/04 (BV, EVA, EVB, LCA, LCB & RL), 2004/05 and 2005/06 (EVA, LCA, LCB, RL, CO, WH & WL).

Grapes were hand-harvested at commercial maturity where it was aimed to keep the soluble solid levels in the berries between 13-15 ºBaumé. Approximately 200 kg of whole bunches was obtained from each vineyard site by randomly selecting bunches from vines throughout a block. Bunches were brought back to the laboratory and randomly distributed into three 60 kg replicates for winemaking. A 10kg subsample was also selected at this time for processing into a uniform sample for chemical and sensory analysis. To produce this sample, whole grapes were squeezed into cooled stainless steel containers to remove the flesh and seeds from the peel. The flesh/seed mix was then strained in order to separate the flesh and juice from the seeds and the seeds were then discarded. The juice and flesh was added back to the berry skins and blended into a smooth homogenate. Aliquots of the slurry were sealed in cut-down cask bladders and stored at -80°C until further use.

Subsection A2: Berries from the cultivar Riesling were machine harvested from a commercial vineyard in Eden Valley, South Australia, in the 2006 vintage. Bunches were de-stemmed and pressed and the free-run juice settled at 4°C for four days after the addition SO_2 (50 ppm). Aliquots were flash frozen in cut-down wine cask liners using liquid N_2 and stored at -80°C until required. Bunches of Cabernet Sauvignon berries, grown in a commercial vineyard in Waikerie, South Australia, were collected by hand in the 2008 vintage. Berries were de-stemmed by hand and flash frozen in liquid N_2 before storage at -80°C until needed. When required, Cabernet Sauvignon berries were ground in a blender under liquid N_2 after which SO2 (50 ppm) was added. The resulting powder was allowed to thaw at 4°C overnight after which it was centrifuged (4000 x g for 15 mins) to remove pomace (ground seeds, skins, pulp etc), producing clarified juice for fermentation.

Subsection A3: Riesling juice from Subsection A2 above was used for the experiments in this section that required whole juice.

Subsection A4: Cabernet Sauvignon grapes were harvested from Willunga, South Australia in March 2008 and 2010 and Riesling grapes were harvested from Charleston, Adelaide Hills, South Australia in March 2008 and 2010. After transportation, bunches were stored overnight at 4°C before the berries were de-stemmed and flash frozen in liquid N_2. Frozen berries were stored at –40°C until further use.

Subsection A5: Shiraz and Cabernet Sauvignon grapes were handpicked from Barossa valley (Nuriootpa, South Australia) and Riverland (Oxford Landing, South Australia), respectively, and transported to the winery in Adelaide, South Australia. Upon receipt, the grapes were kept in a cold room (4°C) until the following day. Independent replicated fermentations (3 x 50 kg) from crushed Shiraz grapes (°Brix = 27.2, pH = 3.9) and Cabernet Sauvignon grapes (°Brix = 24.1, pH = 3.6) were carried out under small-lot commercial conditions as outlined below.

Subsection B1: Cabernet Sauvignon grapes were sampled in triplicates (200 g per field replicate) from Willunga, South Australia. Berries collected in 2006-07 vintage season (Cab07) were 5-10 m away from Eucalyptus trees. The 2006-07 vintage, sampling served as a preliminary study to assess the sampling start-time and sampling interval. In the 2007-08 vintage, Cabernet Sauvignon grapes were collected from an adjacent block at different distances from Eucalyptus trees, 5-10 m away for Cab08Near and 240-250 m away for Cab08Far, to assess the effect of Eucalyptus trees proximity on volatile compounds evolution during berry development. Grape berries were collected fortnightly from two weeks post-flowering (2wpf). Weeks post-flowering (wpf) was counted from the time of a minimum of eighty percent cap-fall. Grape berries, still on rachis, were transported to the laboratory on ice for berry weight and total soluble solids (°Brix) measurements. In the laboratory, berries were removed from their rachis and immediately flash-frozen in liquid nitrogen prior to storage in a -80°C freezer. Samples were kept in the freezer until the end of the vintage season when analysis for volatile compounds commenced.

Riesling and Cabernet Sauvignon grapes were sampled in the 2007-08 vintage in triplicate (200 g per field replicate) from a commercial vineyard at Waikarie, South Australia. Grape berries were randomly sampled from different grapevines (n>100 grapevines) at fortnightly intervals from 2 weeks post-flowering (2wpf). Weeks post-flowering was counted from the time of a minimum of eighty percent cap-fall. Both Riesling and Cabernet Sauvignon were grown in vineyard blocks in close proximity to each other. Berries were sampled and stored as outlined in the section immediately above.

Subsection B2: Cabernet Sauvignon berries were obtained from a commercial vineyard in the Sunraysia region of north-west Victoria. The vines were own-rooted and irrigated. Grapes were hand-harvested at five different stages of maturity between February 6 and March 5 in 2008, February 6 and March 24 in 2009 and between February 4 and March 3 in 2010. Approximately 300 kg of whole bunches was obtained at each harvest date by randomly selecting bunches from vines throughout the block. Bunches were brought back to the laboratory and randomly distributed into six 50 kg replicates for winemaking with and without sugar addition and a subsample frozen in liquid N_2 and stored at -80°C pending further analysis.

Subsection B3: The Cabernet Sauvignon berries for this study were obtained from the same vineyard as those obtained for subsection B3 above. However, for this experiment, k-means clustering of data underlying a yield map of the block and PCD imagery obtained in 2004 and 2005 (Bramley and Hamilton 2005) was used to identify zones of low or high yield/vigour. These areas were used for subsequent sampling of vines for assessment of vine and fruit attributes and small-lot winemaking in the 2005, 2006 and 2007 vintages.

Subsection B4: The Riesling grapes and juice used in this study came from a single vineyard in Eden Valley. The range of elevation amongst the Riesling vines is approximately 35 m, and the grapes were normally harvested into a number of parcels, six of which were included in the experiments described in this report. Samples were obtained as free-run juice from the commercial partner in the 2005 and 2006 vintages.

Subsection B5: Two sites have been used for this work. The first is a commercial vineyard in Sunraysia with a one hectare experimental plot within a larger block of own-rooted Cabernet Sauvignon vines. Three drip irrigation treatments have been imposed since 2002: a well-watered control, regulated deficit irrigation (RDI) and a prolonged preveraison deficit (PD). The PD treatment represents an irrigation strategy where a standard regulated deficit irrigation regime (RDI) was extended in both time and severity, with a two to three week period of no irrigation immediately following the end of the RDI period. The second utilised twelve year old Cabernet Sauvignon vines on Ramsey rootstocks made available for irrigation trials at a commercial vineyard in Waikarie in the South Australian Riverland. In seasons 2006-2007, 2007-2008 and 2008-2009 the vines were irrigated from mid September until after berry harvest. Five different irrigation treatments were applied to the vines in groups of five rows, one central (experimental) row and two buffer rows on each side. Irrigation rates varied from year to year, but included the standard vineyard rate, one treatment double that and three treatments with less than the standard rate. For both irrigation experiments the grapes were hand-harvested at commercial maturity where it was aimed to keep the soluble solid levels in the berries between 13-15 ºBaumé. Approximately 200 kg of whole bunches was obtained from each vineyard site by randomly selecting bunches from vines throughout an appropriate treatment section of the block. Bunches were brought back to the laboratory and randomly distributed into three 60 kg replicates for winemaking.

Subsection B6: This trial manipulated fruit yield using Cabernet Sauvignon vines at a commercial vineyard at Willunga in the Southern Vales of South Australia. To alter yield, inflorescences were removed immediately prior to cap-drop to produce vines of high, medium and low fruit yield, thereby having no secondary effect on vine canopy. The “high” treatment was effectively the control as vines were left to produce their normal crop load of approximately 130 bunches per vine. The “medium” treatment aimed to reduce this to ~ 60 bunches and the “low” treatment to ~30 bunches. The high and medium treatment consisted of 18 vines and the low treatment consisted of 36 vines randomly assigned across three rows of the vineyard plot. Samples were taken from each treatment at two week intervals from 1 to 15 wpf. Berries were separated from the stems and immediately frozen in liquid nitrogen and stored at -80°C. For each sample, 30 fresh berries were randomly selected for measurement of berry weight and total soluble solids (°Brix) at each time point. At harvest, bunches were brought back

to the laboratory and randomly distributed into three 60 kg replicates for winemaking.

Gas chromatography-mass spectrometry (GC-MS)

Several GC-MS methods were employed during this project depending on the nature of the material being analysed. These techniques are used for the identification and quantification of volatile and semi-volatile compounds which are the compounds that contribute to the flavour and aroma of grapes and wine as perceived in the olfactory bulb.

Headspace volatile analysis (SPME-GC-MS) of grapes.

The frozen grapes, sampled as described above, were ground to powder with addition of liquid nitrogen and 7.5 g of grape powder was then transferred into a 20 mL vial (Supelco, Bellefonte, PA, USA). An internal standard (20 µL D_{13}-hexanol; 920 mg/L) was immediately added. Vials were sealed and placed in a cold room (4 °C) overnight for cold stabilization and equilibration of volatile compounds formation.

Solid-phase microextraction – gas chromatography – mass spectrometry (SPME-GC-MS) was used to analyse volatile compounds based on our previous methods (Kalua and Boss 2008) using a Hewlett-Packard 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler. The Gerstel MPS2 autosampler was operated in the SPME mode with a divinylbenzene-carboxen-polydimethylsiloxane fiber (2 cm, 23-Gauge, 50/30 µm DVB-CAR-PDMS fiber, Supelco). Volatile compounds were extracted with sample agitation (250 rpm) for 30 min at 40°C with a prior incubation time of 5 min. The injection temperature was 220°C in split-less mode for 3 min and thereafter the fiber was cleansed in split mode for 7 min at the injection port before re-use. The injection port was lined with a 0.75 mm ID Supelco glass liner. Separation was achieved on a ZB-Wax column (length 30 m, 0.25 mm ID, film thickness 0.25 µm) using helium carrier gas at a flow rate of 1.5 mL/min (constant flow). The column temperature program was as follows: 35°C for 0.5 min, increasing at 7.0°C/min to 245°C with a final isothermal period of 4.5 min (total run time = 35 min). The temperature of the transfer line, interfacing the GC and MS, was set at 250°C. Positive ion electron impact spectra at 70 eV were recorded in the scan mode in the range of m/z 35 – 350 (4.46 scans/s).

The identity of detected volatiles was determined by comparing mass spectra with those of authentic standards and spectral libraries. A laboratory generated library (328 compounds) as well as the US National Institute of Standards and Technology-05a (NIST-05a) and the Wiley Registry 7th Edition mass spectral libraries were used for identification purposes. Compounds were considered positively identified after matching of both mass spectra and linear retention indices (LRI) with that of authentic samples. LRI was calculated from a compounds retention time relative to the retention of a series of n-alkanes (C10-C26). The peak area of each detected compound (selected ions used) in each sample was corrected relative to the amount of internal standard (D_{13}-hexanol) added.

Headspace volatile analysis (SPME-GC-MS) of wines.

SPME-GC-MS was used to analyse the volatile constituents of the wines produced from the small scale winemaking or fermentation of the model must/grape juice mixtures produced in the laboratory. Aliquots were analysed at two different concentrations, 1 in 100 or 1 in 2 diluted with H_2O to a final volume of 10 mL. In all cases, NaCl (3 g) was added to each SPME vial (20 mL) prior to sample addition. Samples were spiked with D_{13}-hexanol as an internal standard (1 in 100 dil.: 1.15 µg; 1 in 2 dil.: 9.20 µg) prior to SPME-GC-MS analysis.

SPME-GC-MS was carried out using an Agilent 6890 gas chromatograph equipped with a Gerstel MP2 auto-sampler and using an Agilent Technologies 5973N mass spectrometer for peak detection and compound identification. The auto-sampler was operated in SPME mode usually utilizing a divinylbenzene-carboxen-polydimethylsiloxane fiber (2 cm, 23-Gauge, 50/30 µm DVB-CAR-PDMS, Supelco, Bellefonte, Pennsylvania, USA) for extraction, although other fibers were used on some occasions. Volatile compounds were extracted using agitation (250 rpm) at 35°C for 90 mins. Chromatography was performed using a ZB-Wax column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm) using helium as a carrier gas at 1.2 mL/min (constant flow). Volatiles were desorbed from the fiber in the GC-inlet (220°C) for 1 min and separated using the following temperature program: 35°C for 1.5 min, increasing at 7°C/min to 245°C, held isothermally at 245°C for 4.5 min. The temperature of the transfer line connecting the GC and MS was held at 250°C. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range: m/z 35-350, scan rate: 4.45 scans/s). The identification of detected volatiles and quantification were carried out as outlined in the method section immediately above.

"Twister" volatile analysis (SBSE-GC-MS) of grapes.

The grape homogenates, prepared as described above, were removed from -80°C storage and thawed for 3 h before use. A 5 g of aliquot of each homogenate was transferred to a 15 mL screw cap glass vial with an aluminium liner and 2g of NaCl added. Two internal standards were added to the homogenates; 10 µL of 1.04 g L^{-1} (*E*)-2-pentenal and 10 µL of 0.96 g L^{-1} 4-methyl-2-pentanol. The grape slurries were then stirred with a PDMS-coated stir bar (0.5 mm film thickness, 10 mm length, Twister; Gerstel, Mülheim an der Ruhr, Germany) for 1 h at room temperature at 1000 rpm using a Gerstel twister stirrer. The stir bar was then removed from the sample, rinsed with distilled water, dried with lint-free cloth, and transferred into a thermal desorption tube.

In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: desorption temperature, 240°C; desorption time, 5 min; cold trap temperature, −150°C; helium inlet flow, 24 mL min^{-1}. The desorbed compounds were then separated in a Hewlett-Packard 6890 gas chromatograph coupled to a 5973N mass spectrometer (Palo Alto, CA). The GC was fitted with a 30 m, 0.25 mm internal diameter, 0.25 µm ZB-Wax capillary column (Phenomenex, Sydney, Australia). The carrier gas was helium (Ultrahigh Purity; Air Liquide, Adelaide, Australia) and the flow rate 1.2 mL min^{-1} (constant flow). The oven was held at an initial temperature of 50°C for 1 min, then increased to 240°C at 5°C min^{-1}, and held at this temperature for 10 min. The mass spectra were recorded in scan mode in the range of 35-350 amu. The identification of detected volatiles and quantification were carried out as outlined in the grape SPME-GC-MS section above.

Natural products/separations chemistry techniques

Berry extractions

Samples of Cabernet Sauvignon and Riesling berries (~1.5 kg of each) were extracted before subsequent fractionation. The frozen berries were ground using a 5L pre-cooled industrial blender. Acetone (500 mL) was added to the resulting pulp and left to extract for 24 h at 4°C. The supernatant was filtered three 3MM paper and stored at 4°C (extract 1). The remaining solids were further extracted for 24 h with acetone (250 mL), then filtered and stored as above (extract 2). A final acetone extraction (250 mL) was conducted on the remaining solids with acetone for 24 h. The supernatant was collected by filtration and stored (extract 3).

Cyclic loading and fractionation of grape extracts.

HP-20 beads (~250 mL) were loaded into a glass chromatography column and then sequentially equilibrated with methanol and acetone (800 mL of each). Grape extracts 2 and 3 from above were combined and passed through the column. The collected eluent was diluted with water (500 mL) to make an ~ 50% aqueous acetone solution. This was passed through the column again and the collected eluent was further diluted with water to make an ~25% aq. acetone solution. Extract 1 was diluted to ~25% aq. acetone and combined with the diluted extracts 2 and 3. This solution was passed through the column again. The eluent was further diluted to ~10% aq. acetone and passed through the column one final time. The eluent was collected and stored at 4°C. The loaded column was then washed with water (800 mL), before being sequentially eluted with 20%, 40%, 60%, 80% aq. acetone, followed by a 100% acetone elution and finally an ethyl acetate elution (800 mL of each). Each eluate was collected separately to provide 5 distinct fractions (the final acetone and ethyl acetate washes were collected together), which were stored at 4°C prior to analysis.

A portion of each fraction (300 mL) was evaporated under reduced pressure to remove organic solvents, and then freeze-dried to provide dry, solid subsamples of each fraction. The solid samples were then acid hydrolysed using the method of Janusz et al.(2003) or added to musts in micro-scale winemaking experiments (see below) to identify suitable precursor fractions. Suitable fractions were then further fractionated using a combination of HPLC and MLCCC chromatographic separation protocols to work towards the isolation of individual components from the mixtures.

Micro-scale winemaking

Media

Synthetic media were prepared based on recipes previously published (e.g. Henschke & Jiranek 1993, Bely et al. 1990, Varela et al. 2004) with slight modifications. D-Glucose and D-fructose (at concentrations to match levels in the respective grape juices used for various experiments), 5 g D/L-malic acid, 5 g tartaric acid, 1.7 g yeast nitrogen base (YNB) without ammonium sulphate growth medium (MP Biomedicals, Santa Ana, CA, USA), 0.2 g citric acid, 15 mg ergosterol, 5 mg sodium oleate, 2 mg nicotinic acid and 0.5 mL Tween 80 were dissolved in 1 L water. The pH of the resulting medium was corrected to match that of the grape juice by addition of KOH. The synthetic medium was sterilized by filtration (0.20 µm Disposable sterile filter units, Nalgene, Rochester, New York, USA). Ammonium chloride (15 g) was dissolved in water (250 mL) and sterilized by filtration.
Nutrient deficient must was prepared in a similar manner using 120 g each of D-glucose and D-fructose, 5 g D/L-malic acid, 5 g tartaric acid, and 0.2 g citric acid dissolved in 1 L water after which the pH was corrected to 3.2 by addition of KOH. The must was sterilized by filtration.

Yeast

Yeast starter cultures were prepared by adding ~0.25 g of wine yeast (strain EC1118, Prise de Mousse, Lallemond, Australia) to synthetic medium (25 mL), which was incubated (28°C) overnight with shaking. The culture was then spun at ~5000 × g for 5 min and the pellet of yeast washed with sterile water. This was repeated twice before resuspending the yeast in sterile water to OD_{600} = 1.

Fermentation

All ferments (50 mL) were prepared under sterile conditions. Increasing amounts of grape juice was added to synthetic medium (0, 5, 10, 20, 50 and 100% v/v grape juice) after which ammonium chloride (15 mg) and Synthetic Complete (Hopkins) amino acid supplement mixture (400 mg, Sunrise Scientific Products, San Diego, CA, USA) was added to all the medium/juice mixtures. Each ferment was then inoculated with yeast starter culture (1 mL, adjusted to 2.0 AU at 600 nm by addition of synthetic media). Water air-locks were used to maintain an anaerobic environment. Fermentations were allowed to proceed to dryness at 22°C until no further mass loss was noted. Wines were harvested by removing yeast cells by centrifugation (615 × g for 2 mins).

Small-scale winemaking

Red wine

Small scale wine lots were produced according to the following protocol by the Provisor winemaking services (Adelaide, South Australia). Grapes (50kg) were crushed and de-stemmed and SO_2 levels adjusted to 40 ppm by the addition of $K_2S_2O_5$. Samples of must were analysed for pH, titratable acidity and °Baumé and pH adjusted to between 3.3 pH and 3.7 pH using tartaric acid if required. The assimilable nitrogen content of the must was supplemented by the addition of 200 ppm $(NH_4)HPO_4$, and

yeast strain EC1118 (Lallemand Australia, Adelaide, South Australia) was inoculated into the must at a concentration of 200 ppm. Fermentation was carried out on the skins with an aim to reduce sugar levels by 1-2 °Baumé per day with temperatures adjusted accordingly. In general, the ferments were conducted at 18-20°C and the cap was plunged twice a day. Ferments were drained and pressed when the °Baumé reached 2°, and the free run juice and pressings further fermented to dryness when the wine was then racked off the gross lees. The SO_2 levels were adjusted to 40 ppm by $K_2S_2O_5$ addition to prevent spoilage and malolactic fermentation and the wine cold stabilised at 0°C for 21 d. The wine was then racked off fining lees, SO_2 levels adjusted to 80 ppm with $K_2S_2O_5$, filtered through a 45 µm membrane and bottled in 375 mL bottles using screw-cap closures.

White wine

Small scale wine making was conducted by the Provisor winemaking services (Adelaide, South Australia) in triplicate using the following protocol. Harvested fruit were placed at 0°C for 12 h, the SO_2 levels were adjusted to 80ppm during crushing and de-stemming using $K_2S_2O_5$. The grapes were immediately drained and pressed, and SO_2 levels readjusted to 25 ppm with $K_2S_2O_5$ and pectinase added at a rate of 30 µL/L (Ultrazyme-CPL; Novozymes, Bagsvaerd, Denmark). Yeast strain EC1118 was added to 250ppm, 150ppm diammonium phosphate was added and tartaric acid added where required to normalize must pH to 3.1-3.3. The fermentations were conducted at 12-16°C, and diammonium phosphate added to a maximum of 500ppm as required. When the must was fermented to dryness it was racked off gross lees, $K_2S_2O_5$ added to 60ppm and the wine was cold stabilised at 0°C for 21 days. The wine was again racked and SO_2 levels adjusted to 25 ppm with $K_2S_2O_5$ before filtering followed by bottling with Stelvin closures.

Sensory analysis

Difference testing

Difference tests were performed with a panel of untrained assessors using the constant reference duo-trio method (Meilgaard et al. 1991). The assessors were Provisor staff and other staff located around the University of Adelaide Waite campus with experience in previous difference tests. In each test comparison, the first sample presented was a reference sample, followed by two other samples, one of which was identical to the reference. The instructions to panellists were to smell and taste the samples and identify the same sample as the reference. The panellists were also asked to comment on the reasons for their choice. Three 375 mL bottles of each fermentation replicate from the vineyard treatments were carefully combined and blended into a large glass bottle to make a representative sample of each wine treatment. The wine samples were presented in three-digit coded ISO standard tasting glasses (30 mL) and assessed at room temperature under white fluorescent lighting.

Descriptive sensory analysis

Descriptive sensory analysis was conducted at the sensory laboratories of CSIRO or Provisor, both of which comply with international standards for the design of test rooms (ISO 8589: 1988). Descriptive analysis was conducted by Provisor's or CSIRO's trained sensory panel which consisted of members that had been screened for sensory acuity. For each sensory profile the panel underwent 12 two hour training

sessions with wine samples. The panel assessed the wines using a standard wine assessment protocol to ensure uniformity in the assessment procedure (Jackson 2002). Processed white bread/water biscuits and water were consumed between samples to minimise carryover effects and an inter-stimulus interval of four minutes was chosen as a suitable time between samples. Using a standard approach the panel generated a standard list of vocabulary terms to profile the differences between the wines for appearance, aroma, flavour, texture and aftertaste of the wine samples (ISO 8586-1:1993). Reference standards were developed to help clarify some of the sensory attributes and ensure full agreement across assessors. For each year, descriptive analysis was carried out in triplicate with panel members tasting up to 7 samples per day. Panellists received a sample volume of 30 ± 1.5ml served at room temperature in 214ml standardised tasting wine glasses (ISO 3591:1977). Each wine glass was covered with a glass petri dish cover to prevent headspace loss and samples were poured immediately before serving to the assessor. Samples were blind-coded with random 3-digit and the order of sample assessment was randomised to account for first order and carryover effects. The experimental design was produced using the design generation package – CycDesigN (Release 2.0; CycSoftware, Hamilton, New Zealand). Attributes were rated on 100mm unstructured line scales anchored at 5 and 95%, respectively, with extremes for each descriptive term. Data were recorded and stored using the Compusense sensory data acquisition software (Version 4.6, 2004; Compusense Inc., Guelph, Ontario, Canada).

Results and discussion

Further details for some subsections of this chapter are available. As noted in the text, interested persons should contact GWRDC for more information.

A. Links between grape and wine composition

The identification of grapevine precursors contributing to wine flavour and aroma was a key outcome of this work. Determining the relationship between grape compounds and the volatiles in wine will ultimately provide chemical targets for research teams studying viticultural effects on wine composition (see section B below) to enable more focus to their work. It will also provide targets for the development of tools to measure grape flavour potential and will relate grape composition to sensory attributes defined as being important for consumer preference. Due to the importance of this work, several approaches were being taken to meet this challenge and these are outlined below.

Subproject A1: Identification of associations between the sensory attributes and volatile composition of Cabernet Sauvignon wines and the volatile composition of the grapes used for their production.

The work presented in this section has been published in the manuscript Forde et al. (2011).

In this study we set out to identify associations between grape chemical composition, wine chemical composition and wine sensory attributes for the variety Cabernet Sauvignon. In order to obtain grape and wine samples with a broad range of chemical and sensory characteristics, experiments were conducted for three vintages across multiple vineyards in both South Australia and Victoria. Amongst the samples were two taken from one vineyard where regions were differentiated based on measurements of vine vigour (Bramley and Hamilton 2007), and another two were taken from two blocks in a single vineyard that were separated by less than 100 m, but which had been consistently graded at different quality levels. In all but two cases samples were taken from the same site for two vintages or more. The winemaking procedure was controlled across the three vintages to minimise fermentation variables, and no malolactic fermentation was conducted on the small scale wines to remove this variable from the study. Thus the sample set encompasses a range of Cabernet Sauvignon wines with the differences being due predominantly to inter-vineyard and intra-vineyard variation as well as vintage effects.

Thirty two wine sensory attributes were scored across the wines using a trained panel. Of these, two were visual attributes, ten were odour and ten were flavour characteristics, eight related to aftertaste and two measured wine body (mouthfeel). Visual and wine mouthfeel attributes were measured although they are unlikely to be directly influenced by volatile compounds. However, it is possible that our study may identify volatile compounds that are associated with these sensory characters and thus

act as predictors in the grapes or wine. Any relationship may also indicate common control of the production of non-volatile and volatile metabolites contributing to specific wine sensory attributes. Eleven of the other attributes did not show any association with volatile metabolites measured in either the berries or the wine.

Using SBSE we quantified 48 compounds (Table 1) in the 20 wine slurries. Methoxypyrazines are known to be important for the flavour and aroma of Cabernet Sauvignon wines, but as they are present in low ng/kg amounts in the berries. To measure such small amounts we used a stable isotope dilution assay to quantify isobutyl methoxypyrazine levels in the grape slurries as well as the wine samples. For the general analysis of the wine volatile chemical composition we used headspace SPME to quantify 101 compounds (Table 2).

Table 1. Grape compounds measured by SBSE-GCMS in this study.

Retention index[a]	Compound	Method of identification[b]	Quantify ion or scan (Sc)
<1000	butanal	A	Sc
<1000	ethyl acetate	A	Sc
1028	methyl benzene	A	Sc
1060	hexanal	A	Sc
1123	GVunknown1	C	Sc
1176	heptanal	A	Sc
1198	3-methylbutanol	A	Sc
1214	(*E*)-2-hexenal	A	Sc
1228	2-pentyl furan	A	Sc
1280	octanal	A	84
1291	1-octen-3-one	A	Sc
1310	2-heptanol	A	Sc
1314	(*E*)-2-heptenal	A	Sc
1345	1-hexanol	A	Sc
1357	(*E*)-3-hexen-1-ol	A	Sc
1379	(*Z*)-3-hexen-1-ol	A	Sc
1387	nonanal	A	Sc
1403	(*E*)-2-hexen-1-ol	A	Sc
1412	(*Z*)-2-hexen-1-ol	A	Sc
1423	(*E*)-2-octenal	A	Sc
1446	1-octen-3-ol	A	Sc
1451	1-heptanol	A	Sc
1461	(*E*,*Z*)-2,4-heptadienal	A	Sc
1483	GVunknown2	C	Sc
1486	2-ethyl-1-hexanol	A	Sc
1486	(*E*,*E*)-2,4-heptadienal	A	81
1492	decanal	A	Sc
1507	6-hepten-1-ol	A	Sc
1511	acetic acid	A	60
1518	benzaldehyde	A	Sc
1528	(*E*)-2-nonenal	A	Sc
1553	1-octanol	A	Sc
1579	(*E*,*Z*)-2,6-nonandienal	A	Sc
1615	(*E*)-2-octen-1-ol	A	Sc
1633	GVunknown3	C	Sc
1640	phenylacetaldehyde	A	91
1643	1-nonen-4-ol	B	83
1692	α-terpineol	A	121
1844	*trans*-geraniol	A	Sc
1883	hexanoic acid	A	60
1877	benzyl alcohol	A	108
1910	phenyl ethanol	A	Sc
1945	benzothiazole	A	Sc
2029	(*E*)-2-hexenoic acid	A	73

Retention index[a]	Compound	Method of identification[b]	Quantify ion or scan (Sc)
2035	4-hydroxy-2,5-dimethyl-3(2H)-furanone	B	85
2200	nonanoic acid	A	73

[a]The retention index is based on a series of n-alkanes (C10–C26) on ZB-Wax + (30 m, 0.25 mm, 0.25 µm). [b]Method of identification: A, identities confirmed by comparison of mass spectra and retention index with those of authentic standards; B, identities tentatively assigned based on the comparison with those from either the NIST05 and Wiley Registry 7th edition mass spectral libraries or literature; C, unidentified compound.

Table 2. Wine compounds measured by SPME-GCMS in this study.

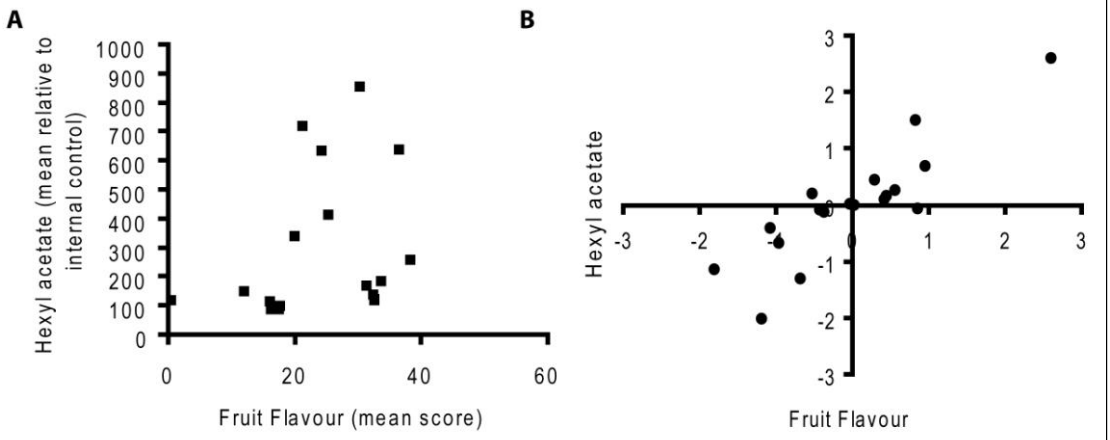
Retention index[a]	Compound	Method of identification[b]	Quantify ion or scan (Sc)
<1000	acetaldehyde	A	Sc
<1000	ethyl acetate	A	61
<1000	ethanol	A	43
<1000	ethyl propanoate	A	102
<1000	ethyl 2-methylpropanoate	A	116
<1000	2,3-butadione	A	86
1020	2-methylpropyl acetate	A	73
1038	ethyl butanoate	A	88
1042	methylbenzene	A	91
1042	1-propanol	A	59
1050	ethyl 2-methylbutanoate	A	102
1057	2,3-pentadione	A	100
1062	ethyl 3-methylbutanoate	A	88
1079	2,2,5-trimethylhexane-3,4-dione	B	85
1096	2-methyl-1-propanol	A	Sc
1110	3-methylbutyl acetate	A	Sc
1125	*p*-xylene	B	106
1146	ethyl pentanoate	A	85
1155	alpha-terpinene	A	121
1160	1-butanol	A	56
1181	3-methylbutyl propanoate	A	99
1228	ethyl hexanoate	A	99
1223	3-methylbutan-1-ol	A	70
1252	ethenyl benzene	A	104
1258	*o*-cymene	A	119
1266	terpinolene	A	136
1266	hexyl acetate	A	84
1265	WVunknown1	C	125
1287	ethyl pyruvate	A	116
1295	ethyl-(*E*)-3-hexenoate	A	142
1310	WVmonoterpene1	C	93
1310	propyl hexanoate	A	117
1315	4-methyl-1-pentanol	A	56
1320	2-heptanol	A	83
1325	ethyl heptanoate	A	113
1328	3-methyl-1-pentanol	A	56
1339	ethyl (*E*)-2-hexenoate	A	97
1349	ethyl 2-hydroxypropanoate	A	103
1354	1-hexanol	A	69
1365	WVunknown2	C	140
1366	(*E*)-3-hexen-1-ol	A	82
1367	*n*-heptyl acetate	A	98
1385	methyl octanoate	A	87
1379	3-ethoxy-1-propanol	A	86
1387	(*Z*)-3-hexen-1-ol	A	82
1422	WVunknown3	C	119
1433	ethyl octanoate	A	127
1454	3-methylbutyl hexanoate	A	99
1451	1-octen-3-ol	A	85
1456	1-heptanol	A	83
1472	octyl acetate	A	84
1487	acetic acid	A	60

Retention index[a]	Compound	Method of identification[b]	Quantify ion or scan (Sc)
1483	ethyl 7-octenoate	B	88
1490	2-ethyl-1-hexanol	A	83
1503	WVmonoterpene2	C	121
1505	WVunknown4	C	83
1509	3-ethyl-4-methylpentanol	B	84
1519	propyl octanoate	B	145
1517	vitispirane I	B	192
1520	vitispirane II	B	121
1536	ethyl nonanoate	A	88
1545	WVunknown5	C	125
1545	(2,3)-butanediol	A	75
1553	2-methylpropyl octanoate	A	127
1548	linalool	A	93
1557	1-octanol	A	84
1587	ethyl 8-nonenoate	B	138
1593	(2,3)-butanediol	A	75
1593	2-methyl propanoic acid	A	88
1593	methyl decanoate	A	87
1599	1,2-propane diol	A	61
1612	*β*-cyclocitral	B	152
1624	ethyl 2-furoate	A	95
1641	ethyl decanoate	A	101
1659	3-methylbutyl octanoate	A	127
1660	1-nonanol	A	98
1678	diethyl butanedioate	A	101
1714	WVnorisoprenoid1	C	192
1719	3-(methylthio)-1-propanol	A	106
1736	1,1,6-trimethyl-1,2-dihydronaphthalene	B	157
1741	ethyl undecanoate	A	88
1763	1-decanol	A	112
1767	*β* -citronellol	A	123
1785	ethyl phenyl acetate	A	91
1801	megastigmatrienone	B	190
1808	ethyl 4-hydroxybutanoate	B	88
1814	phenyl ethyl acetate	A	104
1816	*β* -damascenone	A	121
1844	ethyl dodecanoate	A	101
1869	hexanoic acid	A	60
1864	3-methylbutyl decanoate	A	155
1881	benzyl alcohol	A	107
1904	WVunknown6	C	129
1915	phenyl ethanol	A	122
2026	*γ*-nonalactone	A	Sc
2044	WVunknown7	C	161
2052	ethyl tetradecanoate	A	88
2084	octanoic acid	A	60
2192	nonanoic acid	A	73
2258	ethyl hexadecanoate	A	88
2300	decanoic acid	A	87

[a]The retention index is based on a series of n-alkanes (C10–C26) on ZB-Wax + (30 m, 0.25 mm, 0.25 μm). [b]Method of identification: A, identities confirmed by comparison of mass spectra and retention index with those of authentic standards; B, identities tentatively assigned based on the comparison with those from either the NIST05 and Wiley Registry 7th edition mass spectral libraries or literature; C, unidentified compound.

Our samples were obtained across three vintages and involved repeated sampling at some sites. This introduces vintage and vineyard effects into the data set and these were indeed seen in the data. While these effects are important for differentiating wine styles, they may mask underlying associations that exist between sensory attributes of wine and the chemical composition of both the wine and the grapes that were used to make the wine. Associations between chemical variates and sensory variates were identified using non-orthogonal analysis of variance and a forward selection regression

Figure 1. Underlying relationships between data sets: Graphs showing the observed relationship between the sensory attribute "fruit flavour" and the levels of hexyl acetate in the wine samples. The data plotted in A represent means of both "fruit flavour" and hexyl acetate measurements without correction for vintage or regional effects. The data plotted in B represent the residuals from models for the "fruit flavour" and hexyl acetate variates after year and sample effects have been fitted.



procedure. This removes vintage and regional effects so that underlying associations can be detected between chemical and sensory attributes (Figure 1). The statistical treatment allows us to observe connections between x and y variates, allowing for the possibility that years and samples (regions) could have a differential effect on the two variates that could have the potential to disrupt any underlying relationship.

Two visual descriptors (colour intensity and viscosity) were scored for each wine by the trained panel. A negative association was seen between both descriptors and the amount of acetaldehyde detected in wine (Table 3). There was a positive association between the concentration of ethyl 7-octenoate and both colour intensity and viscosity, although the relationship was not as strong as that seen for acetaldehyde. Interestingly, as decanal concentrations in grapes decreased the wine viscosity scores increased and this relationship was also seen for the amount of decanol detected in the wine headspace.

Table 3. Wine and grape compounds that were associated with wine visual descriptors.

Wine sensory attribute	**Associated wine components**	**P-value**	**Associated grape components[a]**	**P-value**
Colour intensity	*acetaldehyde*[b]	<0.001	*(Z)-3-hexenol*	<0.001
	ethyl 7-octenoate	0.001	*hexanoic acid*	0.007
Viscosity	*acetaldehyde*	<0.001	*decanal*	<0.001
	ethyl 7-octenoate	0.002	*benzyl alcohol*	0.002
	1-decanol	0.006		

[a] *The order of the components in this column does not imply a relationship to the compounds in the same row of the "wine components" column.*
[b] *Those compounds in italics are negatively correlated to the sensory attribute and those non-italicised are positively correlated.*

The levels of six of the ten odour descriptors used to describe the wines could be

associated with the concentration of certain volatile components of the wine or grapes (Table 4). Overall odour impact was found to be lower in wines with higher ethyl undecanoate levels, but was positively associated with *β*-damascenone concentration in the wine headspace. The amount of trans-geraniol in the grapes was negatively associated with the aroma impact of the wine. Isoamyl propanoate concentrations in wine were higher in those with greater pepper and woody/tobacco odour, and the amount of IBMP in grapes was positively associated with the woody/tobacco aroma of the wine. For the spicy aroma descriptor, it was found that the abundances of hexyl acetate and nonanoic acid were negatively associated, and this was also observed for nonanoic acid in the grape homogenates. Wines with a higher pungent odour were found to have lower amounts of the long chain ethyl esters ethyl undecanoate and ethyl tetradecanoate (Table 4).

Table 4. Wine and grape compounds that were associated with wine aroma sensory attributes.

Wine sensory attribute	**Associated wine components**	**P-value**	**Associated grape components[a]**	**P-value**
Aroma impact	*ethyl undecanoate*[b]	<0.001	trans-*geraniol*	0.002
	β-damascenone	0.007	2-pentyl furan	0.007
Green	-		-	
Pepper	isoamyl propanoate	<0.001	ethyl acetate	0.009
	phenyl ethanol	0.009		
Spicy	*hexyl acetate*	<0.001	*nonanoic acid*	<0.001
	nonanoic acid	0.003	heptanal	0.001
	2-ethyl-1-hexanol	0.005		
Woody/Tobacco	isoamyl propanoate	<0.001	IBMP	0.004
	decanoic acid	0.002		
Earthy	-		-	
Pungent	ethyl 2-hexenoate	<0.001	*decanal*	<0.001
	ethyl tetradecanoate	<0.001		
	ethyl undecanoate	0.006		
Berry	-		-	
Sweet	-		-	
Chemical	-		-	

[a] *The order of the components in this column does not imply a relationship to the compounds in the same row of the "wine components" column.*

[b] *Those compounds in italics are negatively correlated to the sensory attribute and those non-italicised are positively correlated.*

Of the ten flavour attributes used to describe the Cabernet Sauvignon wines, nine showed a relationship with headspace measurements of wine or grape volatile compounds (Table 5). Overall flavour impact was negatively associated with the concentration of ethenyl benzene in the wine and (*Z*)-3-hexenol in the berries. The concentrations of the wine esters ethyl decanoate and ethyl dodecanoate in the wine headspace, along with 1-heptanol, were positively associated with "sweet" wine flavour. Interestingly, as the 1-heptanol concentration in the wine headspace increased, the "acidic" flavour attribute also increased. "Green" flavour was associated with higher amounts of ethyl 3-methyl butanoate extracted from the wines.

Table 5. Wine and grape compounds that were associated with wine flavour sensory attributes.

Wine sensory attribute	Associated wine components	P-value	Associated grape components[a]	P-value
Flavour impact	*ethenyl benzene*[b]	<0.001	*(Z)-3-hexenol*	<0.001
Sweet	1-heptanol	<0.001	*2-heptanol*	0.007
	ethyl dodecanoate	<0.001		
	ethyl decanoate	0.004		
Acidic	1-heptanol	0.001	*2-heptanol*	0.007
Bitter	-		-	
Alcohol	-		-	
Green	ethyl 3-methyl butanoate	0.001	2-ethyl-1-hexanol	0.002
Berry	hexyl acetate	<0.001	(*E*,*E*)-heptadienal isomer	<0.001
	2,3-pentadione	0.001	1491	
			octanal	0.003
Pepper	*β*-damascenone	<0.001	*2-heptanol*	<0.001
	nonanoic acid	0.006	*hexanal*	0.002
			GVunknown3	0.002
Woody/tobacco	*ethanol*	<0.001	IBMP	0.04
Chemical	-		-	

[a] *The order of the components in this column does not imply a relationship to the compounds in the same row of the "wine components" column.*
[b] *Those compounds in italics are negatively correlated to the sensory attribute and those non-italicised are positively correlated.*

In several cases compounds that were found to be related to odour descriptors were also associated with wine flavour descriptors. For example, it was found that higher hexyl acetate concentrations were positively associated with berry flavour (Table 5), having previously been shown to be negatively associated with spicy aroma (Table 4). *β*-Damascenone was again positively associated with a sensory attribute, in this case pepper flavour (Table 5), having previously been shown to be higher in wines with greater aroma impact (Table 4). IBMP concentrations in the grapes were positively associated with woody/tobacco flavour in the wine (Table 5), as they were for woody/tobacco wine aroma (Table 4).

The mouthfeel descriptor "body" refers to the viscosity / thickness of the wine in mouth. This was negatively associated with acetaldehyde abundances in the wine and decanal concentration in the grapes from which the wine was made (Table 6). In support of this relationship seen by the mouth perception of body, grapes with high decanal levels produced wines were also found to be visually low in viscosity (Table 3).

Table 6. Wine and grape compounds that were associated with wine mouthfeel sensory attributes.

Wine sensory attribute	Associated wine components	P-value	Associated grape components[a]	P-value
Body	*acetaldehyde*[b]	< 0.001	*decanal*	<0.001
			1-nonen-4-ol	<0.001
Warming	-		-	

[a]*The order of the components in this column does not imply a relationship to the compounds in the same row of the "wine components" column.*
[b] *Those compounds in italics are negatively correlated to the sensory attribute and those non-italicised are positively correlated.*

Overall aftertaste was lower when higher amounts of ethanol and ethenyl benzene detected in the headspace above the wine and when higher concentrations of *trans*-geraniol were detected in the berry homogenates (Table7). Acidic aftertaste was associated with higher abundances of *β*-cyclocitral and *β*-damascenone, both of which are presumably derived from carotenoid degradation. It was also found that diacetyl was negatively associated with the amount of warming aftertaste imparted by the wine.

Table 7. Wine and grape compounds that were associated with wine aftertaste.

Wine sensory attribute	Associated wine components	P-value	Associated grape components[a]	P-value
Overall aftertaste	*ethanol*[b]	<0.001	trans-*geraniol*	0.002
	ethenyl benzene	0.007		
Sweet	-		-	
Acidic	*β*-cyclocitral	<0.001		
	isobutyl octanoate	<0.001		
	β-damascenone	0.004		
Bitter	-		-	
Berry	-		-	
Woody	-		-	
Warming	*diacetyl*	<0.001	*(Z)-3-hexenol*	0.004
	terpinolene	0.001		
Astringency	*o*-cymene		*decanal*	0.002
	methylbenzene			
	ethyl heptanoate			

[a]*The order of the components in this column does not imply a relationship to the compounds in the same row of the "wine components" column.*
[b] *Those compounds in italics are negatively correlated to the sensory attribute and those non-italicised are positively correlated*

Although correlation does not imply causation, many of the relationships observed in the current study support findings from previous studies or match sensory attributes with secondary metabolites that have relevant descriptors. For example, *β*-damascenone is thought to be derived in wine from non-volatile precursors (Puglisi et al. 2001, 2005) produced in berries from the enzymatic degradation of carotenoids

(Matthieu 2007). Several publications suggest that *β*-damascenone has a high odour activity value in red wine and so has a direct impact on red wine aroma (e.g. Aznar et al. 2001, Ferriera et al. 2000, 2002). Recent work by Pineau et al. (2007) illustrates that *β*-damascenone concentrations in wine are below threshold in that matrix. Nevertheless, the presence of low amounts of *β*-damascenone in wine appeared to enhance the odour impact of other flavour impact compounds related to fruity characters (Pineau et al.2007). The results of this study support the findings of Pineau et al. (2007) as a correlation seen between *β*-damascenone concentration in wine headspace and overall odour impact was observed (Table 1). In another example, berry flavour positively correlated with the abundance of hexyl acetate in the wine headspace (Table 2). Hexyl acetate has been implicated previously as one of a group of esters responsible for fruity characteristics of wine (Lilly et al. 2006). Its origin in wines has been described as being fermentation-derived (Siebert et al. 2005), although recently it has also been implied that grapes contain a metabolite or metabolites essential for hexyl acetate production (Keyzers & Boss 2010). Whilst it would be incorrect to interpret these results as suggesting that hexyl acetate is solely responsible for berry flavour, it is possible that hexyl acetate concentration in wine acts as an indicator that grape composition has favoured the production of "berry flavour" esters during fermentation. As evidence for this from this study, the amount of hexyl acetate in wine correlate with those of the esters 2-methylpropyl acetate (r=0.88), ethyl butyrate (r=0.8), 3-methylbutyl acetate (0.9) and octyl acetate (0.81) all of which are fruity esters. The amount of acetate ester production could be influenced by grape metabolites and this in turn influences the perceived berry flavour in the wine.

In other cases, the correlations observed suggest that different sensory profiles of wine can be linked to changes in berry metabolism, and, in some cases, the presence of higher amounts of specific metabolites is indicative of the fruit being exposed to certain conditions during development. For example, aroma impact was negatively associated with the abundance of the monoterpene *trans*-geraniol in the berries, whereas, as stated above, *β*-damascenone is positively associated with aroma impact. This may indicate that there is a trade off between the production of carotenoids and their C_{13}-norisoprenoid degradation products (including *β*-damascenone precursors), and monoterpenes in the plastids of the berries. Monoterpene production consumes the C_{10} precursor geranyl pyrophosphate, which is also a precursor for carotenoid biosynthesis. Therefore, an increase in monoterpene production in berries, and hence *trans*-geraniol, may limit carotenoid production. It was also found that spicy aroma in the wine correlated positively with ethyl acetate concentration in the berries. Higher ethyl acetate levels would suggest that anaerobic metabolism has been elevated in some of the berry samples at some stage during ripening (Dixon and Hewitt 2001). It has been seen that post-harvest drying increases the abundance of ethyl acetate and alcohols in grapes (Franco et al. 2004) and it is possible that grapes may undergo similar changes in volatiles on the vine if they are heat- and/or water-stressed. Ethyl acetate concentration in berries correlated with the amounts of octanal (r = 0.82), (*E*,*E*)-heptadienal (r = 0.75), (*E*)-2-octen-1-ol (r = 0.77), (*E*)-2-decanal (r = 0.79), which are compounds that originate from the oxidation of unsaturated fatty acids (Frankel 1985). Interestingly, similar types of compounds were shown to increase in abundance in bell peppers during hot-air drying (Luning et al. 1995), and may indicate water and/or heat stress experience by some of the grape samples. The correlation between the abundance of these grape metabolites and spicy aroma (Table 4) suggests

that water and/or heat stresses on berries may lead to the production of Cabernet Sauvignon wines with higher spicy aromas.

Some correlations observed suggest that conditions in some fermentations have favoured certain chemical changes in the wine. As the wine-making variables were controlled in this study, these chemical differences are presumable due to differences in the composition of the grapes. For example, it was shown that the wine colour was negatively associated with the amount of acetaldehyde in the wine headspace (Table 3). It has been demonstrated that the stable adduct pigments vitisin B and *p*-coumaroylvitisin B are formed from acetaldehyde and the anthocyanins malvidin-3-O-glucoside or malvidin-3-O-(6-O-*p*-coumaroyl)-glucoside respectively (Morata et al. 2007). Therefore, lower concentrations of acetaldehyde in the wine suggests that more has been consumed in the production of these stable adduct pigments, resulting in a positive effect on wine colour. This also suggests that it may be possible to predict the colour stability of wine by measuring acetaldehyde. In another example, acidic aftertaste correlated positively with *β*-cyclocitral and *β*-damascenone concentration extracted from the wine. These compounds are derived from enzymatic or chemical degradation of carotenoids (Ferreira et al. 2008; Vogel et al. 2008) and, in the case of *β*-damascenone, are produced from non-volatile and aglycone precursors in the fruit and wine (Daniel et al. 2008). It has been shown that pH influences the rate of *β*-damascenone production from suitable precursors in model systems (Daniel et al. 2008) and this may explain the correlation seen between acidic aftertaste and the levels of this compound in the headspace of the wine.

Other associations arose out of this analysis that represent links between sensory attributes and wine or grape composition where potential explanations for the relationships are not immediately apparent. For example, the amount of isoamyl propanoate in the wines correlated with both pepper and woody aroma (Table 3). Isoamyl propanoate is an ester produced by the yeast during fermentation probably from isoamyl alcohol and propanoic acid. However, the descriptors for this isoamyl propanoate are fruity, suggesting that the positive correlation with pepper and woody aroma is not causal. Similarly, green flavour was positively correlated with ethyl 3-methyl butanoate concentrations in the wine (Table 4). This ester is also more associated with fruity characters in wine than with green descriptors and so any correlation with green flavour is presumably indirect. Exploring these correlations is the subject of future work.

Viticulturalists and wine makers evaluate the quality of grapes throughout the growing season based on a series of learned sensory parameters and incorporating a high degree of experiential knowledge of the grapes, region, and climate accumulated over many years. This approach is dependent on the subjective rating of vineyard managers and can be biased by external parameters such as historical information about the site or other factors unrelated to the flavour potential of the grapes. For more objective measures of grape potential there needs to be a robust understanding of the biochemistry of flavour development in the grape and the subsequent sensory attributes of wines made from these grapes. Recently, research has focussed on developing standard approaches to evaluating the perceived quality of grapes and a move towards an integration of sensory strategies with chemical measures for the management of grape quality (Winter et al. 2004). These approaches tend to be cumbersome and although they attempt to remove some of the subjectivity from vineyard grape

assessments, they do not endeavour to explain the evolution of flavour in the grape or the subsequent production of flavour in the finished wine. An understanding of flavour development in the grape and the synergies and antagonisms that control the production of important flavour compounds will facilitate viticultural practices based on empirical evidence and allow for tighter control of desirable flavour precursors. The current study provides an objective approach to understanding some of the defining flavour characteristics of Cabernet Sauvignon and acts as a link between the composition of the grape, the volatile profile of the wine and the important perceived properties of finished wines.

Subproject A2: Changes in volatile production in fermentations made from musts with increasing grape content.

The work presented in this section has been published in the manuscript Keyzers and Boss (2010).

As we were conducting the research outlined in subproject A1 above, it became obvious that wines made from the same grape variety can be distinguished based on the geographical location of the vineyards. This suggests that changes in berry composition other than that imposed by genetics may affect the sensory properties of the resulting wine. While some of the differences seen in these wines could be attributed to changes in compounds responsible for varietal character, there was good evidence that compounds that are considered to be fermentation-derived (i.e. esters) can vary in wines made from different grape parcels even when wine-making conditions are controlled.

As fermentation volatiles are produced by yeast from primary metabolites, this can be modelled in artificial solutions or musts containing sugars, amino acids, and various vitamins and micronutrients (e.g. Henschke & Jiranek 1993, Bely et al. 1990, Varela et al. 2004). Model musts or model grape juice media (MGJM) have proven to be important tools for the examination of the effects of nutrients or fermentation conditions on the production of volatiles produced by yeast. However, these experiments often assume that the major role the grape plays in the production of wine acids, esters and alcohols produced during fermentation is as a source of sugars, amino acids and nitrogen.

The research discussed in this section involved a set of experiments in which small-scale fermentations were conducted where the major variable was the amount of grape juice in the fermentation. As such this study also represents the test of a quick and reproducible method to establish those wine volatile compounds that are significantly altered by grape juice constituents. Whilst volatiles already present in grape juice can be readily identified using SPME-GC-MS (Kalua & Boss 2009), the use of model musts spiked with grape juice enables the identification of those compounds that are absent from juice but are found following fermentation and are significantly altered by changing the amount of grape juice present. To identify which compounds are especially grape-dependent, these experiments were designed to determine the relative concentration of volatile compounds in the final wine as a function of juice concentration. In order to do this, ferments of equal volume but varying in the amount

of grape juice were prepared. This was achieved by diluting the grape juice with varying amounts of model must. Six different compositions were chosen to measure (0, 5, 10, 20, 50 and 100% v/v grape juice), three of which were at low juice concentrations (0-20%) in order to have a greater opportunity to highlight significant changes that may occur with only small addition of juice and hence with minimal changes in yeast assimilable nitrogen (YAN). In fact, the levels of YAN were not significantly different for the Riesling fermentations with 0, 5 and 10% grape juice added or the Cabernet Sauvignon fermentations with up to 20% grape juice added (Table 8). Volatile components were analysed in these wines to identify those that increase as the proportion of grape juice in the musts was increased, indicating that their production is dependent or enhanced by the presence of grape components in the fermentation.

Table 8. pH and YAN measurements of the MGJM/grape juice mixtures before fermentation[a]

	% Grape juice					
	0%	**5%**	**10%**	**20%**	**50%**	**100%**
Riesling						
pH	2.97 ± 0.02	2.94 ± 0.02	2.95 ± 0.01	2.98 ± 0.02	2.99 ± 0.06	2.98 ± 0.03
YAN (mgN/L)	785 ± 11 d	798 ± 7 d	807 ± 10 cd	829 ± 10 c	870 ± 20 b	945 ± 22 a
Cabernet Sauvignon						
pH	3.98 ± 0.03	3.93± 0.04	3.92 ± 0.03	3.97± 0.03	3.92± 0.05	3.90± 0.02
YAN (mgN/L)	789 ± 8 d	787 ± 10 d	798 ± 14 d	810 ± 2 d	880 ± 13 b	963 ± 16 a

[a] *Values represent means ± standard error (n=3) and different letters denote significant differences between treatments at* $p < 0.05$

There were a large number of identified compounds (94 for Riesling and 108 for Cabernet Sauvignon) that showed significant differences ($p < 0.05$) in concentration in the headspace of the wines in response to differences in the amount of grape juice present in the fermentations. To identify if there were any common trends in the way the relative amounts of the wine volatiles fluctuated with variation in the percentage of grape juice, the data were analysed by k-means clustering. Data were normalized within each dilution series by dividing the means of each compound by the maximum value. This procedure allows the relative response of all volatiles to be compared across each series of treatments independent of their differing abundances within a sample. The compounds were then clustered using a Euclidean distance metric to group those that behave in a similar manner within each dilution series. The ten clusters formed for each cultivar are shown in Figures 2 and 3 for Riesling (R-1 to R-10) and Cabernet Sauvignon (CS-1 to CS-10), respectively, whilst the individual compounds in each cluster are listed in Tables 9 and 10.

The initial hypothesis was that any grape-derived metabolites would be absent from ferments carried out with no grape juice and their levels would then increase linearly in proportion to the amount of grape juice added. A cluster containing volatiles showing such a trend was noted in both cultivars (Figure 2: R-1; Figure 3: CS-1). However, it should be noted that not all of the members of these clusters were unable to be detected in the 0% grape juice samples, but were present in relatively low levels compared to other samples. Amongst the 26 compounds that fall into the R-1 cluster in Riesling, half were found to be terpenoids or nor-isoprenoids (e.g. linalool, ocimene, terpinolene, α-terpinene, TDN; Table 3). Although not all of these terpene or nor-

isoprenoid compounds could be identified as volatile components of the Riesling juice used in this study (data not shown), many have been shown to exist as glycosidically bound precursors (Williams et al. 1982, Winterhalter et al. 1990) and this is likely to be the major source of these volatiles during fermentation. The compounds in these clusters that could not be detected in the wine made with MGJM alone were (*E*)- and (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, ethyl (*E*)-3-hexenoate, ethyl 2-furoate and *β*-damascenone (Tables 9 and 10). These compounds that increase in concentration with respect to the amount of grape juice may do so because they represent the increasing concentration of a wine volatile precursor in the grape or, more simply, because that compound is itself found in the grape juice and so the pattern seen represents the dilution effect of adding MGJM to the juice.

To examine if the variation noted was purely due to dilution of actual grape juice components, samples of the Riesling and Cabernet Sauvignon juice used in the fermentation experiments were analysed by SPME-GC-MS. The volatile head-space of both juices was dominated by hexanal, (*E*)-2-hexenal, hexanol, and both (*E*)- and (*Z*)-3-hexenol to varying degrees (data not shown) as has been previously reported (Kalua & Boss 2009) and so dilution would account for the changes seen in the levels of both (*E*)- and (*Z*)-3-hexenol in both fermentation series. There was a complete absence in the grapes of any significant quantities of the (*Z*)-3-hexenyl acetate and ethyl (*E*)-3-hexenoate esters, which are presumably derived from (*E*)- and (*Z*)-3-hexenol. Therefore, the production of these compounds during fermentation is dependent on the interaction of yeast metabolism and grape components that are not found in the MGJM.

Hexanol and hexyl acetate were also grouped in these clusters and both were detected to some extent in the fermentations conducted with no grape juice (Tables 9, 10 and 11). However, the level of these volatiles increased markedly as the proportion of grape juice in the fermentations increased (Table 11), with hexyl acetate levels being 48- and 141-fold higher in the wines made from 100% grape juice compared to those produced from MGJM alone for the Riesling and Cabernet Sauvignon series respectively. Hexanol in wines has been thought to originate directly from grapes or via yeast metabolism (Schreier 1979), and previous research has suggested that hexyl acetate in wines originates predominantly from grapes (Killian & Ough 1979, Molina et al. 2007). This study has shown that the level of these compounds increases considerably as the proportion of grape juice present in fermentations is increased (Table 11) providing strong evidence that grapes provide the major source of hexanol and precursors to hexyl acetate in wines. Certain inferences can be made from these results that may have implications for winemaking. For example, hexyl acetate is considered to contribute to “fruity” flavour and aroma in wines (e.g. Coelho et al. 2009, Gomez-Miguez et al. 2007). However, there is a complete absence of hexyl acetate in any of the fruit juice samples. Conversely, hexanol is deemed to have “grassy” or “green” influences on wine, which are often undesirable (Culler et al. 2004, Gurbuz et al. 2006). Both these compounds increase directly in proportion to the amount of grape juice present and, as hexanol could be considered a direct precursor of hexyl acetate, it suggests that one cannot have the desired component without the initial presence of the unwanted component. In “Subproject A3” below, experiments that establish if there is a direct link between these two compounds in wines are discussed.

Figure 2. Results of k-means clustering analysis with Riesling fermentations (clusters R1-R10). Normalized concentrations for individual compounds are shown in grey while the cluster mean is shown in black.

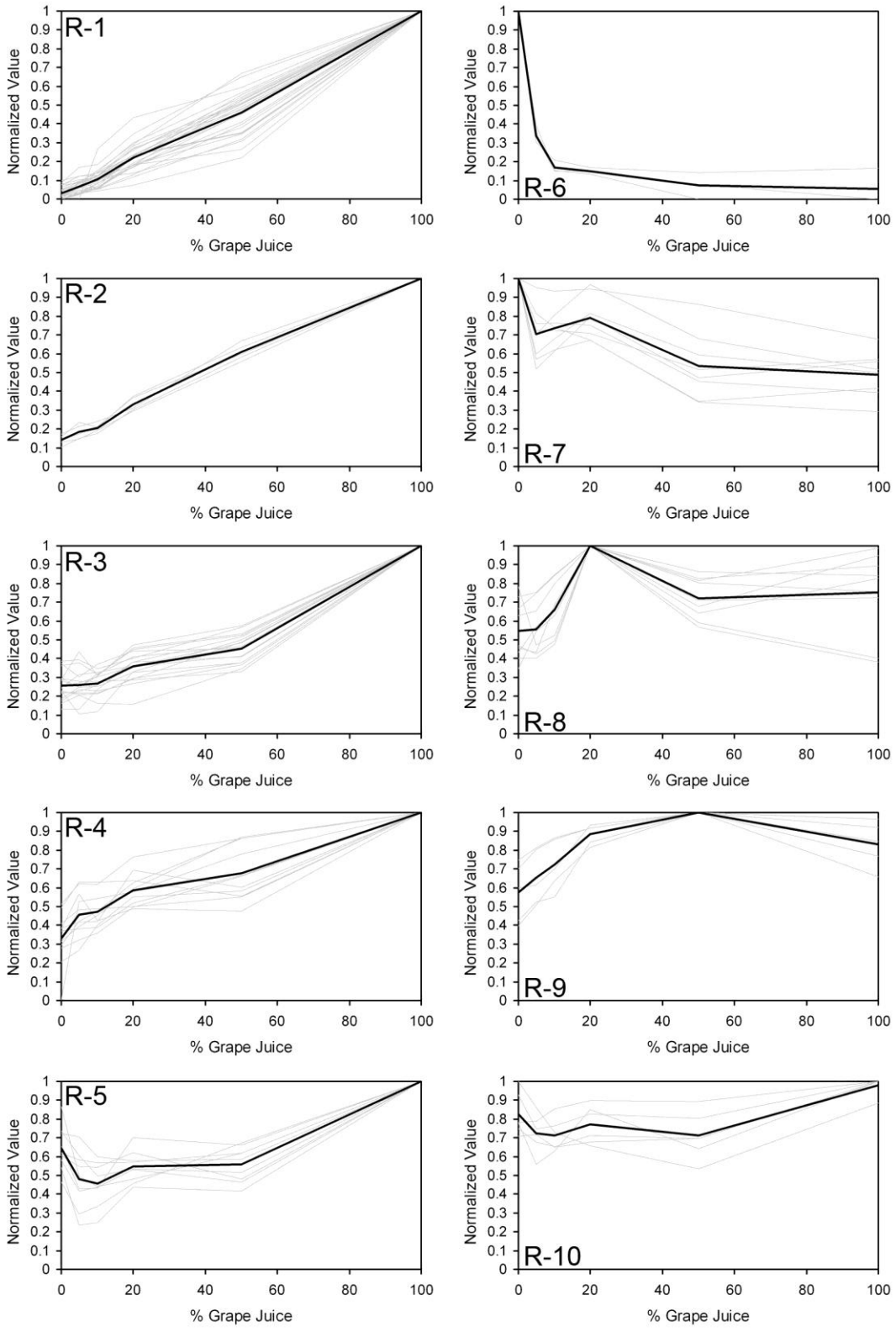


Table 9. Riesling compounds grouped in each cluster from Figure 2.

LRI[a]	COMPOUND	METHOD OF ID[b]
	CLUSTER 1	
	ALIPHATICS	
1344	Hexanol	A
1356	(E)-3-Hexenol	A
1380	(Z)-3-Hexenol	A
1988	Dodecanol	A
	ESTERS	
1143	Methyl hexanoate	A
1251	Hexyl acetate	A
1283	Ethyl (*E*)-3-hexenoate	A
1292	(Z)-3-Hexenyl acetate	A
1391	Methyl octanoate	A
1625	Methyl decanoate	A
1652	Ethyl 2-furoate	A
2107	Ethyl 3-hydroxytridecanoate	B
	TERPENOIDS	
1110	Myrcene	A
1125	α-Terpinene	A
1256	Terpinolene	A
1315	Linalyl ethyl ether	B
1464	α-Terpinyl ethyl ether	B
1484	Nerol oxide	A
1569	Linalool	A
1637	Hotrienol	B
	NOR-ISOPRENOIDS	
1549	Vitispirane 1	B
1552	Vitispirane 2	B
1663	Riesling acetal	B
1778	1,1,6-Trimethyl-1,2-dihydronapthalene (TDN)	B
1855	β-Damascenone	A
	AROMATICS	
2190	(*p*)-Vinyl guaiacol	B
	CLUSTER 2	
	ESTERS	
2046	γ-Nonalactone	A
	TERPENOIDS	
1148	Limonene	A
1221	Ocimene	A
1242	(*p*)-Cymene	B
2057	Nerolidol	A
	CLUSTER 3	
	ALIPHATICS	
1370	3-Ethoxypropanol	A
1389	2-Nonanone	A
1536	2-Nonanol	A
	ESTERS	
963	2-Methylpropyl acetate	A
1018	Butyl acetate	A
1072	3-Methylbutyl acetate[c]	A
1118	Ethyl 2-butenoate	B
1128	Pentyl acetate	B
1659	3-(Methylthio)propyl acetate	B
1707	Diethyl succinate	A
1852	Phenylethyl acetate[c]	A
1987	2-Phenylethyl butanoate	B
	CARBOXYLIC ACIDS	

LRI[a]	COMPOUND	METHOD OF ID[b]
	CLUSTER 4 (cont.)	
	TERPENOIDS	
1702	β-Farnesene	A
1762	α-Farnesene	A
1786	α-Farnesene isomer	B
1790	Geranyl acetate	A
	CLUSTER 5	
	ALIPHATICS	
1026	Hexanal	A
1139	2-Heptanone	A
1627	2-Undecanone	A
	ESTERS	
1205	Ethyl hexanoate[c]	A
1304	Ethyl heptanoate	A
1372	Heptyl acetate	A
1727	Ethyl 9-decenoate	B
	CARBOXYLIC ACIDS	
1879	Hexanoic acid[c]	A
2169	Nonanoic acid	A
	CLUSTER 6	
	ALIPHATICS	
1436	(E)-2-Octenal	A
1557	(E)-2-Nonenal	A
	AROMATICS	
1545	Benzaldehyde	A
	CLUSTER 7	
	ALIPHATICS	
1843	2-Tridecanone	A
2039	2-Pentadecanone	A
	ESTERS	
1680	Ethyl decanoate[c]	A
1695	3-Methylbutyl octanoate	A
1881	Ethyl dodecanoate	A
1897	3-Methylbutyl decanoate	A
	CARBOXYLIC ACIDS	
1467	Acetic acid	A
	CLUSTER 8	
	ALIPHATICS	
1102	Butanol	A
1748	3-(Methylthio)propanol	A
	ESTERS	
1492	Octyl acetate	A
1561	Ethyl nonanoate	A
2066	Ethyl tetradecanoate	A
2125	Ethyl pentadecanoate	B
2242	Ethyl hexadecanoate	A
2398	Ethyl octadecanoate	A
	TERPENOIDS	
1696	α-Terpineol	A
	CLUSTER 9	
	ALIPHATICS	
1578	Octanol	A
1793	Decanol	A
	ESTERS	
1659	γ-Butyrolactone	A
	TERPENOIDS	

2305	9-Decenoic acid	B	1797	β-Citronellol	A
	TERPENOIDS			**CLUSTER 10**	
1532	Geranyl ethyl ether	A		*ALIPHATICS*	
1832	Nerol	A	1177	2-Methylbutanol/3-methylbutanol[c]	A
	AROMATICS			*ESTERS*	
1905	Benzyl alcohol	A	873	Ethyl acetate[c]	A
	CLUSTER 4		984	Ethyl butanoate	A
	ALIPHATICS		1455	Ethyl octanoate[c]	A
1558	*Rac*-2,3-Butanediol	A	1474	3-Methylbutyl hexanoate	A
1751	2-Undecanol	A	2412	Ethyl (Z)-9-octadecenoate	A
	CARBOXYLIC ACIDS				
2077	Octanoic acid[c]	A			
2257	Decanoic acid[c]	A			
2419	Dodecanoic acid	B			

[a] LRI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}); [b] A: Identity confirmed by matching mass spectra and LRI with that of authentic standards; B: Tentative assignment based upon comparison with mass spectral libraries and published LRI; [c] Samples identified and quantified in 1 in 100 dilution.

An important observation made in these experiments was that the production of many fermentation esters was enhanced when grape juice levels were increased (Tables 9 and 10; and Figures 2 and 3). This was more apparent in the Cabernet Sauvignon experiment than in the Riesling fermentations. Seven acetate esters markedly increased in the Cabernet Sauvignon fermentations as the proportion of juice in the must increased (Tables 10 and 11). Furthermore, another 16 esters clustered in CS-2, where many of the compounds were two-fold higher in the fermentations with 5% grape juice compared to those with MGJM alone (Table 12) and increased up to 8-fold when 100% grape juice was fermented. The esters in this category were not only acetate esters but also include those with longer acyl chains (Table 12). Other trends observed in these fermentations (CS-3, 4 and 5; Figure 3) support the suggestion that whilst some esters are produced by yeast in MGJM alone, the presence of grape juice in the fermentation can enhance their production. Although the overall patterns are subtly different, all these clusters share the fact that the 0% juice samples produce a measurable amount of these compounds and the levels of these compounds then show a general trend upwards as the percentage of juice in the fermentations increases. In contrast, the Riesling series of wines did not show such a marked increase in ester production as the juice content of the fermentations increased. Some acetate esters grouped in cluster R-3 and some ethyl esters in R-5 (Figure 2 & Table 9).

Whilst it could be speculated that the differential ability of the Riesling and Cabernet Sauvignon juices to enhance ester production is varietal, there are other possible causes. First, free run juice was used for the Riesling experiment, whereas the Cabernet Sauvignon juice was obtained from macerated whole berries, to best replicate winemaking practices for these cultivars. Second, it is possible that the differences observed are due to changes in berry composition caused by vineyard and environmental variables and the developmental stage at which the grapes were harvested. Determining the source of these differences in juice composition that, in turn, effect ester production, is discussed in "Subproject 4" below.

As well as the predicted increasing trends discussed above, clusters exhibiting more unexpected trends were also found. For example, benzaldehyde was found to decrease in an exponential manner relative to juice concentration in both cultivars (clusters R-6 and CS-6; Figures 2 and 3).

Figure 3. Results of k-means clustering analysis with Cabernet Sauvignon fermentations (clusters CS1-CS10). Normalized concentrations for individual compounds are shown in grey while the cluster mean is shown in black.

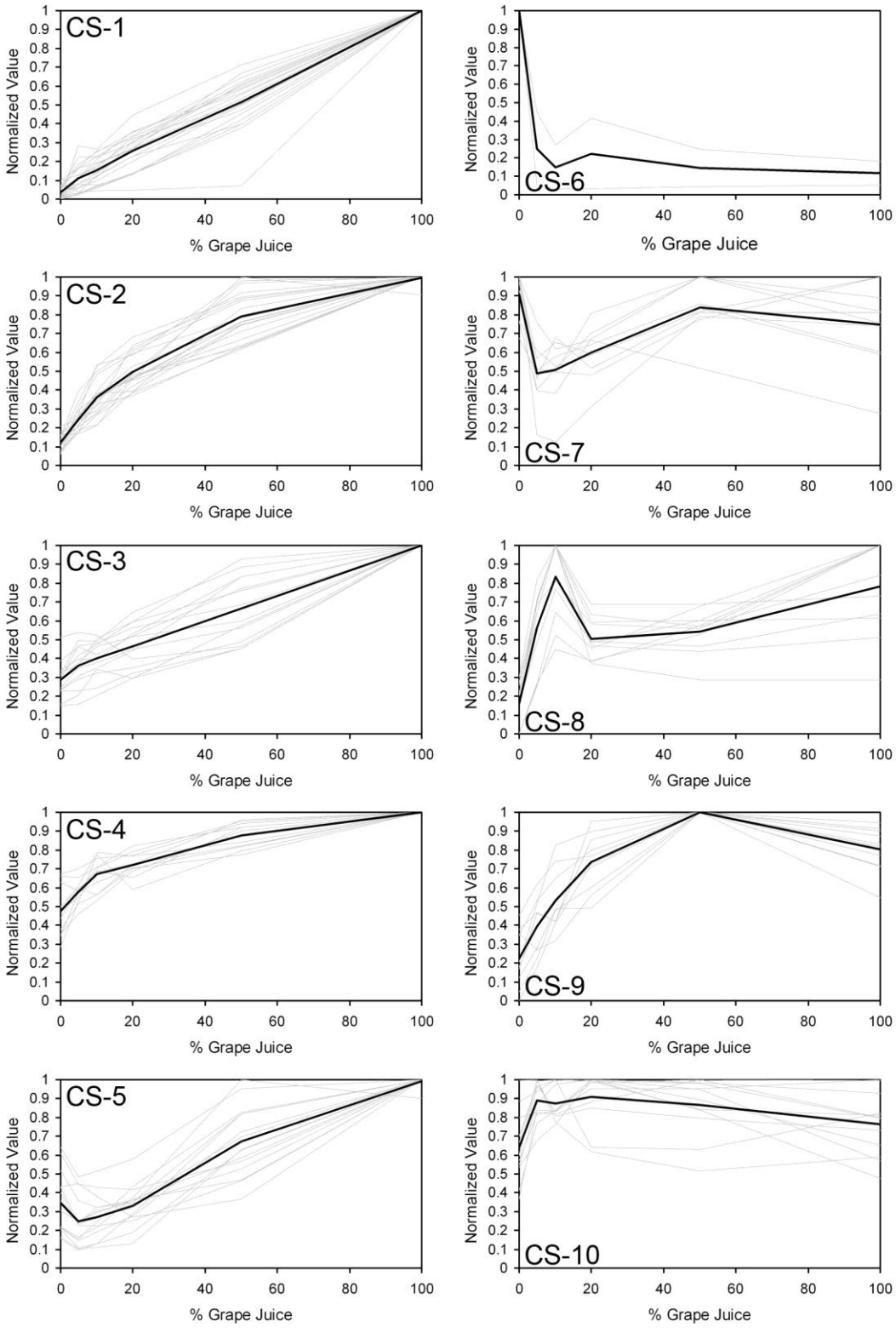


Table 10. Cabernet Sauvignon compounds grouped in each cluster from Figure 3.

LRI[a]	COMPOUND	METHOD OF ID[b]
	CLUSTER 1	
	ALIPHATICS	
1223	3-Octanone	A
1273	3-Hydroxy-2-butanone	A
1346	Hexanol	A
1359	(*E*)-3-Hexenol	A
1384	(*Z*)-3-Hexenol	A
1461	1-Octen-3-ol	A
	ESTERS	
924	Propyl acetate	A
956	2-Methylpropyl acetate	A
1013	Butyl acetate	A
1239	2-Heptyl acetate	B
1251	Hexyl acetate	A
1288	Ethyl-(*E*)-3-hexenoate	A
1292	(*Z*)-3-Hexenyl acetate	A
1335	Ethyl (*E*)-2-hexenoate	A
1655	Ethyl 2-furoate	A
1764	Benzyl acetate	A
	NOR-ISOPRENOIDS	
1855	β-Damascenone	A
	AROMATICS	
1913	Benzyl alcohol	A
	CLUSTER 2	
	ESTERS	
1071	3-Methylbutyl acetate[c]	A
1115	Ethyl 2-butenoate	A
1125	Pentyl acetate	A
1167	2-Methylpentyl acetate	B
1241	3-Methylbutyl butanoate	A
1304	Propyl hexanoate	A
1472	3-Methylbutyl hexanoate	A
1489	Octyl acetate	A
1538	Propyl octanoate	A
1576	2-Methylpropyl octanoate	A
1660	3-(Methylthio)propyl acetate	B
1708	Diethyl succinate	A
1852	Phenylethyl acetate[c]	A
	CARBOXYLIC ACIDS	
1994	2-Ethylhexanoic acid	A
	TERPENOIDS	
1693	β-Citronellyl acetate	A
1742	Neryl propanoate	B
	CLUSTER 3	
	ALIPHATICS	
984	Propanol	A
1047	2-Methylpropanol	A
1104	Butanol	A
1305	2-Heptanol	A
1387	2-Nonanone	A
1537	2-Nonanol	A
1751	2-Undecanol	A
	ESTERS	
912	Ethyl propanoate	A
1382	2-Ethylhexyl acetate	A
	CLUSTER 5	
	ALIPHATICS	
1568	*Rac*-2,3-Butanediol	A
1607	*Meso*-2,3-Butanediol	A
1625	2-Undecanone	A
2035	2-Pentadecanone	A
	ESTERS	
1692	3-Methylbutyl octanoate	A
1724	Ethyl 9-decenoate	B
1893	3-Methylbutyl decanoate	A
2061	Ethyl tetradecanoate	A
2078	3-Methylbutyl dodecanoate	B
2233	Ethyl hexadecanoate	A
2254	Ethyl 9-Hexadecenoate	A
2335	2-Phenylethyl octanoate	B
2391	Ethyl octadecanoate	A
	CLUSTER 6	
	AROMATICS	
1547	Benzaldehyde	A
	OTHERS	
1552	2-Methyl-dihydro-3(2H)-thiophenone	B
	CLUSTER 7	
	ESTERS	
1322	Ethyl heptanoate	A
1557	Ethyl nonanoate	A
1678	Ethyl decanoate[c]	A
1772	Ethyl undecanoate	A
1875	Ethyl dodecanoate	A
2119	Ethyl pentadecanoate	B
2406	Ethyl (Z)-9-octadecenoate	A
	AROMATICS	
1947	2-Phenylethanol[c]	A
	CLUSTER 8	
	TERPENOIDS	
1697	β-Farnesene	A
1780	α-Farnesene isomer	B
2054	Nerolidol	A
2245	2,3-Dihydrofarnesol	B
2314	Farnesol	A
	CLUSTER 9	
	ALIPHATICS	
1023	Hexanal	A
1755	3-(Methylthio)propanol	A
	ESTERS	
1592	Ethyl 3-(methylthio)propanoate	A
1621	Methyl decanoate	A
1833	Methyl dodecanoate	A
	CARBOXYLIC ACIDS	
1689	Butanoic acid	A
2091	Octanoic acid[c]	A
	TERPENOIDS	
1788	Geranyl acetate	A
2239	Farnesyl acetate	A
	AROMATICS	
2197	(*p*)-Vinyl guaiacol	B

LRI[a]	COMPOUND	METHOD OF ID[b]	LRI[a]	COMPOUND	METHOD OF ID[b]
1712	Decyl acetate	A		**CLUSTER 10**	
1766	9-Decenyl acetate	B		*ALIPHATICS*	
1820	Ethyl phenylacetate	A	1173	2-Methylbutanol/3-methylbutanol[c]	A
	TERPENOIDS		1580	Octanol	A
2211	Cadalene		1794	Decanol	A
	CLUSTER 4			*ESTERS*	
	ALIPHATICS		918	Ethyl 2-methylpropanoate	A
1223	Pentanol	A	1009	Ethyl 3-methylbutanoate	A
	ESTERS			*CARBOXYLIC ACIDS*	
863	Ethyl acetate[c]	A	1496	Acetic acid	A
978	Ethyl butanoate	A	1626	2-Methylpropanoic acid	A
1204	Ethyl hexanoate[c]	A		*TERPENOIDS*	
1369	Heptyl acetate	A	1570	Linalool	A
1454	Ethyl octanoate[c]	A	1799	β-Citronellol	A
1842	Ethyl 4-hydroxybutanoate	B	1833	Nerol	A
	CARBOXYLIC ACIDS		1879	Geraniol	A
2321	9-Decenoic acid	B		*AROMATICS*	
2432	Dodecanoic acid	A	1985	Benzothiazole	A
	AROMATICS				
2040	Phenol	A			
2417	Benzophenone	A			

[a] LRI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}); [b] A: Identity confirmed by matching mass spectra and LRI with that of authentic standards; B: Tentative assignment based upon comparison with mass spectral libraries and published LRI; [c] Samples identified and quantified in 1 in 100 dilution.

Table 11. Cluster means for R-1 and CS-1 and peak areas of selected volatiles grouped in these clusters relative to the 0% grape juice samples.

Compound	**% Grape juice[a]**					
	0%	**5%**	**10%**	**20%**	**50%**	**100%**
			Riesling			
R-1 mean	1	2.2	3.4	7.1	14.8	32.1
Hexanol	1.0 f	2.7 e	4.8 d	9.1 c	20.8 b	31.2 a
Hexyl acetate	1.0 e	2.0 d	2.5 d	5.8 c	15.5 b	48.5 a
Linalool	1.0 f	2.0 e	2.9 d	5.4 c	11.4 b	20.8 a
			Cabernet Sauvignon			
CS-1 mean	1	3.1	4.4	7.3	14.6	28.4
Hexanol	1.0 f	2.1 e	3.4 d	5.9 c	11.9 b	19.1 a
Hexyl acetate	1.0 f	4.0 e	9.3 d	18.8 c	53.6 b	140.2 a
Linalool[b]	1.0 d	1.5 c	1.6 ab	1.7 a	1.2 bc	1.2 d
Butyl acetate	1.0 f	2.5 e	3.5 d	5.2 c	9.9 b	15.2 a
Propyl acetate	1.0 e	3.1 d	4.7 c	6.0 c	9.4 b	18.5 a
2-Heptyl acetate	1.0 e	3.6 d	5.2 d	9.9 c	17.3 b	42.7 a
2-Methylpropyl acetate	1.0 e	2.7 d	3.7 cd	5.2 c	8.5 b	14.6 a

[a]Values are geometric means (n=3) of the peak areas relative to the 0% juice sample and, for each compound, different letters denote significant differences between treatments at $p < 0.05$. [b]The linalool values from Cabernet Sauvignon are included for comparison, but the compound was placed in cluster CS-10.

Other unusual patterns include those seen in clusters R-7 to 10/CS-7 to 10 where the levels of volatiles do not display a constant trend across the dilution series, and often peak in fermentations that have an intermediate level of grape juice added (Figures 2 and 3). These trends are indicative of the complex interplay between grape composition and yeast metabolism that determines the volatile composition of wine. Whilst the causes of these patterns are beyond the scope of this work, the grouping of

compounds in clusters suggests that the regulation of their production is linked and this is supported by the observation that compounds of similar classes are grouped in specific clusters (Figures 2 and 3; Tables 9 and 10).

Table 12. Cluster means for CS-2, 3 and 4 and peak areas of selected volatiles grouped in these clusters relative to the 0% grape juice samples.

Compound	**% Grape juice[a]**					
	0%	**5%**	**10%**	**20%**	**50%**	**100%**
			Cabernet Sauvignon			
CS-2 mean	1	2.0	3.0	4.1	6.5	8.2
Pentyl acetate	1.0 f	2.6 e	3.9 d	5.6 c	9.0 b	14.4 a
Octyl acetate	1.0 f	1.5 e	2.1 d	3.4 c	5.4 b	7.3 a
2-Methylpentyl acetate	1.0 f	2.0 e	3.2 d	3.9 c	6.5 b	8.5 a
3-Methylbutyl acetate	1.0 f	2.6 e	3.8 d	4.7 c	6.7 b	9.9 a
3-Methylbutyl butanoate	1.0 d	2.3 c	2.9 bc	3.6 b	5.3 a	5.6 a
3-Methylbutyl hexanoate	1.0 c	1.2 c	1.4 c	2.3 b	4.5 a	4.7 a
CS-3 mean	1	1.3	1.4	1.6	2.3	3.5
Ethyl propanoate	1.0 d	1.3 cd	1.6 bc	1.7 bc	2.0 b	3.4 a
2-Ethylhexyl acetate	1.0 e	1.4 d	1.5 cd	1.8 c	2.3 b	4.1 a
Ethyl phenylacetate	1.0 e	1.4 d	2.0 c	2.2 c	3.8 b	6.0 a
CS-4 mean	1	1.2	1.4	1.5	1.8	2.1
Ethyl acetate	1.0 e	1.4 d	1.6 cd	1.7 bc	1.9 b	2.3 a
Ethyl butanoate	1.0 e	1.7 d	1.9 cd	2.1 bc	2.4 b	3.0 a
Ethyl hexanoate	1.0 d	1.2 c	1.6 b	1.6 b	1.7 ab	2.0 a

[a] *Values are geometric means of the relative peak areas (n=3) and, for each compound, different letters denote significant differences between treatments at $p < 0.05$.*

Given that yeast require grape juice as a source of nutrients to allow growth and reproduction during fermentation, any metabolite produced by the yeast can be considered as being of grape derivation. We therefore feel that the currently applied terminology of "grape-derived" versus "fermentation-derived" to be somewhat artificial and misleading. Foremost, the experiments described in this study have indicated that the biosynthetic origins of various volatile components cannot purely be assigned as grape- or fermentation-derived. For example, the terpenoids from Riesling ferments were largely shown to be derived from the grape juice although there were notable exceptions. Conversely, many terpenoids from Cabernet Sauvignon were shown to be independent of grape juice concentration. Such analyses highlight that the biosynthetic origin for each compound or compound class may differ in a wine depending on different experimental variables. We therefore suggest that new terms be coined to describe the various trends noted in this study. Compounds that are absent when no juice is present and are found to increase in concentration with respect to the amount of grape juice are grape-dependent (e.g. R-1/CS-1, Figures 2 and 3). Those volatiles that are found at measurable levels when no juice is present, but which increase in proportion to the concentration of juice in the ferment, are grape-enhanced (e.g. R-2 to R-5/CS-2 to CS-5, Figures 2 and 3). Wine volatiles that decrease in concentration, such as benzaldehyde which decreases in an exponential manner in proportion to the concentration of juice in the ferment (e.g. R-4/CS-4, Figures 2 and 3), are grape-modulated. Finally, those compounds whose concentration is invariant across the treatments, rather than the traditionally used fermentation-derived phrase, are grape-independent components. These terms better encapsulate the variation of these compounds in respect to their biosynthetic origin. However, the terms must be

moderated for each volatile compound based upon experimental variables.

The experiments described in this section have been used to identify volatiles dependent on, or enhanced by, the presence of grape juice during fermentation in a model system. This is a simple method that is able to highlight not only those compounds present in wine that are found directly and unaltered in the juice itself, but more importantly those compounds that are grape-dependent but not present in the native juice. The data obtained in this study indicate that there are compounds that increase in concentration as the level of grape juice in the fermentations is increased, several of which are formed during the fermentation process itself, and are therefore examples of grape-derived compounds produced by the action of yeast upon certain precursors found only in the juice itself. The production of many esters was also found to increase as the amount of juice was increased in the Cabernet Sauvignon fermentations, although these compounds were produced in MGJM samples alone. This suggests that the juice contributes significantly to the pool of substrates the yeast uses to produce these compounds in addition to those components present in the MGJM. Alternatively, the grape juice may contain compounds that stimulate the production of these compounds by the yeast without being direct precursors of the final volatile compound. It is acknowledged that the complexity of the system (i.e. the fermentation process) is such that whilst the grape juice provides many substrates and co-factors, properties of different yeasts, such as their ability to transport compounds into the cell and the substrate preference of their enzymes (Verstrepen et al.2003, Pak et al. 2009, Van Belle & Andre 2001), will no doubt determine the final volatile profile in the wine. Nevertheless, the approach described here has been used to identify fermentation volatiles that may be influenced by grape composition. Bearing in mind that even minor variation in the concentration of esters, even when significantly below their reported odour thresholds, can have a major sensory impact (Pineau et al.2009), investigation into the origins and fates of such esters will yield valuable information regarding the factors that control these important impact odorants. Further experimentation will determine the exact nature of the influence grape juice components have on wine volatiles either as raw materials or as regulators of yeast metabolism and to confirm the results of these small scale experiments in a winery situation. The identification of the grape substrates or factors that enhance the production of these volatiles in fermentations is currently underway (Subprojects A3 & A4) and will be important for the prediction of grape quality, directed grape breeding for new varieties with specific flavour properties and the use of viticultural treatments to manage wine flavour outcomes.

Subproject A3: Confirmation of links between grape compounds and wine volatiles through the spiking of specific compounds into model musts.

The experiments discussed in “Subproject A1” suggested that some wine esters were influenced by grape composition, as several esters were found to be associated with differences in wine sensory attributes even when fermentation conditions were standardised. Following this, the experiments in “Subproject A2” enabled us to identify those volatiles present in wine whose concentrations depend on the grape juice used as a feedstock for fermentation thus highlighting those compounds that may benefit from

further investigation of compound biosynthesis. One group of compounds that was highlighted by this study are compounds produced by the lipoxygenase pathway, the so-called C6 volatiles and their derivatives whose presence in wine showed a direct dependence on grape juice concentration. These compounds are produced via the lipoxygenase pathway in the degradation of linoleic and linolenic acids as part of wound response in the grape berry (Dunlevy et al. 2009).

Some of the C6 compounds formed in the grapes by the action of the lipoxygenase pathway persist during fermentation and contribute to the pool of compounds found in wine. Furthermore, they also seem to be substrates for yeast activity and are converted into other volatile compounds that are present in wines.

The significance of these findings goes beyond the C6 compounds shown in the tables above. It is most likely that the array of alcohols found in grape berries (Schreier 1979) can be utilised as substrates for ester production by yeast. Alternatively, grape juice may contribute to the levels of acyl-CoA substrates. These could be in the form of carboxylic acids, Co-A precursors or factors required for their production such as biotin. For example, changes in the levels of both panthothenic acid and biotin in model fermentation conditions have been shown to influence the production of carboxylic acids and some ethyl esters (Bohlscheid et al. 2007, Wang et al. 2003). However, substrate availability does not always explain experimental data concerning the production of esters (Verstrepen et al. 2003), and so scenarios can be envisaged where the grape juice contains regulators of certain genetic and biochemical pathways in yeast that enhance the production of specific compounds or the importation of substrates during fermentation. These regulators are more likely to be discovered using the approach described below.

(Unpublished data to support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Subproject A4: Discovery of grape compounds contributing to wine volatile composition using a separations chemistry approach.

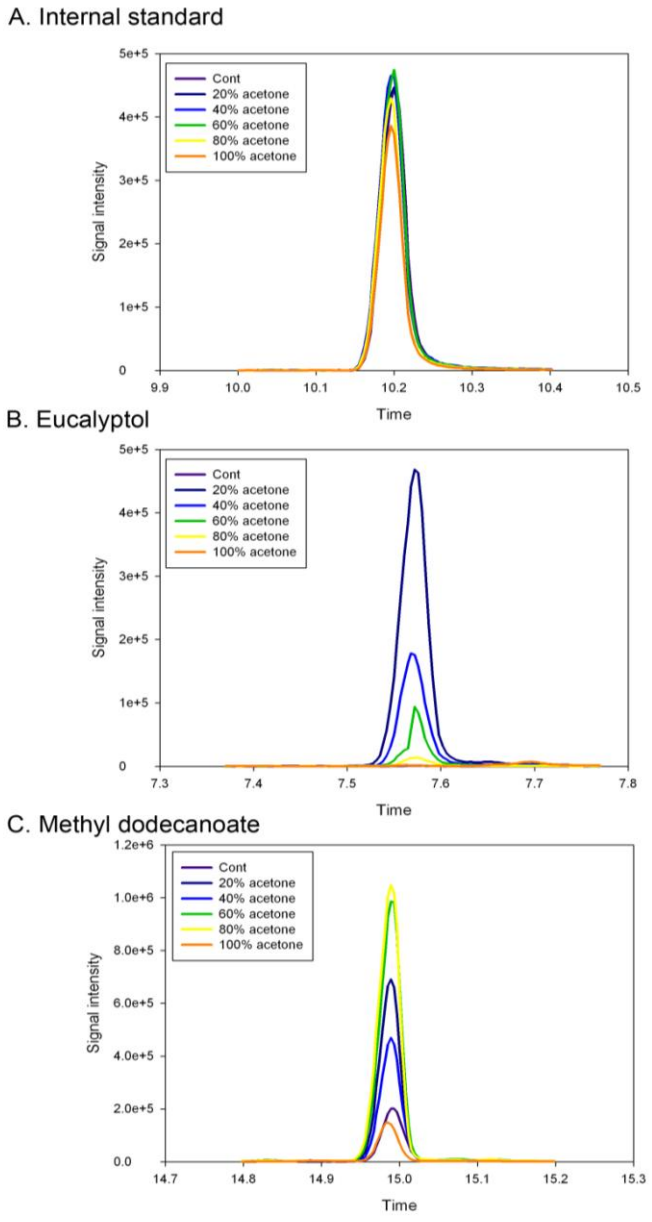
An alternative approach to the discovery of chemical compounds of a certain biological interest involves natural products or separations chemistry. In brief, this methodology involves the extraction and fractionation of a range of compounds from a biological tissue and the testing of these fractions for certain activities or functional groups. Fractions containing compounds of interest then undergo repeated rounds of fractionation allowing the eventual isolation of pure compounds responsible for the targeted activity. For this subproject, we were targeting non-volatile components of grape must that have the potential to contribute to or alter wine volatile profiles.

The first step in the process is the extraction of precursors from the grape tissue. This is somewhat problematic as several variables must be taken into consideration. First, the polarity of the solvent will determine what compounds are extracted. Second, this solvent will also affect the subsequent cyclic loading procedure and the nature of the column matrix required for separation of components of the grape tissue. Third, it needs to be remembered that grape tissue is biologically active, and so the solvent needs to be biochemically inert so that it does not produce artifacts and/or result in the

loss of some non-volatile precursors. With these factors in mind, extractions were first conducted on both Riesling and Cabernet Sauvignon grapes methanol as the solvent.

The techniques showed much promise in that the ability to extract and fractionate wine flavour precursors from grapes was clearly demonstrated. Figure 4 depicts compound peaks from chromatograms produced from the acid hydrolysis of five Riesling juice fractions eluted from a silica gel column with increasing concentrations of acetone.

Figure 4. Comparison of the levels of eucalyptol and methyl dodecanote released after hydrolytic cleavage of acetone fractions of Riesling juice.



These chromatograms demonstrate the ability of the technique to separate flavour precursors into separate fractions. For eucalyptol, the precursor has been eluted from the column with only 20% acetone, whereas the precursor to methyl dodecanoate is eluted more with the 60% and 80% acetone eluents compared to the other solutions. Therefore, differential fractionation of the precursors to these two potential wine volatiles has been achieved by the cyclic loading technique.

These eluates were further fractionated using a variety of chromatographic techniques, including both normal- and reversed-phase stationary phases, in low, medium and high pressure systems (LP-,MP-, HPLC and MLCCC). Fractions of interest were selected by their ability to release volatile compounds after enzyme or acid hydrolysis. However, after undergoing rounds of iterative fractionation, NMR analysis of the material failed to identify fractions of pure compounds in the time frame of the project. This work is therefore continuing in a GWRDC funded PhD project (GWR Ph1006).

Nevertheless, it has been important to develop these techniques as this approach to the discovery of wine volatile precursors in grapes is novel. Studies on glycosidic grape volatile precursors have been conducted in the past by a general survey of the compounds released from the hydrolysis of non-volatile grape extracts (e.g. Sefton 1998). In many cases, researchers have then predicted the chemical structure of the precursor and then tested its properties after chemically synthesising the compounds (e.g. Puglisi et al. 2001, 2005). With a separations chemistry approach, the aim is to directly identify the grape compounds responsible for the volatile precursors. Furthermore, by using micro-fermentation to characterise the fractions produced, we can target precursors released during the winemaking process, and not in the artificial system created when conducting acid or enzyme hydrolysis. This means it is also possible to identify compounds that indirectly affect wine volatile production, which may be more important than chemical precursors if their action on yeast metabolism is profound. Identification of these precursors or modulators of yeast activity will allow their measurement in grape samples to predict the volatile composition and sensory attributes of the resulting wine. An eventual understanding of their synthesis in the berries and the variables that affect their production will enable the development of strategies to alter their levels in berries through vineyard management or in novel cultivars.

(Unpublished data to support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Subproject A5: Examination of the changes in volatile compound profiles of musts and wines during the fermentation process.

The hypothesis behind these experiments was that it may be possible to observe a decrease in some volatile compounds as others increase suggesting some metabolic link between them during the fermentation process. Most studies on wine flavour development during alcoholic fermentation have targeted the production of specific compounds. The objective of this study was to explore, in a non-targeted manner, possible formation pathways and associated chemical reactions for the formation of volatile compounds in wine during fermentation.

These experiments were conducted with both Shiraz and Cabernet Sauvignon grapes and triplicate 50 kg fermentations conducted under controlled conditions. Liquid samples (50 mL) were taken from the fermentations at the same time daily for a week – from crushing until pressing. After the first week of vinification, liquid samples were taken once every two days until the completion of primary fermentation. Thereafter, samples were collected at each unit operation. Volatile compounds formed during alcoholic fermentation were monitored using SPME-GC-MS. A combination of analysis of variance (ANOVA) and stepwise linear discriminant analysis (SLDA) was used to select volatile compounds that significantly changed during fermentation and those compounds that discriminated particular fermentation stages.

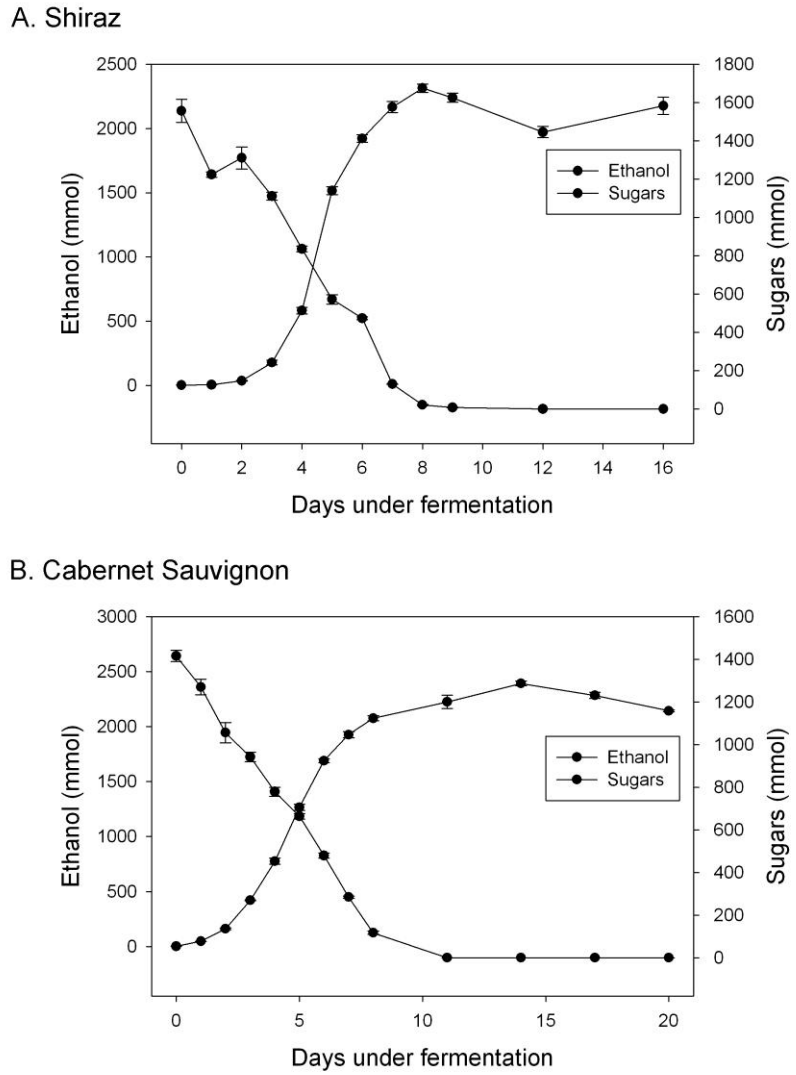
Besides the major product, ethanol, alcoholic fermentation of grapes also produces an array of volatile compounds linked to wine flavour (Mauricio et al. 1997; Rapp, 1998). The concurrent alcoholic fermentation and flavour development showed three distinct stages and are defined in the context of this study as flavour initiation (lag) phase: 0 – 2 days; flavour growth (development) phase: 3 – 8 days; and flavour consolidation phase: 9 – 20 days. The initial 2-day lag followed by an exponential growth on day-3 (Figure 6) was consistent with earlier studies (Stashenko et al., 1992; Vianna et al., 2001).

During the initiation phase, the fermentation contained very few volatile compounds but these grew in number and amount with the progression of fermentation (Table 20 and Figure 7). Through the flavour consolidation phase some compounds disappeared (e.g. methyl esters and Z-3-hexen-1-ol) while some were formed (e.g. ethyl esters and 2,3-butanediol, Table 20 and Figure 7). Flavour developmental rates peaked on the fourth day coinciding with the intercept between alcohol formation and sugar depletion of the fermentation curves (Figure 6). This could entail that flavour development is at equilibrium and this equilibrium point could be estimated from the intercept of the alcohol formation and sugar depletion (Figure 6). Indeed, it is at this equilibration point when most of the changes from grape to wine flavour occur.

Grape volatile profiles were dominated by C6-aldehydes/alcohols while esters and alcohols dominated wines (Table 20 and Figure 7). The change from grape to wine flavour is neither direct nor instantaneous and usually occurs within a two-week period in phases. During this period, many potential wine flavour precursors were detected (Figure 7), most of which have received minimal attention in wine flavour research. The flavour initiation phase (0 – 2 days) was characterised with the reduction of aldehydes to alcohols. The dominant volatile compounds were hexan-1-ol, *E*-2-hexen-1-ol and *Z*-3-hexen-1-ol (Table 20 and Figure 7), consistent with an earlier study (Herraiz et al. 1990). Towards the end of this phase, C6-esters (ethyl hexanoate and hexyl acetate) started to appear and their abundances increased thereafter (Peaks 7 and 8; Figure 7).

The flavour growth phase (3 – 8 days) started with the formation of the methyl-branched alcohols, derived from amino acids (Schwab et al. 2008), and appearance of phenyl derivatives (Table 20). Later during this phase, there was a generation of C8 – C12 carboxylic acids and accumulation of methyl esters of the same carbon length (Table 20).

Figure 6. Fermentation curves for Shiraz (A) and Cabernet Sauvignon (B) showing the changes in ethanol and sugars (glucose + fructose) during vinification. Error bars represent standard errors for independent triplicate ferments.



Among the carboxylic acids in wine, hexanoic (C6) and octanoic (C8) acids have been shown to be dominant (Torija et al. 2003), consistent with our observations during alcoholic fermentation (Table 20). It has been suggested that carboxylic acid constituents originally present in grapes might bear some relationship with wine quality and some of these acids might distinguish different grape varieties (Yunoki et al. 2004). Observations from our study (Table 20 and Figure 7), suggests that most of these carboxylic acids react quickly forming methyl esters as evidenced through the simultaneous appearance of these two groups of volatile compounds during alcoholic fermentation. As well as the formation of methyl esters during early fermentation, there was also a progressive appearance of acetate esters followed by ethyl esters. Towards the end of this flavour growth phase, methyl esters were less abundant than ethyl esters while acetate esters were not depleted in the fermentations (Table 20).

Accumulation of C8 – C12 ethyl esters concurrent with the depletion of respective methyl esters and carboxylic acids characterised the flavour consolidation phase (Table 20). During this phase, esters and alcohols were the major volatile classes. Additionally, it was observed that the major compounds detected throughout alcoholic fermentation possessed even-numbered carbon chains (Figure 7, Tables 21 and 22), consistent earlier observations (Nykanen 1986). This suggests that the major biochemical pathways leading to the synthesis of these volatiles utilise the C2 acetyl-groups as building blocks or involve the cleavage of long chain compounds common in plants (Schwab et al. 2008). This might imply that most of the volatile compounds in wine derive from plant precursors with yeast as a processing aid. Indeed, it has been suggested that formation of diketones occurs outside the yeast cell (Suomalainen & Ronkainen 1968), which is hypothesized (in this work) as an active area for flavour development during alcoholic fermentation. This is opposed to the ideology of flavour formation totally within the yeast cell (Nykanen, 1986; Saerens et al. 2008; Swiegers et al. 2005). A closer examination of this concept and the probable changes and mechanisms of grape to wine flavour development was subsequently explored by studying the quantitative trends observed during alcoholic fermentation.

Straight chain aldehydes/alcohols are the major components of the volatile profile for crushed grapes – the starting raw material for the winemaking process (Figure 7). The majority of these straight chain aldehydes/alcohols (Table 20) are C6-compounds formed through the lipoxygenase pathway, which have been explored in other work described in this report (Kalua et al. 2009).

Aldehydes were common in freshly crushed grapes and conceivably reduced to alcohols with the action of alcohol dehydrogenase (ADH) as is observed by the increase of their respective alcohols as the fermentation progresses (Tables 22 and 22). The reduction of C6-aldehydes to C6-alcohols during alcoholic fermentation has been reported previously (Joslin et al., 1978), and this is consistent with our observations (Tables 20-22). A reduction in E-2-hexenal (a major volatile compound in fresh crushed grapes) corresponds to an increase in hexanal levels from day-0 to day-2 (Tables 21 and 22), a probable indication of reduction from E-2-hexenal to hexanal. Possible further reduction of hexanal and E-2-hexen-1-ol is suggested from the corresponding increase of hexan-1-ol after day-2 (Tables 21 and 22). Eventually, it can be hypothesized that E-2-hexenal, hexanal, and E-2-hexen-1-ol were all reduced to hexan-1-ol within the first four days of alcoholic fermentation and carried through from grapes to wine (Tables 21 and 22). Consequently, among the C6-compounds, hexan-1-ol concentrations significantly increased during alcoholic fermentation of both Shiraz and Cabernet Sauvignon grapes (Tables 21 and 22), which is consistent with an earlier observation (Herraiz et al. 1990) but contrary to another report where hexan-1-ol concentrations declined within a few days of the beginning of the alcoholic fermentation (Mauricio et al. 1997). The attenuation of the leafy/grassy odour in fermented grapes has been attributed to this corresponding increase of hexan-1-ol and a depletion of the C6-aldehydes and unsaturated alcohols (Herraiz et al., 1990) since the C6-aldehydes have generally lower odour threshold values than hexan-1-ol (Kalua et al. 2007; Rapp 1998). It is also envisaged that the C6-compounds contribute to the production of the fruity smelling hexyl acetate and hexyl hexanoate (from the hexan-1-ol precursor) after the flavour initiation phase (Table 20).

Table 20. Volatile compounds and classes explaining the alcoholic fermentation progression of both Shiraz and Cabernet Sauvignon grapes.

	Volatile Compounds and Classes Discriminating Vinification Stages							
Flavour Development Phase	***Alcohols***	***Aldehydes***	***Carboxylic Acids***	***Phenyl Derivatives***	***Acetate Esters***	***Methyl Esters***	***Ethyl Esters***	***Miscellaneous Esters***
Flavour Initiation (0 – 2 days)	Ethanol Hexan-1-ol E-2-Hexen-1-ol Z-3-Hexen-1-ol Octan-1-ol	Hexanal E-2-Hexenal	Acetic acid					
Flavour Growth (3 – 8 days)	Ethanol 2-Methyl-1-propanol 3-Ethoxy-1-propanol 3-Methyl-1-butanol Hexan-1-ol Z-3-Hexen-1-ol Heptan-1-ol	Hexanal	Acetic acid Hexanoic acid Octanoic acid	Benzyl alcohol 2-Phenyl ethanol 2-Phenylethyl acetate	3-Methylbutyl acetate Hexyl acetate Heptyl acetate	Methyl octanoate Methyl decanoate Methyl tetradecanoate	Ethyl butyrate Ethyl hexanoate Ethyl heptanoate Ethyl octanoate Ethyl nonanoate Ethyl decanoate Ethyl undecanoate Ethyl dodecanoate Ethyl tetradecanoate Ethyl-9-decenoate Ethyl-E-2-hexenoate	3-Methylbutyl hexanoate 3-Methylbutyl octanoate 3-Methylbutyl decanoate Hexyl hexanoate 2-Methylpropyl octanoate Propyl octanoate Propyl decanoate
Flavour Consolidation (9 – 20 days)	Ethanol 2,3-Butanediol 2-Methyl-1-propanol 3-Methyl-1-butanol 3-Methyl-1-pentanol Hexan-1-ol Heptan-1-ol		Acetic acid Hexanoic acid	Benzyl alcohol 2-Phenyl ethanol 2-Phenylethyl acetate	3-Methylbutyl acetate Hexyl acetate Heptyl acetate	Methyl octanoate Methyl decanoate	Ethyl butyrate Ethyl hexanoate Ethyl heptanoate Ethyl octanoate Ethyl nonanoate Ethyl decanoate Ethyl dodecanoate Ethyl tetradecanoate Ethyl-9-decenoate Ethyl-E-2-hexenoate	3-Methylbutyl octanoate 3-Methylbutyl decanoate 2-Methylpropyl octanoate Propyl octanoate Propyl decanoate
Bottled Wine (224 & 231 days)	Ethanol 2,3-Butanediol 2-Methyl-1-propanol 3-Methyl-1-pentanol Hexan-1-ol Heptan-1-ol		Acetic acid Hexanoic acid	2-Phenyl ethanol 2-Phenylethyl acetate	3-Methylbutyl acetate Hexyl acetate		Ethyl hexanoate Ethyl heptanoate Ethyl octanoate Ethyl decanoate Ethyl-E-2-hexenoate	

Figure 7. Chromatograms illustrating progression of flavour development at different fermentation stages for Shiraz: (A), Initial stage before fermentation; (B), Four days into fermentation; (C), Sixteen days into fermentation. (1) Ethyl acetate; (2) Ethanol; (3) Hexanal; (4) 3-Methylbutyl acetate; (5) E-2-Hexenal; (6) 3-Methyl-1-butanol; (7) Ethyl hexanoate; (8) Hexyl acetate; (9) [$^{2}H_{13}$]hexanol (Internal standard); (10) hexan-1-ol; (11) Methyl octanoate; (12) Z-3-Hexen-1-ol; (13) E-2-Hexen-1-ol; (14) Ethyl octanoate; (15) Acetic acid (16) Methyl decanoate; (17) Ethyl decanoate; (18) 3-Methylbutyl octanoate; (19) Ethyl-9-decenoate; (20) Methyl dodecanoate; (21) 2-Phenylethyl acetate; (22) Ethyl dodecanoate; (23) 2-Phenyl ethanol; (24) Octanoic acid; (25) Decanoic acid.

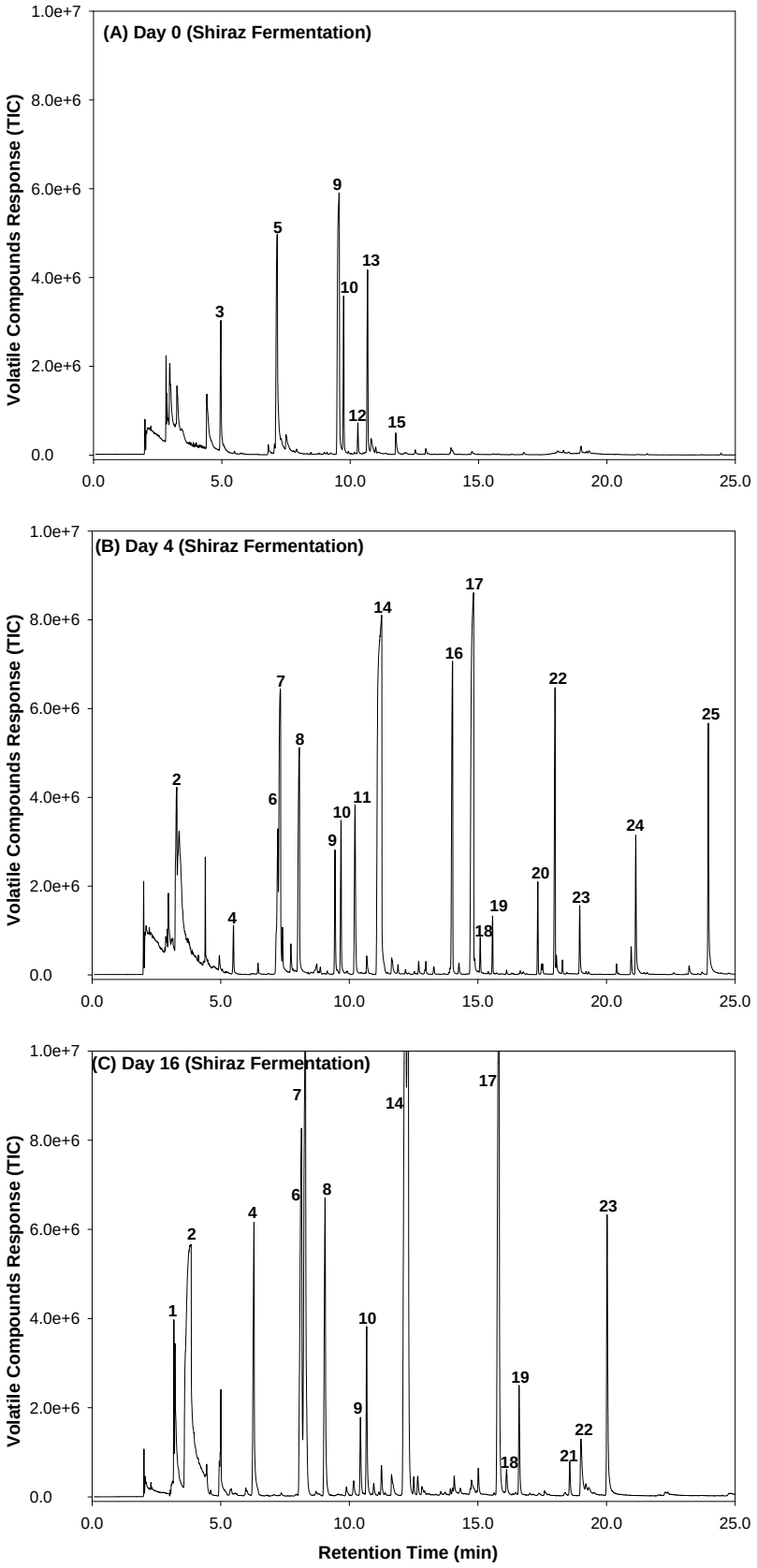


Table 21. Shiraz - Levels of major volatile compounds during alcoholic fermentation progression

Volatile Compounds and Classes	***Progress of Alcoholic Fermentation (Concentrations [A] at specified days)***						
	0	***2***	***4***	***8***	***12***	***16***	***224***
Aldehydes							
Hexanal	7.6 ± 0.8 c	1.2 ± 0.2 a	5 ± 2 b	ND	ND	ND	ND
E-2-Hexenal	17 ± 1 b	1.0 ± 0.2 a	ND	ND	ND	ND	ND
Alcohols							
Hexan-1-ol	6.4 ± 0.2 a	29.3 ± 0.2 b	32 ± 5 b	44 ± 5 c	55 ± 3 d	57 ± 4 d	62 ± 2 d
E-2-Hexen-1-ol	8.29 ± 0.09 b	0.14 ± 0.02 a	ND	ND	ND	ND	ND
Heptan-1-ol	ND	0.16 ± 0.01 a	ND	3.26 ± 0.09 b	4.2 ± 0.4 c	4.9 ± 0.6 c	5.2 ± 0.3 c
Octan-1-ol	ND	0.10 ± 0.01	ND	ND	ND	ND	ND
3-Methyl-1-butanol	ND	9.6 ± 1.0 a	64 ± 9 b	ND	ND	250 ± 14 c	ND
Carboxylic Acids							
Hexanoic acid	ND	0.48 ± 0.08 a	4 ± 1 b	10.7 ± 1.0 c	12 ± 1 c	2.3 ± 0.8 ab	3.9 ± 0.2 b
Octanoic acid	ND	0.4 ± 0.1 a	18 ± 1 b	22 ± 3 b	21 ± 3 b	1.2 ± 0.7 a	1.5 ± 0.5 a
Decanoic acid	ND	< 0.4	27 ± 4	13 ± 4	4 ± 1	ND	ND
Phenyl Derivatives							
Benzyl alcohol	ND	0.14 ± 0.03 a	0.4 ± 0.2 b	1.08 ± 0.07 d	0.79 ± 0.09 c	ND	ND
2-Phenyl ethanol	ND	1.0 ± 0.2 a	17 ± 7 a	141 ± 13 c	152 ± 7 c	96 ± 7 b	94 ± 2 b
2-Phenylethyl acetate	ND	ND	1.6 ± 0.7 a	17 ± 2 d	13.9 ± 1.0 c	6.5 ± 0.5 b	5.7 ± 0.4 b
Acetate Esters							
Hexyl acetate	< 0.05	6.4 ± 0.5 a	57 ± 5 b	101 ± 10 d	95 ± 14 cd	88 ± 6 cd	71 ± 13 bc
Heptyl acetate	ND	ND	0.41 ± 0.09	ND	ND	ND	ND
Octyl acetate	ND	ND	1.19 ± 0.07 ab	1.3 ± 0.1 b	1.10 ± 0.9 a	ND	ND
Z-3-Hexenyl acetate	ND	0.11 ± 0.02 a	1.3 ± 0.2 b	2.8 ± 0.2 d	2.1 ± 0.4 c	2.5 ± 0.1 cd	ND
3-Methylbutyl acetate	0.15 ± 0.06 a	0.5 ± 0.3 a	21 ± 13 a	72 ± 10 b	75.3 ± 0.8 b	79 ± 1 b	79 ± 8 b
Methyl Esters							
Methyl hexanoate	ND	0.15 ± 0.05 a	1.1 ± 0.3 b	ND	ND	ND	ND
Methyl octanoate	ND	2.1 ± 0.2 a	20 ± 4 b	5.2 ± 0.2 a	3 ± 1 a	2.7 ± 0.6 a	1.8 ± 0.2 a
Methyl decanoate	ND	0.7 ± 0.1 a	31 ± 6 d	13.3 ± 0.3 c	9.6 ± 0.3 bc	3.9 ± 0.6 ab	2.1 ± 0.3 a
Ethyl Esters							
Ethyl butyrate	ND	ND	< 1.0	12 ± 3 b	11 ± 2 ab	6.8 ± 0.3 a	ND
Ethyl hexanoate	ND	0.55 ± 0.09 a	80 ± 8 b	247 ± 31 c	198 ± 14 c	217 ± 16 c	256 ± 29 c
Ethyl heptanoate	ND	ND	0.54 ± 0.08 a	3.7 ± 0.1 c	3.9 ± 0.3 c	3.6 ± 0.1 c	2.8 ± 0.5 b
Ethyl octanoate	<0.05	1.3 ± 0.2 a	150 ± 30 b	389 ± 23 cd	443 ± 33 d	361 ± 67 cd	290 ± 32 c
Ethyl nonanoate	ND	ND	1.07 ± 0.05 a	4.2 ± 0.4 b	5 ± 1 b	0.91 ± 0.07 a	0.52 ± 0.07 a
Ethyl decanoate	ND	<0.5	103 ± 12 a	287 ± 27 c	279 ± 23 c	175 ± 26 b	79 ± 15 a
Ethyl dodecanoate	ND	ND	25 ± 3 b	66 ± 10 c	54 ± 4 c	17 ± 4 ab	6 ± 1 a
Ethyl tetradecanoate	ND	ND	1.4 ± 0.5 a	3.2 ± 0.7 b	0.80 ± 0.02 a	ND	ND
Miscellaneous Esters							
3-Methylbutyl hexanoate	ND	ND	3.6 ± 0.5 a	5.8 ± 0.7 b	5.4 ± 0.7 b	3.8 ± 0.6 a	ND
3-Methylbutyl octanoate	ND	ND	3.5 ± 0.4 ab	12 ± 2 c	13 ± 1 c	5 ± 1 b	1.9 ± 0.5 a
3-Methylbutyl decanoate	ND	ND	0.9 ± 0.1 a	4.4 ± 0.9 b	7.3 ± 0.4 c	ND	ND
Hexyl hexanoate	ND	ND	1.5 ± 0.2 b	1.6 ± 0.3 b	0.7 ± 0.1 a	ND	ND
Propyl octanoate	ND	ND	1.8 ± 0.2 b	1.7 ± 0.2 b	ND	0.54 ± 0.05 a	ND

Different letters in a row represent significantly ($p<0.05$) different means ± standard error ($n=3$ independent ferments). ND represents not detectable at S/N=3. [A] Concentration (μmol/kg of ferment based on 21.25μmol of d13-Hexanol per kilogram of ferment)

Table 22. Cabernet Sauvignon - Levels of major volatile compounds during alcoholic fermentation progression

Volatile Compounds and Classes	***Progress of Alcoholic Fermentation (Concentrations [A] at specified days)***						
	0	***2***	***4***	***8***	***12***	***16***	***231***
Aldehydes							
Hexanal	5 ± 1 b	1.0 ± 0.2 a	1.6 ± 0.3 a	ND	ND	ND	ND
E-2-Hexenal	19 ± 2 b	0.7 ± 0.2 a	1.4 ± 0.5 a	ND	ND	ND	ND
Alcohols							
Hexan-1-ol	5.4 ± 0.3 a	21.1 ± 0.7 b	26.3 ± 0.9 c	30 ± 2 d	29.3 ± 0.6 cd	28.2 ± 0.2 cd	36 ± 2 e
E-2-Hexen-1-ol	5.6 ± 0.2 b	0.24 ± 0.05 a	ND	ND	ND	ND	ND
Heptan-1-ol	ND	0.11 ± 0.01 a	1.0 ± 0.1 b	ND	9.8 ± 0.4 d	2.7 ± 0.4 c	3.3 ± 0.2 c
Octan-1-ol	ND	0.39 ± 0.05 a	ND	0.70 ± 0.07 c	0.56 ± 0.02 b	ND	ND
3-Methyl-1-butanol	ND	ND	79 ± 3 a	106 ± 8 a	312 ± 1 b	ND	ND
Carboxylic Acids							
Hexanoic acid	<0.05	0.8 ± 0.2 a	6.3 ± 0.5 bc	8 ± 1 c	9.1 ± 0.5 cd	11.4 ± 0.8 d	4 ± 2 b
Octanoic acid	0.12 ± 0.05 a	2.4 ± 0.6 a	17 ± 1 b	14 ± 4 b	18.2 ± 0.5 b	27 ± 2 c	4.6 ± 0.4 a
Decanoic acid	ND	3.1 ± 0.9	19 ± 2	ND	ND	ND	ND
Phenyl Derivatives							
Benzyl alcohol	< 0.5	< 0.5	1.1 ± 0.3 a	1.9 ± 0.2 b	1.76 ± 0.04 b	2.7 ± 0.2 c	1.4 ± 0.2 ab
2-Phenyl ethanol	1.1 ± 0.3 a	3.7 ± 0.7 a	54 ± 8 b	163 ± 4 cd	181 ± 5 d	219 ± 10 e	156 ± 6 c
2-Phenylethyl acetate	0.18 ± 0.03 a	0.30 ± 0.04 a	3.1 ± 0.3 b	7.0 ± 0.4 c	7.8 ± 0.4 c	9.9 ± 1.0 d	3.0 ± 0.2 b
Acetate Esters							
Hexyl acetate	ND	12.6 ± 0.4 a	39.3 ± 1.0 e	33 ± 1 d	33.5 ± 0.3 d	28 ± 1 c	20 ± 2 b
Heptyl acetate	ND	ND	ND	1.58 ± 0.08 a	1.93 ± 0.02 b	1.7 ± 0.1 a	ND
Octyl acetate	ND	ND	0.6 ± 0.1	ND	ND	ND	ND
Z-3-Hexenyl acetate	ND	0.09 ± 0.02	ND	ND	ND	ND	ND
3-Methylbutyl acetate	ND	4 ± 2 a	27 ± 9 b	33 ± 1 b	36.6 ± 0.1 b	37 ± 3 b	57 ± 13 c
Methyl Esters							
Methyl hexanoate	ND	0.26 ± 0.09 a	0.7 ± 0.2 b	ND	ND	ND	ND
Methyl octanoate	ND	4.6 ± 0.4 c	12.3 ± 0.2 d	3.0 ± 0.3 b	2.5 ± 0.2 b	4.8 ± 0.3 c	1.4 ± 0.6 a
Methyl decanoate	ND	2.6 ± 0.3 ab	16 ± 1 e	5.7 ± 0.3 c	5.0 ± 0.5 bc	10 ± 2 d	1.5 ± 0.1 a
Ethyl Esters							
Ethyl butyrate	ND	ND	4.0 ± 0.6 a	7.2 ± 0.7 b	6.8 ± 0.4 b	9.9 ± 0.3 c	6.5 ± 0.6 b
Ethyl hexanoate	ND	12 ± 1 a	63 ± 3 ab	153 ± 3 bc	163 ± 3 c	176 ± 6 c	140 ± 7 bc
Ethyl heptanoate	ND	ND	0.9 ± 0.1 a	4.04 ± 0.09 c	5.01 ± 0.05 d	5.3 ± 0.4 d	2.6 ± 0.2 b
Ethyl octanoate	<0.5	21 ± 4 a	197 ± 23 b	414 ± 8 d	413 ± 20 d	312 ± 68 c	183 ± 4 b
Ethyl nonanoate	ND	0.12 ± 0.02 a	1.5 ± 0.3 c	2.4 ± 0.1 d	2.3 ± 0.2 d	3.9 ± 0.2 e	0.67 ± 0.09 b
Ethyl decanoate	<0.5	7.8 ± 0.7 a	97 ± 9 c	182 ± 1 d	210 ± 14 d	272 ± 30 e	49.4 ± 0.6 b
Ethyl dodecanoate	<0.05	0.9 ± 0.2 a	20 ± 2 b	40 ± 2 cd	37 ± 4 c	48 ± 7 d	1.7 ± 0.7 a
Ethyl tetradecanoate	ND	<0.05	1.0 ± 0.2 a	2.0 ± 0.3 b	1.6 ± 0.3 ab	1.5 ± 0.2 ab	ND
Miscellaneous Esters							
3-Methylbutyl hexanoate	ND	0.13 ± 0.03 a	2.8 ± 0.2 b	5.5 ± 0.2 d	ND	4.2 ± 0.5 c	ND
3-Methylbutyl octanoate	ND	0.23 ± 0.04 a	5.6 ± 0.3 b	9.8 ± 0.2 c	9.6 ± 0.7 c	12 ± 2 d	1.4 ± 0.2 a
3-Methylbutyl decanoate	ND	ND	1.9 ± 0.2 a	5.3 ± 0.1 b	6.5 ± 0.8 bc	8 ± 1 c	ND
Hexyl hexanoate	ND	0.10 ± 0.02 a	0.75 ± 0.01 c	0.45 ± 0.06 b	ND	ND	ND

Volatile Compounds and Classes	*Progress of Alcoholic Fermentation (Concentrations [A] at specified days)*						
	0	***2***	***4***	***8***	***12***	***16***	***231***
Propyl octanoate	ND	ND	1.7 ± 0.3 b	2.0 ± 0.2 b	0.96 ± 0.03 a	0.73 ± 0.09 a	ND

Different letters in a row represent significantly ($p<0.05$) different means ± standard error ($n=3$ independent ferments). ND represents not detectable at S/N=3. [A] Concentration (μmol/kg of ferment based on 21.25μmol of d13-Hexanol per kilogram of ferment)

Early into the flavour growth phase of fermentation (day-3), branched-chain aldehydes/alcohols, phenyl derivatives, and carboxylic acid appeared in the fermentations (Table 21). This appearance coincided with the increase in the levels of ethanol (Figure 7) suggesting that the action of yeast during alcoholic fermentation is important for their formation. Branched-chain aldehydes/alcohols have been reported to originate from amino acids valine (R1 = $CH(CH_3)2$), leucine (R1 = $CH_2CH(CH_3)2$), and isoleucine (R1 = $CH(CH_3)CH_2CH_3$), in Equation 1 forming 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-methyl-1-butanol, respectively (Schwab et al. 2008). The formation of these alcohols from the corresponding amino acids occurs in three steps (Equation 1): de-amination (i) of the amino acids with the aid of aminotransferase provides 2-keto acids; which decarboxylate (ii) to form aldehydes; which are then reduced (iii) to the alcohols.

$$\underset{\text{Amino acid}}{R_1CH(NH_2)COOH} \xrightarrow{(i)} \underset{\text{2-Keto acid}}{R_1COCOOH} \xrightarrow{(ii)} \underset{\text{Aldehyde}}{R_1CHO} \xrightarrow{(iii)} \underset{\text{Alcohol}}{R_1CH_2OH} \qquad (1)$$

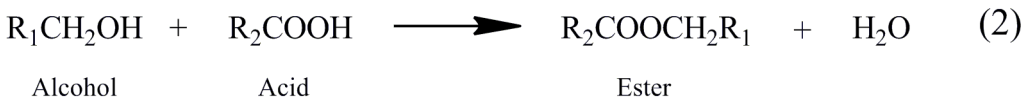
It is not clear if grapes play a role in this transformation. The observed initiation of this process after the onset of alcoholic fermentation indicates that the reaction needs a trigger from the fermentation process (Tables 21 and 22). This could be release of amino acids or catalysts from the grape material or yeast. Keto acids and branched-chain aldehydes were not detected in this study and this could be due to either low volatility or fast reaction rate/conversion to form other flavour compounds that are stable in the grape/wine matrix. Grapes have the potential to reduce aldehydes to alcohols during physiological development (Kalua et al. 2009) but whether the enzymes that are responsible for such reactions are active in fermentations is unknown. This potential for grapes to reduce aldehydes to alcohols does not eliminate the possibility that it is solely the yeast that drive the conversion of amino acids to alcohols (Equation 1). Indeed, it has been suggested that yeast metabolism and degradation of dead yeast could release enzymes and hydrolyse grape material releasing volatile compounds precursors such as amino acids (Perez-Serradilla & de Castro, 2008).

There was a significant increase in the amount of 3-methyl-1-butanol (from leucine, Equation 1) after the onset of alcoholic fermentation in both Shiraz and Cabernet Sauvignon (Tables 21 and 22). A similar observation was made for 2-phenyl ethanol (Tables 21 and 22), which is reported to originate from the aromatic amino acid phenylalanine (R1 = CH_2(Phenyl) in Equation 1) during alcoholic fermentation (Lamikanra et al. 1996; Schwab et al. 2008). Overall, the formation of phenyl derivatives appeared to be predominantly from the vinification process, although trace amounts were present in grape juice (Tables 21 and 22). Levels of the phenyl

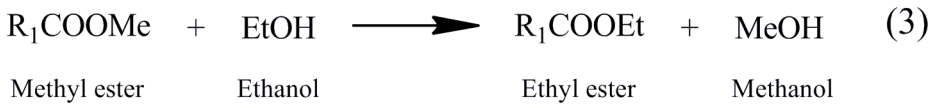
derivatives peaked at 16 days (Tables 21 and 22), consistent with an earlier observation of 2-phenyl ethanol (Lamikanra et al. 1996).

The hydrolysing effect of yeast (Perez-Serradilla et al. 2008) could explain the presence of carboxylic acids in the ferment (Tables 21 and 22). Levels of carboxylic acids increased at the onset of alcoholic fermentation and decreased thereafter (Tables 21 and 22), consistent with an earlier report (Molina et al. 2007). It is not clear whether these carboxylic acids originate from direct synthesis via acyl-CoA intermediates in the yeast or from hydrolysis of other fatty acid containing compounds such as triglycerides and simple phospholipids from grapes and dead yeast cells (lees). Indeed, phospholipids have been reported as major structural components of yeast cells and there are phospholipases that are activated in presence of aqueous ethanol (Suomalainen 1971; Swiegers et al. 2005). However, it is still unclear whether these alcohols and carboxylic acids that appear at the onset of alcoholic fermentation are direct products of yeast metabolism or formed from the interactions of yeast metabolites with the grape material.

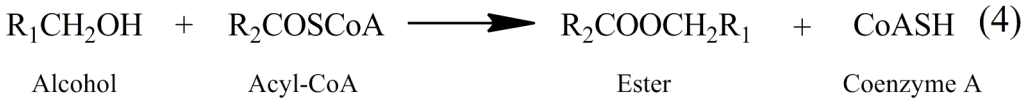
During the early flavour growth phase (day-4), methyl-, acetate-, and ethyl esters appear consecutively in the fermentations (Tables 21 and 22). Methyl esters peaked before acetate- and ethyl esters. The different flavour development patterns for carboxylic acids and methyl esters from the ethanol development curve could indicate that carboxylic acids and methyl esters are formed from an indirect influence of the vinification process. Methyl esters could potentially form from the esterification of methanol and carboxylic acids (Equation 2). Carboxylic acids could be released from the yeast or grape tissue during early fermentation alluded to above whereas methanol is believed to derive from the enzymatic degradation of pectin in grapes (Gnekow & Ough 1976; Vilanova et al. 2007). However, this process is not expected to be rapid and would require catalysis by an enzyme (see below).



Methyl esters were not detected at the beginning of alcoholic fermentation but significantly increased to a maximum at day-4 (Tables 21 and 22) prior to decreasing to undetectable levels in the finished wine. This decrease in methyl esters that preceded the accumulation of ethyl- and acetate esters (Tables 21 and 22) suggests a possible two-step mechanism for the formation of some wine ethyl esters through methyl esters, similar to observations made in studies into fermented dairy products (Liu et al. 2004). This two step mechanism involves esterification (Equation 2) and alcoholysis (Equation 3), culminating in the loss of methyl esters and accumulation of ethyl esters.



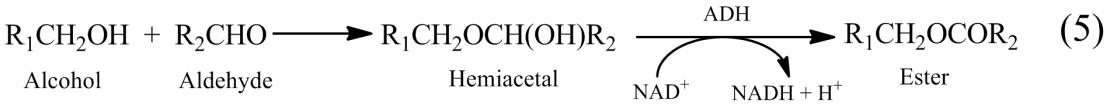
To the best of our knowledge, this is the first time that a two step ester formation mechanism has been proposed for ethyl ester flavour development in wines. In wines, it is believed that fatty acid ethyl esters are formed from the reaction of acyl-CoA with ethanol catalysed by alcohol acetyl transferase (AAT) during alcoholic fermentation of grape juice (Equation 4; Herraiz et al., 1993; Nykanen, 1986; Webb, 1967). This concept of direct ethyl ester synthesis through acetyl- and acyl-CoA (Equation 4) does not account for the presence of methyl esters in the fermentation (Tables 21 and 22). It is possible that both pathways exist, although the relative importance of both is unknown. However, the mechanism for methyl ester production is most likely catalysed by AAT (Equation 4) rather than via the non-catalysed condensation reaction depicted in Equation 2.



Acetate esters significantly increased with the progression of alcoholic fermentation (Tables 21 and 22). In general, the concentration of acetate and ethyl esters increased during the exponential growth phase of alcoholic fermentation, up to the mid-point of the exponential phase, with a slight decrease during the stationary phase, consistent with earlier observations (Molina et al. 2007; Vianna et al. 2001). It is unlikely that acetate esters follow a two-step mechanism since the substitution should occur with the alcohol moiety of the ester (Equation 3). Acetate esters are most likely formed following Equation 4 (R2 = CH3) with the acetyl-CoA potentially coming from yeast metabolism or released from the grape material. The catalytic activity for ester synthesis is more likely to come from yeast alcohol acetyl transferase (AAT). However, it has been suggested that both enzymatic and spontaneous chemical ester formation are practically possible during fermentation with the enzymatic ester formation reported to be more significant than the latter (Liu et al. 2004).

With most of the flavour compounds formed during the flavour growth phase (first week of alcoholic fermentation), a cooling-off in the rates of formation was observed during the flavour consolidation phase (> day-8). Early during this flavour consolidation phase (day-9), miscellaneous esters showed a brief significant increase, only to decrease to trace levels in the finished wines (Tables 21 and 22). Miscellaneous esters (Table 20), in the context of this study, refer to majorly branched-chain and non-aliphatic esters.

Most of the miscellaneous esters are likely to originate from the de-amination, decarboxylation, and reduction of amino acids and compounds in the grape material initially forming alcohols (Equation 1). The alcohols formed could react with Co-A esters of carboxylic acids (Equation 4) produced during the early stages of alcoholic fermentation. It is therefore not surprising that these miscellaneous esters appear in the ferment at the same time as their respective alcohols and carboxylic acids (Tables 21 and 22). On the other hand, minor straight chain miscellaneous esters, such as hexyl hexanoate, could be formed from the oxidation of hemiacetals (Equation 5), as has been suggested for some microbial ester formation (Park et al., 2009).



(5)

Apart from C6-aldehydes, other aldehydes were rare in the fermentations (Table 20). This suggests that the formation of esters through oxidation of the hemiacetal with nicotinamide adenine dinucleotide (NAD+) as a hydrogen acceptor (Equation 5) is less likely to occur than from an alcohol and an acyl-CoA (Equation 4). Alternatively, miscellaneous esters could be formed from alcoholysis (Equation 3) with minor alcohols, such as 3-methyl-1-butanol. It should also be noted that ester accumulation is an equilibrium between ester formation and hydrolysis (Liu et al. 2004; Swiegers et al. 2005; Vianna et al. 2001). In our study, the rate of ester formation peaked between four and six days into alcoholic fermentation and generally declined thereafter. The decrease in levels of esters (Tables 21 and 22) later during fermentation could indicate that the rate of ester hydrolysis is greater than the rate of ester formation. The final equilibrium concentration of esters could thus depend on the thermodynamic and kinetic stability of different flavour compounds in the wine matrix. Thermodynamic stability takes precedence in freshly produced wine whereas kinetic stability is attained with aging. Such kinetic stability phenomena could be associated with the reported decrease of acetate esters during wine maturation (Rapp 1998).

This study shows that the formation of volatile compounds in wine during alcoholic fermentation could be explained in the context of potential precursors in grapes. While ethanol is the major product of alcoholic fermentation, it is apparent that several volatile compounds with flavour potential are formed alongside ethanol. Phenyl-, branched and straight chain alcohols (apart from ethanol) are potentially derived from the reduction of aldehydes present in the grapes. The straight chain aldehydes and minor quantities of phenyl aldehydes were originally present in grapes while branched chained and the majority of phenyl aldehydes were released during alcoholic fermentation. Fresh crushed grapes had aldehydes as major volatile compounds but the fermentation was dominated by alcohols early in the process. Esters could be formed either from direct esterification (Equation 2 & 4) or through alcoholysis (Equation 3) with methyl esters as intermediates. Esters dominated the growth and consolidation phases and were the major volatile compounds in the finished wine.

The presence of methyl esters during alcoholic fermentation is rarely reported whereas the detection of carboxylic acids is consistent with earlier studies (Edwards et al. 1990; Yunoki et al. 2005). The findings of this study suggest that methyl esters may act as precursors for ester formation (Equations 3). However, formation of esters through acyl-CoA (Equation 4) is likely to be the major pathway to ester production. Nevertheless, the relative importance of each to wine ester concentrations needs further exploration. It is still unclear the extent to which the carboxylic acids and alcohols (apart from ethanol) utilised for ester production are direct products of yeast metabolism or grape-derived. It is also unclear the extent to which these reactions are catalysed by enzymes in the yeast, or can occur outside the cells in the acidic conditions of a fermentation. Evidence from subprojects A1-A4 certainly suggests

that the grape berry is a source of precursors for ester formation and identification of these precursors will enhance our understanding of the effect of grape composition on wine chemical and sensory properties.

B. Grape flavour development and the effects of vineyard variables on wine volatile composition and sensory attributes.

This section describes the results from field trials aimed at generating robust knowledge about the timing of flavour development in the berry and the impact of environmental or viticultural management practices on wine flavour and aroma. Current industry concerns were the focus of this work, namely effects of irrigation regimes, yield and harvest timing on wine composition. Work was also conducted with the precision viticulture team led by Dr Rob Bramley (CSIRO Ecosystem Sciences) to further highlight the potential benefits that could be gained by vineyard mapping and selective harvesting of parcels of grapes, and this has been now put in the context of wine sensory outcomes. These studies feed directly into the work described in section A, that is, the linking of grape composition to wine chemical and sensory properties.

Subproject B1: Volatile production in berries during development

The work presented in this section has been published in the manuscripts Kalua and Boss (2009), and Kalua and Boss (2010).

The grapes themselves represent the largest raw material used in the production of any wine and as such play an important role in determining the sensory attributes of the final product. Importantly, the grapes not only supply sugars to the yeast for ethanol production, but also provide other organic compounds that the yeast may utilise in the production of volatile aroma compounds. Other grape compounds may persist through to the wine after undergoing minimal alteration during the winemaking process and therefore have an impact on wine flavour and aroma. Some of these grape compounds are volatile in the berries and may be either tasted in the grapes or detected in the headspace above a sample of crushed grapes.

Evolution of volatile compounds from fruit-set to late ripening in most fruits is characterized by an accumulation of fruity esters and terpenes (Beekwilder et al. 2004; Moshonas et al. 1997). Understandably, most studies have focused on the changes in the volatile profiles of crushed berries postveraison (e.g. Gomez et al. 1995, Park et al.1991) as this is when many physiological changes are occurring in berries. However, it is still unclear what happens to volatile compounds and their precursors prior to veraison and whether some potential aroma compounds are synthesized or sequestered during this period. Furthermore, previous studies on the evolution of volatile compounds have focused on terpenes and benzene derivatives (Coelho et al. 2006, 2007; Park et al. 1991) with a few exploring C6-volatile compounds (Garcia et al. 2003). These studies are characterized by the subjective selection of compounds or a group of compounds based on prior experience. In this

study, we used a multivariate statistical technique to identify volatile compounds that are significantly changing during grape berry development.

In general, the understanding of the evolution of volatile compounds evolution during berry development is lacking, as is a comprehensive understanding of the links between grape and wine aroma. The established view of the impact of grape-derived volatile compounds on wine sensory attributes is based around grape aroma components that undergo no or minimal alteration during fermentation, such as terpenes and methoxypyrazines (Rapp & Mandery 1986). In combination with the research described in section A of this report, an understanding of changes in secondary metabolism during berry development may provide predictive information about the link between grape and wine aroma.

For the first part of this study, Cabernet Sauvignon grapes were sampled fortnightly from three different sections of a vineyard in the Southern Vales across two vintages. During the 2006-07 vintage, Cab07 berries (Table 23) were collected at fortnightly intervals from 3 weeks post-flowering (3wpf) and were sampled from vines that were 5-10 m away from Eucalyptus trees. Weeks post-flowering (wpf) was counted from the time of a minimum of eighty percent cap-fall. During the 2007-08 vintage, Cabernet Sauvignon grapes were collected from an adjacent block at different distances from Eucalyptus trees, either 5-10 m away for Cab08Near (Table 23) or 240-250 m away for Cab08Far (Table 23), to assess the effect of the proximity of Eucalyptus trees on the evolution of volatile compounds during berry development.

The volatile compounds released from the crushed grapes samples were then analysed using SPME-GCMS (Kalua and Boss 2008). The initial broad observation made was that the berries sampled preveraison consistently produced more volatile compounds than those sampled postveraison (Figure 8). This was somewhat of a surprise given that the ripening phase is often thought of as the time when most flavour and aroma compounds are produced. The difference in the volatile profile at different berry development stages was apparent from GC-MS chromatograms (Figure 8). However, we sought to apply an objective way of recognizing developmental patterns and identifying the volatile compounds associated with these patterns. SLDA bi-plots (Figure 9) recognized and illustrated these berry development patterns in grapes and explained most of the variance (> 99.0 %) with the first two discriminant functions. A bi-plot for all developmental stages (Figure 9A) did not explicitly show the berry developmental stages, apart from showing a similarity in the profiles of the post-veraison samples (11, 13, 14wpf cluster, Figure 9A) and an outlier for the early berry development sample (3wpf). The outlier grape berries were sampled only a few weeks after flowering (Table 23) and, as such, represents berries not long after fruit-set. Excluding this outlier from subsequent analysis revealed a previously hidden berry development pattern (Figure 9B).

Table 23. Sampling details and descriptions (°Brix, berry mass and colour) through berry development

Date [A]	wpf [B]	Berry Mass (g)		°Brix		Sample Description
2006-07 Vintage						
			***Cabernet Sauvignon* 2007 (Cab07)**			
29/11/2006	3	0.13 ± 0.01 a		6.8 ± 0.1 a		Green small (pea-like) berries
13/12/2006	5	0.36 ± 0.01 b		6.2 ± 0.1 a,b		Green small (pea-like) berries
27/12/2006	7	0.42 ± 0.01 b		5.7 ± 0.1 b		Green berries
10/01/2007	9	0.54 ± 0.02 c		10.2 ± 0.7 c		Berries softening and turning colour - veraison
24/01/2007	11	0.77 ± 0.04 d		18.0 ± 0.3 d		Berries (about 90%) pink in colour
7/02/2007	13	0.90 ± 0.04 e		23.1 ± 0.2 e		Uniform pink berries
15/02/2007	14	0.70 ± 0.02 d		25.0 ± 0.2 f		Red berries
21/02/2007	15	0.74 ± 0.02 d		26.8 ± 0.3 g		Red plump berries
2007-08 Vintage						
		Cab08Near [C]	**Cab08Far [D]**	**Cab08Near [C]**	**Cab08Far [D]**	
29/11/2007	2	0.101 ± 0.01 a	0.097 ± 0.008 a	8.2 ± 0.1 c	8.8 ± 0.1 c	Green small (pea-like) berries
13/12/2007	4	0.295 ± 0.01 b	0.288 ± 0.007 b	5.61 ± 0.07 a	6.01 ± 0.04 a	Green small (pea-like) berries
27/12/2007	6	0.37 ± 0.02 c	0.33 ± 0.01 b, c	5.66 ± 0.06 a	5.89 ± 0.03 a	Green berries
10/01/2008	8	0.42 ± 0.02 d	0.37 ± 0.01 c	6.4 ± 0.1 b	6.56 ± 0.06 b	Berries softening and turning colour - veraison
24/01/2008	10	0.81 ± 0.02 f	0.79 ± 0.02 d	14.9 ± 0.2 d	15.9 ± 0.3 d	Berries (about 90%) pink in colour
07/02/2008	12	1.01 ± 0.02 g	1.05 ± 0.03 e	18.1 ± 0.3 e	18.6 ± 0.3 e	Uniform pink berries
21/02/2008	14	0.75 ± 0.02 e	0.75 ± 0.02 d	24.4 ± 0.2 f	24.7 ± 0.2 f	Red shrivelled berries

Different letters in a column represent significantly ($p<0.05$) different means ± standard error (n=30 independent berries)A, Sampling and analysis date;, B,Weeks post-flowering after at least 80 percent cap-fall; C,Berry samples collected close to Eucalyptus trees; D,Berry samples collected far from Eucalyptus trees.

Grape berries at 5, 7, and 9wpf showed a trend (Figure 9B), an indication that certain volatile compounds were progressively changing during this period. After 9wpf, the 11, 13 and 14wpf berries formed a cluster (Figure 9B), but there was no obvious trend, an indication that the volatile profile did not significantly ($p > 0.05$) change across these three weeks. However, leaving the berries longer on the vines to ripen changed their volatile profile, which was evident from the significant discrimination ($p < 0.05$) of the 15wpf grape berries (Figure 9B). The SLDA analysis allows us to distinguish different berry developmental stages: post-fruit set, pre-veraison, veraison, post-veraison, and late ripening (Table 24).

Interestingly, the preveraison samples had the greater levels of terpenes and esters than postveraison samples (Figure 10). This is somewhat unusual as other fruit crops, such as strawberries and bananas generally produce terpenes and esters (which give these fruit their distinctive taste) late in development usually as a means to attract seed dispersal agents.

Figure 8. Chromatograms showing the differences in common and major volatile compounds at different berry developmental stages: preveraison (A), veraison (B), and postveraison (C). (1) Ethyl acetate; (2) Ethanol; (3) Furan, 2-ethyl; (4) Hexanal; (5) Methyl hexanoate; (6) Eucalyptol (1,8-cineol); (7) (E)-2-Hexenal; (8) Hexyl acetate; (9) (Z)-3-Hexenyl acetate; (10) n-Heptan-2-ol; (11) [$^{2}H_{13}$]hexanol (Internal standard); (12) hexan-1-ol; (13) (Z)-3-Hexen-1-ol; (14) (E)-2-Hexen-1-ol; (15) (Z)-3-Hexenyl butanoate; (16) Acetic acid; (17) Benzyl aldehyde; (18) β-Caryophyllene; (19) Ethyl decanoate; (20) 2-Phenyl ethanal; (21) α-Caryophyllene; (22) (-)-α-Cubebene; (23) Benzyl alcohol; (24) 2-Phenyl ethanol

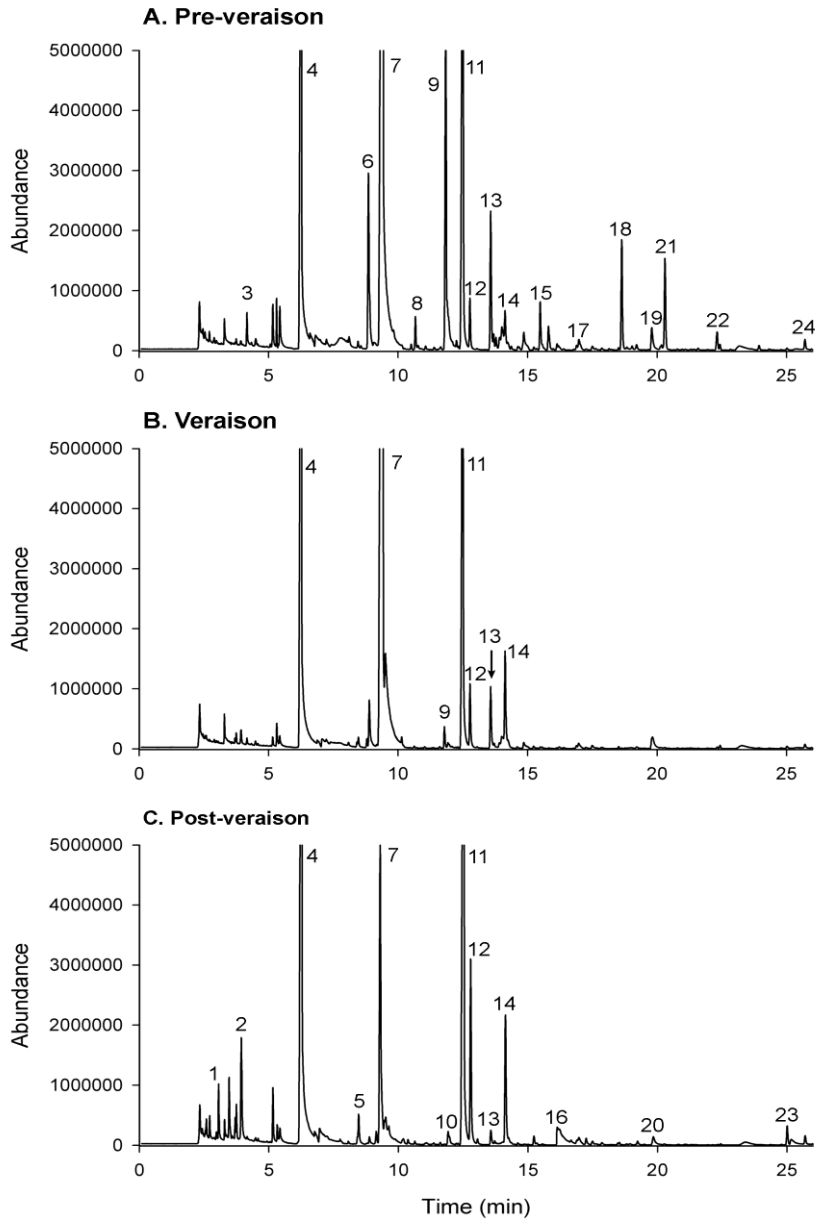
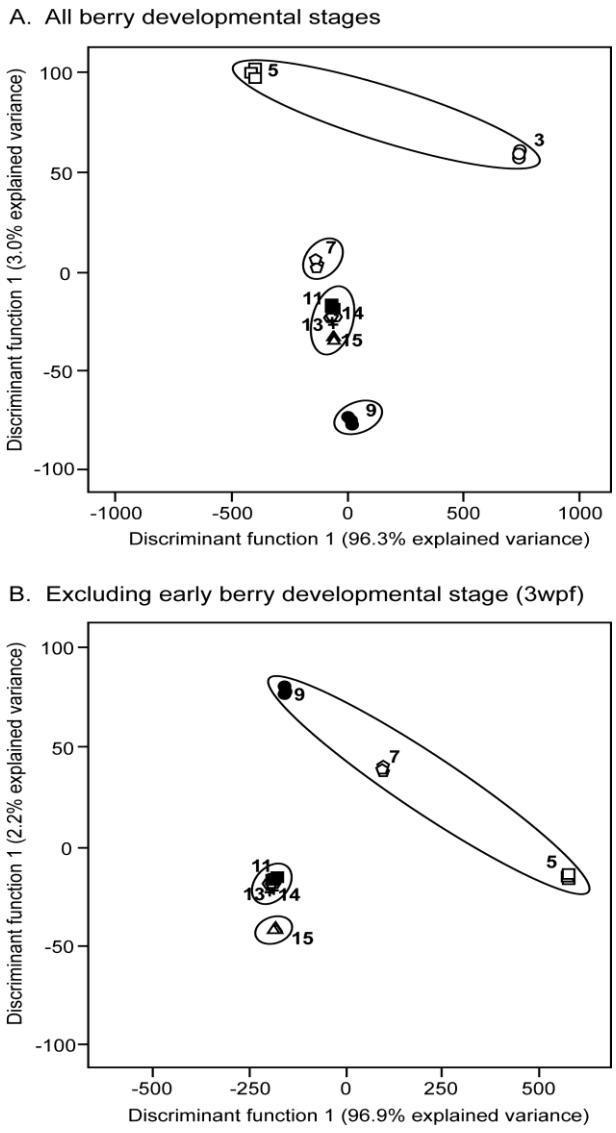


Figure 9. Stepwise linear discriminant analysis (SLDA) bi-plots illustrating a pattern of the berry developmental stages in grapes. Numbers in the bi-plots represent the weeks post-flowering (wpf) for Cabernet Sauvignon grapes (Cab07)



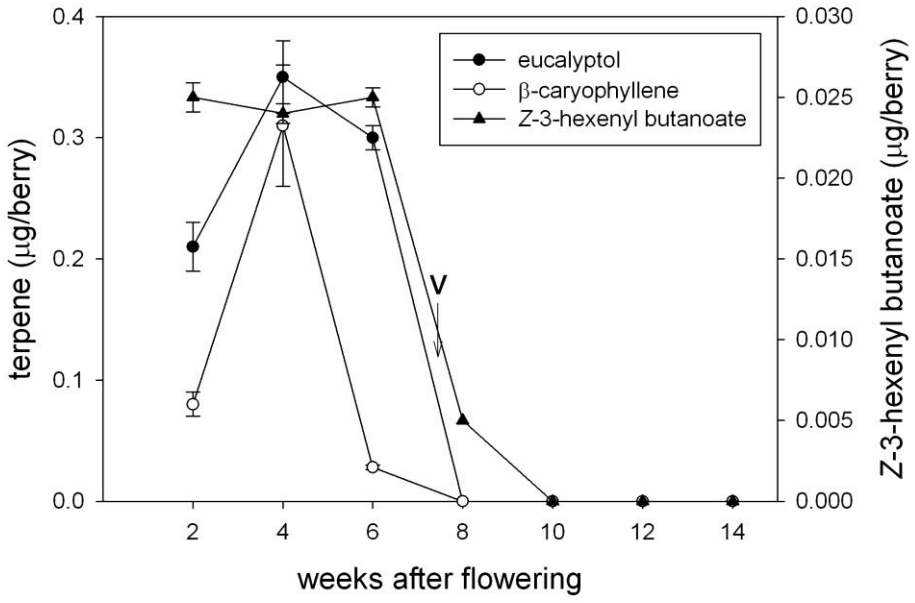
Eucalyptol (1,8-cineol) was only detected during early berry development as were some sesquiterpenes, the family of compounds to which rotundone, the sesquiterpene responsible for Shiraz pepper character, belongs (Wood et al. 2008). The detection of eucalyptol is interesting given that there is speculation that the presence of eucalyptol in wine is due to the proximity of Eucalyptus trees to vineyards (Farina et al. 2005). In the samples we examined, eucalyptol was detected at similar levels in young berries situated immediately next to Eucalyptus trees or at some distance, suggesting that eucalyptol can be produced by grape berries and is characteristic of early berry development. It is known that grapevines have the capability to produce eucalyptol as Gewurtztraminer was shown to possess an enzyme that was shown to produce the compound *in vitro* (Martin and Bohlmann 2004). However, the gene that makes this enzyme was only detected in flowers in that variety (Lucker et al. 2004).

Table 24 Volatile compounds characterizing berry developmental stages in Cabernet Sauvignon grapes.

Developmental Stage	Volatile Compounds Characterizing Berry Developmental Stages			Characteristic Compounds and ***Functional Groups*** [A]
	Cab07	**Cab08Far**	**Cab08Near**	
Post-Fruit Set (≤4 weeks post-flowering)	***Esters*** *(Z)*- 3-hexenyl butanoate ***Aldehydes*** *(E)*-2-Hexenal Heptanal ***Terpenes*** Eucalyptol (1,8-cineol) [B] β-Caryophyllene [B] (-)-α-Copaene β-Cymene α-Muurolene [B]	***Esters*** *(Z)*-3-hexenyl acetate *(Z)*-3-hexenyl butanoate Hexyl acetate ***Aldehydes*** *(E)*-2-Hexenal Heptanal Hexanal Pentanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Terpenes*** Eucalyptol (1,8-cineol) [B] α-Caryophyllene [B] α-Muurolene [B] γ-Muurolene [B] 2,2,6-Trimethyl-cyclohexanone	***Esters*** *(Z)*-3-hexenyl acetate *(Z)*- 3-hexenyl butanoate ***Aldehydes*** *(E)*-2-Hexenal Hexanal Pentanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Benzene Derivatives*** Benzyl alcohol ***Terpenes*** Eucalyptol (1,8-cineol) [B] α-Caryophyllene [B] β-Ionone (-)-α-Cubebene α-Muurolene [B] 2,2,6-Trimethyl-cyclohexanone	***Esters*** *(Z)*- 3-hexenyl butanoate ***Aldehydes*** *(E)*-2-Hexenal Heptanal ***Terpenes*** Eucalyptol (1,8-cineol) [B] β-Caryophyllene[B] α-Caryophyllene (α-humulene) [B] α-Muurolene [B]
Pre-Veraison (5-7 weeks post-flowering)	***Esters*** *(Z)*-3-hexenyl acetate ***Aldehydes*** *(E)*-2-Hexenal 3-Methyl butanal Heptanal Pentanal ***Terpenes*** Eucalyptol (1,8-cineol) [B] β-Caryophyllene [B] β-Cyclocitral [B] (-)-α-Copaene γ-Muurolene [B]	***Esters*** *(Z)*-3-hexenyl acetate ***Aldehydes*** *(E)*-2-Hexenal Heptanal Hexanal Pentanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Terpenes*** Eucalyptol (1,8-cineol) [B] α-Caryophyllene [B] γ-Muurolene [B] 2,2,6-Trimethyl-cyclohexanone	***Esters*** *(Z)*-3-hexenyl acetate ***Aldehydes*** *(E)*-2-Hexenal Hexanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Terpenes*** Eucalyptol (1,8-cineol) [B] (-)-α-Cubebene 2,2,6-Trimethyl-cyclohexanone	***Esters*** *(Z)*-3-hexenyl acetate ***Aldehydes*** *(E)*-2-Hexenal Heptanal Pentanal ***Terpenes*** Eucalyptol (1,8-cineol) [B] β-Cyclocitral [B] β-Caryophyllene [B] α-Caryophyllene (α-humulene) [B] γ-Muurolene [B] 2,2,6-Trimethyl-cyclohexanone
Veraison (8-9 weeks post-flowering)	***Aldehydes*** *(E)*-2-Hexenal Heptanal Pentanal ***Terpenes*** β-Cyclocitral [B]	***Aldehydes*** *(E)*-2-Hexenal Hexanal Heptanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Terpenes*** 2,2,6-Trimethyl-cyclohexanone	***Esters*** *(Z)*-3-hexenyl acetate ***Aldehydes*** *(E)*-2-Hexenal Hexanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Terpenes*** 2,2,6-Trimethyl-cyclohexanone	***Aldehydes*** *(E)*-2-Hexenal Heptanal ***Terpenes*** β-Cyclocitral [B] 2,2,6-Trimethyl-cyclohexanone
Post-Veraison (10-13 weeks post-flowering)	***Aldehydes*** *(E)*-2-Hexenal 3-Methyl butanal Heptanal ***Benzene Derivatives*** 2-Phenyl ethanol 2-Phenyl ethanal	***Esters*** Methyl butanoate ***Aldehydes*** *(E)*-2-Hexenal Hexanal Heptanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Miscellaneous*** 7-Oxabicyclo[4.1.0]heptane **	***Aldehydes*** *(E)*-2-Hexenal Hexanal ***Alcohols*** Ethanol *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Benzene Derivatives*** Benzyl alcohol	***Aldehydes*** *(E)*-2-Hexenal Heptanal ***Benzene Derivatives***
Late ripening (≥14 weeks post-flowering)	***Aldehydes*** Ethanal *(E)*-2-Hexenal Heptanal 3-Methyl butanal ***Alcohols*** n-Heptan-2-ol ***Benzene Derivatives*** 2-Phenyl ethanol 2-Phenyl ethanal	***Esters*** Ethyl acetate ***Aldehydes*** Heptanal Hexanal ***Alcohols*** *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Miscellaneous*** 7-Oxabicyclo[4.1.0]heptane **	***Esters*** Methyl butanoate Ethyl acetate ***Aldehydes*** *(E)*-2-Hexenal Hexanal ***Alcohols*** Ethanol *(Z)*-3-Hexen-1-ol Hexan-1-ol ***Benzene Derivatives*** 2-Phenyl ethanol	***Alcohols*** ***Aldehydes*** *(E)*-2-Hexenal Heptanal ***Benzene Derivatives*** 2-Phenyl ethanol

It is possible that the presence of eucalyptol and sesquiterpenes in young berries may result from the persistence of these compounds in berry tissues that derive from floral tissues. Alternatively, the production of sesquiterpenes and eucalyptol may be induced by viticultural practices that cause wounding to vines or to herbivore attack as has been reported in other plant species (e.g. Delphia et al. 2007). If these changes persist through to harvest, there may be an impact on wine composition. Further studies are required to confirm these speculations and evaluate their influence on berry composition at harvest.

Figure 10. Changes in the levels of free terpenes (eucalyptol and β-caryophyllene) and the ester ¬Z-3-hexenyl butanoate during Cabernet Sauvignon berry development. The arrow and letter "V" denote the timing of veraison at 8 weeks after flowering. Error bars represent ± standard error.



Veraison and the subsequent ripening phase are seen to be the stages when grapes develop their varietal characteristics. However, our findings suggest that this may be a period in Cabernet Sauvignon berry development when volatile compounds are sequestered as non-volatile conjugates or when various volatile compound biosynthetic pathways are actually silenced. As Figure 8 shows, we saw a decrease in the levels of some volatile compounds in the veraison and postveraison berries compared to the preveraison samples. As we have reported previously (Boss *et al.* 2008), the period up to veraison is also the time during which methoxypyrazines are accumulating in Cabernet Sauvignon berries (Figure 11) and there levels decline after this. These findings emphasise the importance of the preveraison berry developmental period in determining fruit composition.

Figure 11. Methoxypyrazine content of Cabernet Sauvignon berries during development. The arrow and letter "V" denote the timing of veraison at 8 weeks after flowering. Error bars represent ± standard error.

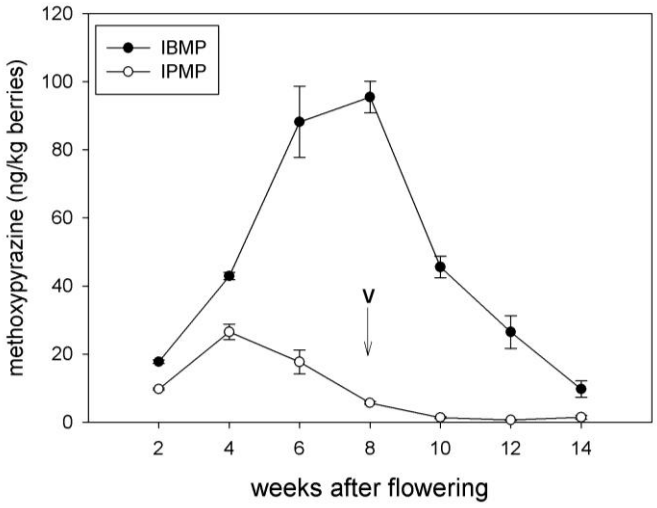


Figure 12. The lipoxygenase pathway.

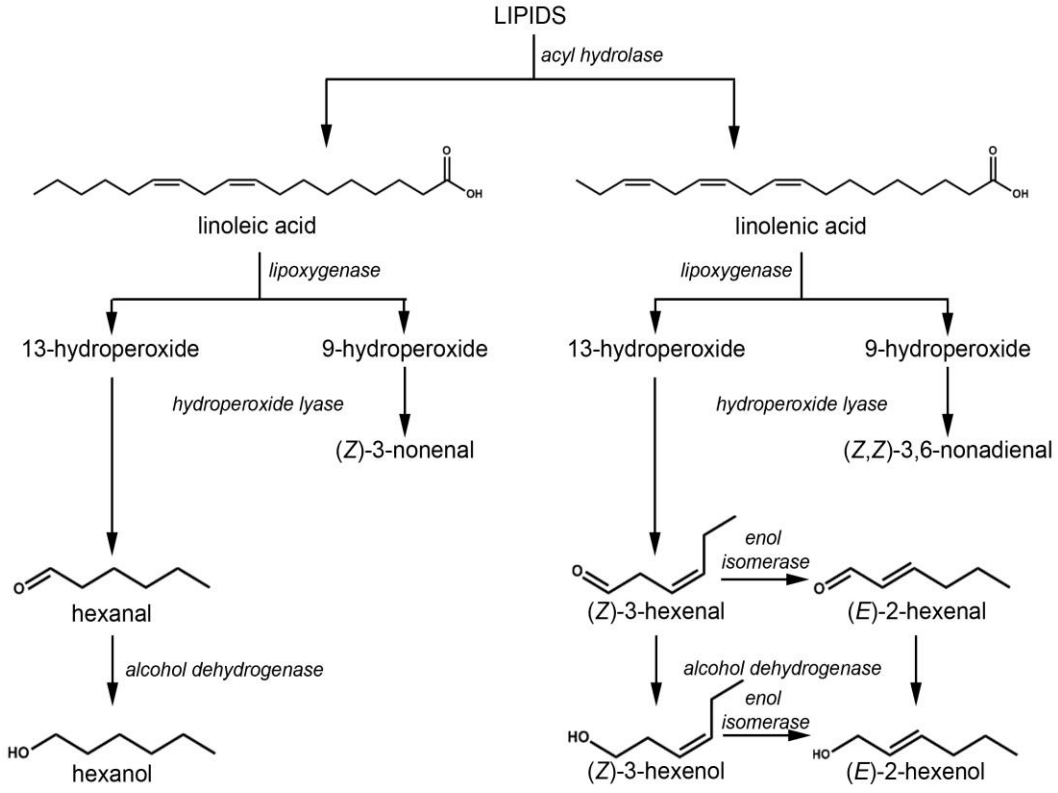
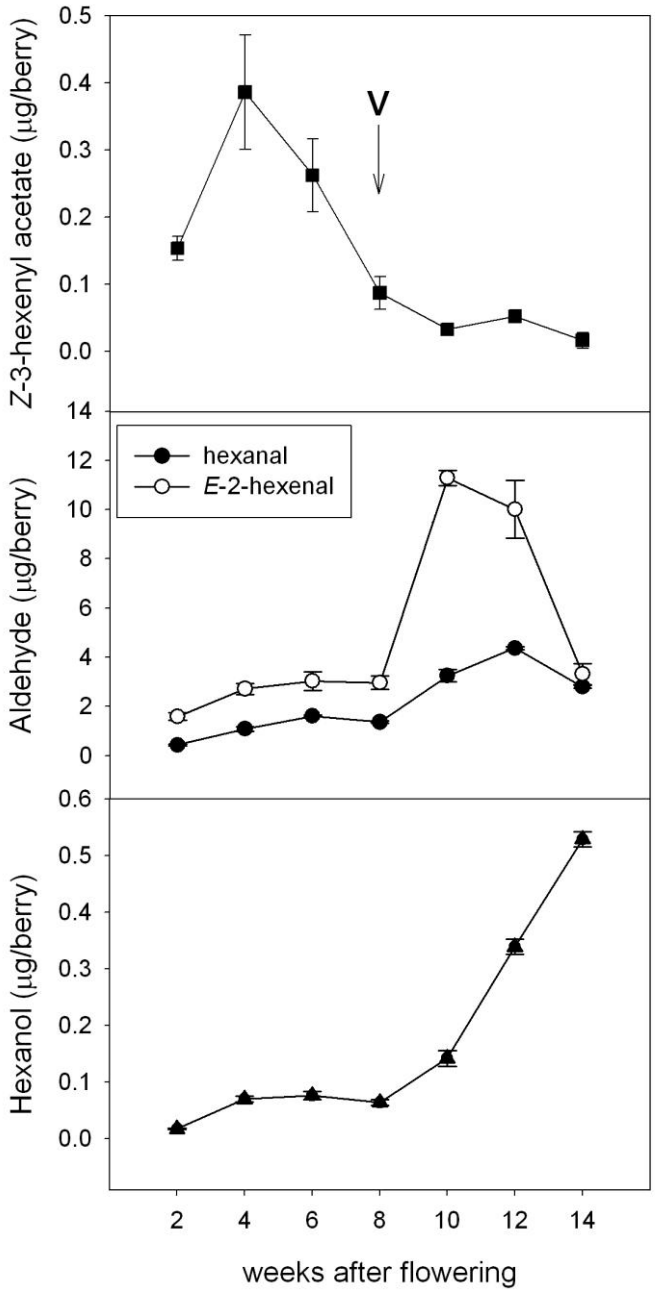


Figure 13. Differences in the generation of C_6-volatile compounds from homogenised grape samples throughout Cabernet Sauvignon berry development. The arrow and letter "V" denote the timing of veraison at 8 weeks after flowering. Error bars represent ± standard error.

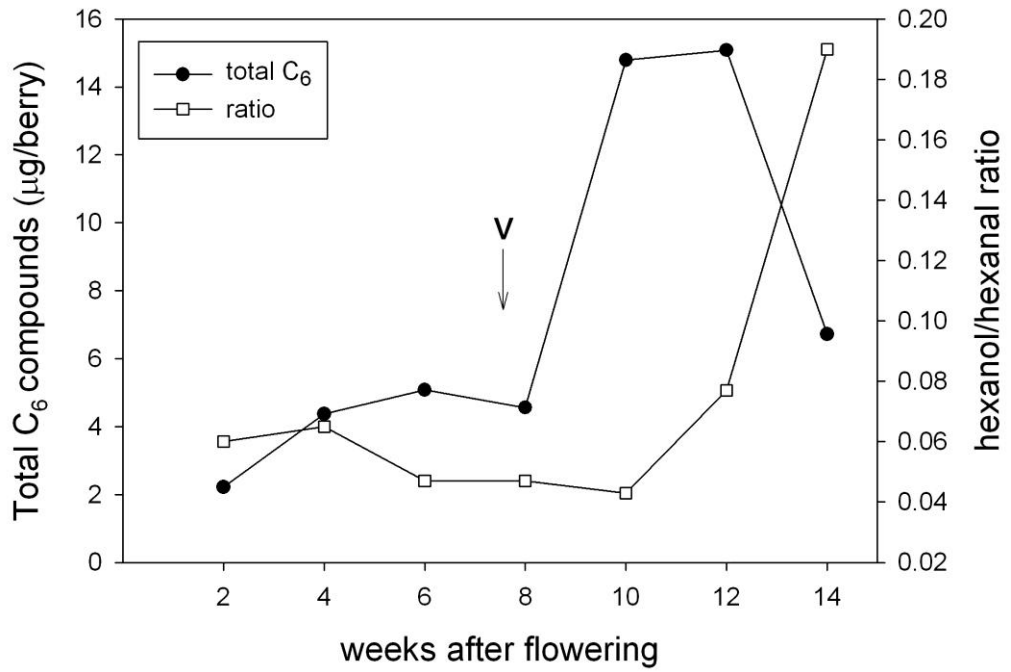


The major volatile compounds detected when berries are crushed are compounds derived from fatty acids. These are produced via the lipoxygenase pathway (Figure 12) and the compounds released are mainly six carbon (C_6) alcohols (hexanol, *Z*-3-hexenol and *E*-2-hexenol) and aldehydes (hexanal and *E*-2-hexenal). These compounds are thought to contribute to green and grassy characters in wine (Allen 2008).

The esters that were found in the young berry samples (*Z*-3-hexenyl acetate and *Z*-3-

hexenyl butanoate) are, in fact, also derived from the alcohols produced from fatty acids by the lipoxygenase pathway. However, berries have less ability to produce these esters after veraison and, as the production of these C_6-derived esters decreases, the levels of the C_6 aldehydes and alcohols increase (Figure 13). As we approach harvest, the levels of the aldehydes themselves then decrease, but the levels of the C_6 alcohol hexanol continue to rise (Figure 13). The total C_6 levels have a trend similar to that of the *E*-2-hexenal during berry ripening (Figure 13) and this measure may be a useful indicator of ripeness if repeated measures over time are made to follow the trend. We suggest that the ratio of hexanol to hexanal may prove to be a more useful ripeness indicator as it highlights a change in metabolism near harvest when the berries begin to produce more volatile alcohols (Figure 14).

Figure 14. Changes in the total amount of C6 volatiles released from crushed grapes during Cabernet Sauvignon berry development and the ratio of hexanol to hexanal in the same samples.

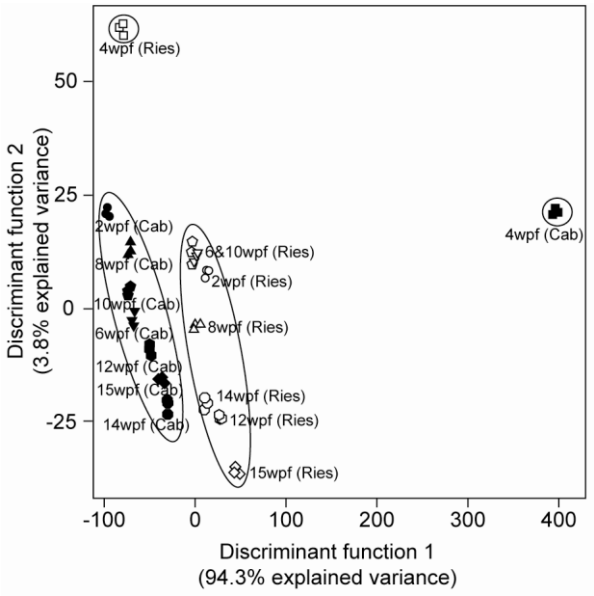


In another developmental study, we compared the progression of volatile production in both Riesling and Cabernet Sauvignon grapes. Cultivars have been grouped based on the level of terpenes into Muscat/floral cultivars (high free monoterpene content), non-Muscat aromatic cultivars (medium free monoterpenes content), and neutral cultivars where monoterpenes do not appear to influence wine aroma (Rapp 1998; Strauss et al. 1986). Under this classification, Cabernet Sauvignon falls in the category of neutral cultivars whereas Riesling is placed in the intermediate group between the Muscat and neutral cultivars (Chisholm et al. 1994; Gomez et al. 1995; Rapp 1998). The flavour differences between wine produced from Riesling and Cabernet Sauvignon grapes are easily discernable by wine consumers

In most cases, there were subtle but significant qualitative and quantitative differences

in the volatile compounds produced by the crushed grapes of both varieties as detected by headspace SPME-GC-MS. These differences between Cabernet Sauvignon and Riesling were revealed through the application of multivariate statistical techniques (Figures 15). The multivariate statistical approach with SLDA bi-plot (Figure 15) clearly showed cultivar differences along the x-axis (Discriminant Function 1) with a higher explained variance (94.3%) than Discriminant Function 2 (3.8%), which discriminates grape maturity. The maturity of both Riesling and Cabernet Sauvignon similarly progresses from positive to negative scores along Discriminant Function 2 (Figure 15).

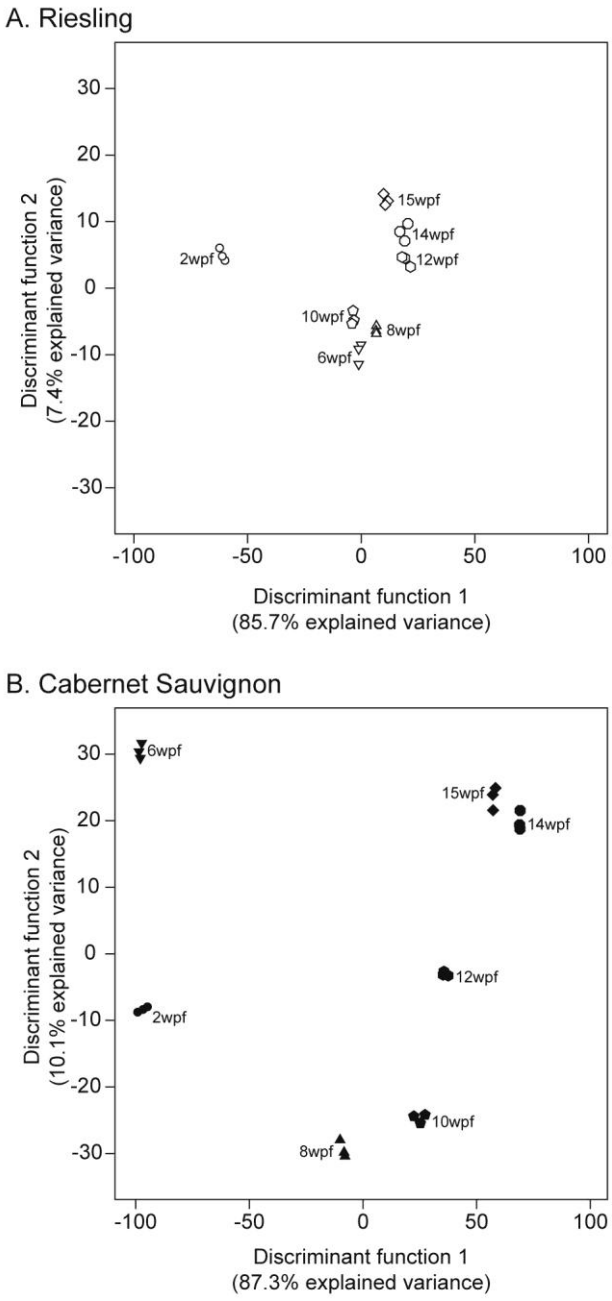
Figure 15. Stepwise linear discriminant analysis (SLDA) bi-plots illustrating overall differences between Riesling and Cabernet Sauvignon grapes along berry development. Numbers in the bi-plots represent the weeks post-flowering (wpf) for Riesling (Ries) and Cabernet Sauvignon (Cab) grapes.



In the SLDA bi-plot from Figure 15, the developmental pattern for each variety was obscured by the vast dissimilarity of the 4wpf grape samples. Removal of the 4wpf grape samples from the multivariate statistical analysis helped to minimize this skewness to visualize the developmental patterns for Cabernet Sauvignon and Riesling grapes based on the volatile compounds released from the crushed grapes (Figure 16). It is apparent that there was better discrimination of the grapes at different developmental stages for the Cabernet Sauvignon samples (Figure 16B) than for the Riesling grapes (Figure 16A). Riesling grapes tended to cluster into groups with increasing maturity along the y-axis (Discriminant Function 2, Figure 16A).

To further explore the volatile components responsible for the relationships observed in the SLDA analyses of the data, compounds and classes characterizing samples or groups of samples of Cabernet Sauvignon and Riesling grapes were extracted from the overall data sets (Table 25) with the application of multivariate statistical techniques and ANOVA. The preveraison berry development stage, in both Riesling and Cabernet Sauvignon, was characterised by the presence of certain monoterpenes,

Figure 16. Stepwise linear discriminant analysis (SLDA) bi-plots illustrating overall differences between Riesling and Cabernet Sauvignon grapes along berry development. Numbers in the bi-plots represent the weeks post-flowering (wpf) for Riesling (Ries) and Cabernet Sauvignon (Cab) grapes.



C_{13}-norisoprenoids, and sesquiterpenes (Table 25). More sesquiterpenes discriminated the Cabernet Sauvignon than Riesling grapes during the preveraison period (Table 25). Previously, a high number of sesquiterpenes have been reported in cultivars other than Cabernet Sauvignon such as "Baga" (Coelho et al., 2006), which could indicate that these compounds may contribute to varietal aroma in some varieties as has been shown to be the case for rotundone in Shiraz (Wood et al.2008). Monoterpenes and sesquiterpenes were still characteristic of Riesling grapes postveraison but were not characteristic of Cabernet Sauvignon grapes (Table 15). In both Riesling and Cabernet

Sauvignon grapes, free C_{13}-norisoprenoids were characteristic of the preveraison period (Table 25).

Table 25. Volatile classes characterising berry development in Riesling and Cabernet Sauvignon grapes.

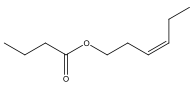
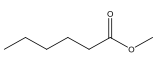
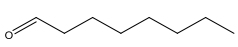
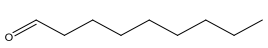
Developmental Stage	Characteristic Volatile Compounds		Differences in Volatile Compounds [A]	
	Riesling	**Cabernet Sauvignon**	**Riesling**	**Cabernet Sauvignon**
Pre-Veraison (2 – 6 weeks post-flowering)	***Aldehydes***	***Aldehydes***	***Esters***	***Aldehydes***
	Hexanal	Hexanal	Hexyl Acetate	Octanal
	E-2-Hexenal	E-2-Hexenal	Z-3-Hexenyl acetate	2,4-Hexadienal
	Alcohols	Octanal		***Alcohol***
	Hexanol	2,4-Hexadienal		Z-3-Hexen-1-ol
	Esters	***Alcohols***		***Ketone***
	Z-3-Hexenyl butanoate	Hexanol		2,2,6-Trimethyl, cyclohexanone
	Hexyl Acetate	Z-3-Hexen-1-ol		***Monoterpenes***
	Z-3-Hexenyl acetate	***Ester***		Eucalyptol
	Monoterpene	Z-3-Hexenyl butanoate		***Sesquiterpenes***
	Geraniol	***Ketone***		γ-Muurolene
	C13-Norisoprenoid	2,2,6-Trimethyl, cyclohexanone		α-Gurjunene [B]
	β-Ionone	***Monoterpenes***		α-Cubebene
	Sesquiterpenes	Eucalyptol		α-Copaene
	α-Caryophyllene	Geraniol		
	α-Muurolene	***C13-Norisoprenoid***		
	Calamenene [B]	β-Ionone		
		Sesquiterpenes		
		α-Caryophyllene		
		α-Copaene		
		α-Cubebene		
		α-Gurjunene [B]		
		γ-Muurolene		
		α-Muurolene		
		Calamenene [B]		
Veraison (7 – 9 weeks post-flowering)	***Aldehydes***	***Aldehydes***		
	Hexanal	Hexanal		
	E-2-Hexenal	E-2-Hexenal		
Post-Veraison (10 – 15 weeks post-flowering)	***Aldehydes***	***Aldehydes***	***Aldehyde***	***Aldehydes***
	Hexanal	Hexanal	Nonanal	Octanal
	E-2-Hexenal	E-2-Hexenal	***Monoterpene***	2,4-Hexadienal
	Nonanal	Octanal	Geraniol	***Alcohols***
	Alcohol	2,4-Hexadienal	***Sesquiterpene***	Z-3-Hexen-1-ol
	Hexanol	***Alcohols***	α-Muurolene	2-Heptanol
	Monoterpene	Hexanol		***Ester***
	Geraniol	Z-3-Hexen-1-ol		Methyl hexanoate
	Sesquiterpene	2-Heptanol		***Benzene Derivatives***
	α-Muurolene	***Ester***		Benzyl alcohol
		Methyl hexanoate		2-Phenyl ethanol
		Benzene Derivatives		
		Benzaldehyde		
		Benzyl alcohol		
		2-Phenyl ethanol		

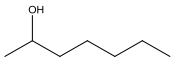
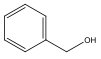
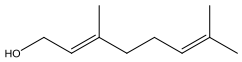
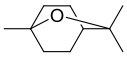
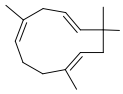
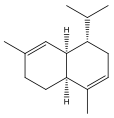
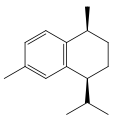
[A] Volatile compounds characteristic of either Riesling or Cabernet Sauvignon only; [B] Tentative identification

A broader range of sesquiterpenes discriminated Cabernet Sauvignon grapes from Riesling grapes during the pre-veraison period (Table 25). Sesquiterpenes are potential wine aroma compounds as has been demonstrated by the identification of rotundone as the compound responsible for "pepper" character in Shiraz wines (Wood et al. 2008). However, many of the sesquiterpenes detected pre-veraison were not detected after veraison. Should these compounds contribute to wine sensory attributes (positively or negatively); understanding their biosynthesis and fate during berry ripening and vinification will enable management of their concentrations in wines. Following a reduction in the terpenes extracted from crushed berries after veraison, there was an increase in some terpenes (e.g. geraniol and α-muurolene, Table 27) late in Riesling berry ripening. Re-emergence of these aroma compounds in Riesling grapes could indicate that these grapes have the potential to biosynthesize these compounds at different stages of maturity (both pre- and post-veraison). Alternatively, it could indicate the potential of the grape to release the stored form of such compounds later during physiological development.

Another distinct difference between Riesling and Cabernet Sauvignon was the release of volatile benzene derivatives (Table 25). Benzene derivatives (benzyl alcohol and 2-phenyl ethanol) discriminated ripe Cabernet Sauvignon berries (Table 25) consistent with an earlier observation that associated a benzene derivative, benzyl alcohol, with certain grape cultivars (Garcia et al. 2003). Benzene derivative concentrations increased towards late maturity with higher concentrations in Cabernet Sauvignon than Riesling (Tables 26 and 27).

Table 26. Cabernet Sauvignon grapes – Trends of common and abundant volatile compounds during berry development

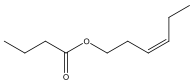
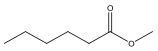
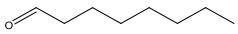
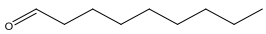
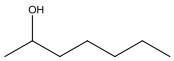
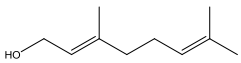
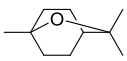
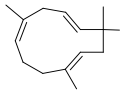
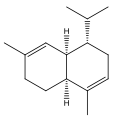
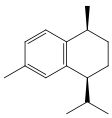
		Concentrations (μg [$^2H_{13}$]hexanol equivalents/mean berry weight in g) at different weeks-post-flowering (wpf)						
		wpf	**2**	**4**	**6**	**8**	**10**	**12**	**14**
		pH	2.64 c (± 0.01)	2.51 a,b (± 0.01)	2.54 b (± 0.01)	2.47 a (± 0.03)	2.98 d (± 0.01)	3.26 e (± 0.03)	3.87 f (± 0.01)
Volatile compounds and classes	**Chemical Structures**	**Brix**	6.2 c (± 0.1)	4.0 a (± 0.1)	4.1 a (± 0.1)	5.5 b (± 0.3)	14.6 d (± 0.2)	18.8 e (± 0.2)	22.7 f (± 0.2)
Esters									
Z-3-hexenyl butanoate			0.10 b (± 0.03)	0.06 a (± 0.01)	<0.02	ND	ND	ND	ND
Methyl hexanoate			ND	ND	<0.01	ND	0.032 a (± 0.008)	0.03 a (± 0.01)	ND
Aldehydes									
Octanal			<0.004	0.009 b (± 0.001)	0.005 a (± 0.002)	ND	.0112 b (± 0.005)	ND	ND
Nonanal			<0.01	ND	ND	0.020 a (± 0.004)	ND	0.04 a,b (± 0.01)	0.046 b (± 0.007)

Volatile compounds and classes	Chemical Structures	Concentrations (µg [$^2H_{13}$]hexanol equivalents/mean berry weight in g) at different weeks-post-flowering (wpf)						
		wpf	**2**	**4**	**6**	**8**	**10**	**12**	**14**
		pH	2.64 c (± 0.01)	2.51 a,b (± 0.01)	2.54 b (± 0.01)	2.47 a (± 0.03)	2.98 d (± 0.01)	3.26 e (± 0.03)	3.87 f (± 0.01)
		Brix	6.2 c (± 0.1)	4.0 a (± 0.1)	4.1 a (± 0.1)	5.5 b (± 0.3)	14.6 d (± 0.2)	18.8 e (± 0.2)	22.7 f (± 0.2)
Alcohol									
2-Heptanol			0.011 a,b (± 0.002)	ND	ND	ND	ND	0.012 a,b (± 0.006)	0.023 b,c (± 0.006)
Benzene Derivatives									
Benzaldehyde			0.006 a (± 0.000)	0.013 a,b (± 0.001)	0.008 a (± 0.000)	0.009 a (± 0.000)	0.022 c (± 0.002)	0.030 d (± 0.007)	0.018 b,c (± 0.001)
Benzyl alcohol			0.013 a (± 0.002)	<0.002	ND	ND	0.024 a (± 0.004)	0.043 b (± 0.008)	0.086 c (± 0.009)
Monoterpenes									
Geraniol			0.003 a (± 0.000)	ND	ND	ND	ND	ND	ND
Eucalyptol (1,8-cineol)			0.04 a (± 0.02)	0.14 b (± 0.01)	0.11 b (± 0.04)	<0.02	ND	ND	ND
Sesquiterpenes									
α-Caryophyllene			0.024 a,b (± 0.005)	0.31 c (± 0.04)	0.06 b (± 0.01)	0.024 a,b (± 0.001)	ND	ND	ND
α-Muurolene			ND	0.004 a (± 0.001)	0.008 b (± 0.001)	ND	ND	ND	ND
Calamenene [A]			0.002 a (± 0.000)	0.040 d (± 0.002)	0.017 c (± 0.003)	0.006 b (± 0.000)	ND	ND	ND

Different letters in a row represent significantly ($p<0.05$) different means ± standard error (n=3 independent field samples). ND represents not detectable at S/N=3. A Tentative identification

These two cultivars showed some similarities in the volatile classes extracted from the headspace of the crushed grapes with aldehydes, alcohols and esters – usually those with a C_6-moiety – common to both Riesling and Cabernet Sauvignon (Table 25). However, qualitative differences were observed between the volatile compounds extracted from the headspace from the crushed grapes of either variety. For instance, Cabernet Sauvignon produced more aldehydes and alcohols than Riesling (Table 25). Additionally, more esters discriminated Riesling than Cabernet Sauvignon (Table 25), which suggests that the Riesling grapes had a greater acyl-transferase activity than Cabernet Sauvignon. While the fruity characteristics of wines from Riesling grapes have been recognised before (Chisholm et al. 1994), the ester production observed in the berries occurred preveraison and so it is doubtful that this will affect wine composition. However, it does indicate that grape berries contain the enzymes required to produce esters and that there is natural variation in the ability of varieties to produce these compounds

Table 27. Riesling grapes – Trends of common and abundant volatile compounds during berry development

Volatile compounds and classes	Chemical Structures	Concentrations (µg [$^2H_{13}$]hexanol equivalents/mean berry weight in g) at different weeks-post-flowering (wpf)						
		wpf	**2**	**4**	**6**	**8**	**10**	**12**	**14**
		pH	2.38 a (± 0.02)	2.39 a (± 0.02)	2.40 a (± 0.01)	2.44 a (± 0.01)	2.74 b (± 0.03)	2.95 c (± 0.03)	3.18 d (± 0.01)
		Brix	4.9 a (± 0.1)	4.2 a (± 0.1)	4.7 a (± 0.1)	6.8 b (± 0.3)	13.7 c (± 0.4)	19.2 d (± 0.3)	20.9 e (± 0.3)
Esters									
Z-3-hexenyl butanoate			0.053 b (± 0.007)	0.067 c (± 0.008)	0.027 a (± 0.000)	ND	ND	ND	ND
Methyl hexanoate			ND	ND	ND	ND	0.016 a (± 0.008)	ND	0.016 a (± 0.005)
Aldehydes									
Octanal			0.003 a (± 0.000)	0.004 a,b (± 0.002)	ND	ND	ND	ND	0.008 b (± 0.004)
Nonanal			<0.01	0.033 a,b (± 0.005)	0.013 a (± 0.006)	0.014 a (± 0.007)	0.02 a (± 0.01)	0.034 a,b (± 0.006)	0.059 a,b (± 0.007)
Alcohol									
2-Heptanol			0.016 a (± 0.008)	0.011 a (± 0.002)	0.021 a,b (± 0.008)	<0.01	0.012 a (± 0.006)	0.044 b (± 0.004)	0.02 a,b (± 0.01)
Benzene Derivatives									
Benzaldehyde			0.009 a (± 0.000)	0.017 a,b,c (± 0.007)	0.010 a,b (± 0.001)	0.03 c (± 0.01)	0.022 a,b,c (± 0.001)	0.030 b,c (± 0.001)	0.020 a,b,c (± 0.002)
Benzyl alcohol			<0.002	0.007 a (± 0.001)	0.007 a (± 0.001)	ND	0.010 a (± 0.005)	0.010 a (± 0.005)	0.029 b (± 0.004)
Monoterpenes									
Geraniol			0.03 a (± 0.01)	<0.005	ND	ND	ND	ND	0.021 a (± 0.004)
Eucalyptol (1,8-cineol)			0.10 b (± 0.04)	0.006 a (± 0.001)	ND	ND	ND	ND	ND
Sesquiterpenes									
α-Caryophyllene			0.078 b (± 0.009)	0.08 b (± 0.02)	0.011 a (± 0.001)	ND	ND	ND	ND
α-Muurolene			ND	0.016 a (± 0.001)	0.013 a (± 0.001)	ND	ND	ND	0.012 a (± 0.006)
Calamenene [A]			0.004 a (± 0.000)	0.017 c (± 0.001)	0.008 b (± 0.001)	ND	ND	ND	ND

Different letters in a row represent significantly ($p<0.05$) different means ± standard error (n=3 independent field samples). ND represents not detectable at S/N=3. A Tentative identification

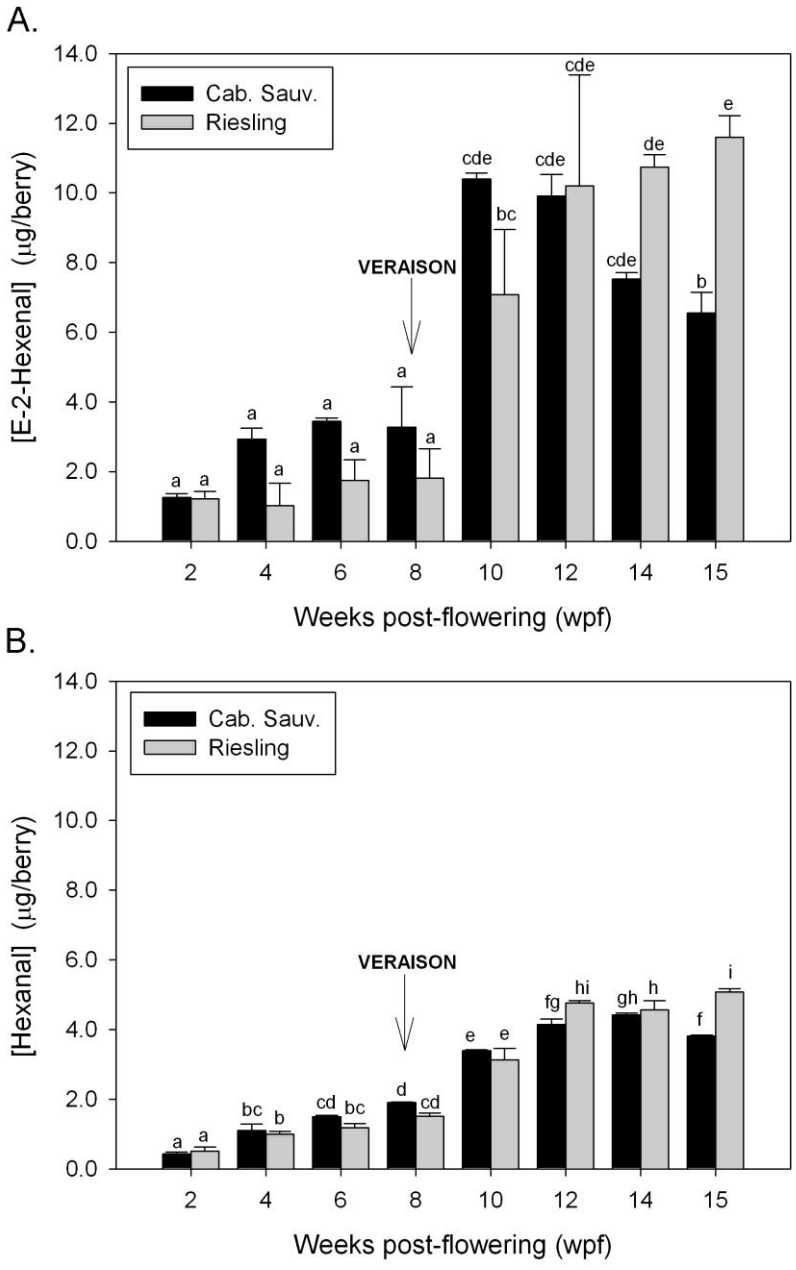
Volatile compounds with a C_6-moiety, usually products of the lipoxygenase pathway (Figure 12), represented the major grape volatile compounds, consistent with earlier findings (Kalua & Boss 2009, Yang et al. 2009). Trends in volatile compound concentrations at the same level of the lipoxygenase pathway (Figure 12) originating from either linolenic acid (C18:3) or linoleic acid (C18:2) were followed to compare the ability of these cultivars to produce these volatile compounds during development. The observed change in the concentration of the C_6 volatile compounds from Cabernet Sauvignon grapes (Figures 17 - 19) obtained from the Riverland grape growing region were similar to the trends from a cooler region reported earlier for the same cultivar (Kalua & Boss 2009). This observation suggests minimal effects from regional and environmental factors on the developmental pattern of volatile compound concentrations from the lipoxygenase pathway.

Aldehydes were the most abundant C_6 volatile compounds produced by the crushed homogenised berries (Figures 17 - 19) and were detected throughout berry development in both Riesling and Cabernet Sauvignon grapes (Figure 17). This suggests that enzymes involved in the production of aldehydes in the lipoxygenase pathway (Figure 12; acyl hydrolase (AH), lipoxygenase (LOX), and hydroperoxide lyase (HPL)) are active throughout berry development. Higher concentrations of *E*-2-hexenal than hexanal have been reported in white grape cultivars (Garcia et al. 2003, Salinas et al. 2004), which was consistent with our observations (Figure 17). This suggests that the C18:3 route (Figure 12) of C_6 volatile compounds production dominates the C18:2 route throughout berry development, either through a predominance of C18:3 substrate or a preference for C18:3 substrate by lipoxygenase (LOX) enzyme.

An examination of the concentrations of *E*-2-hexenal in the headspace of crushed grapes showed trends during physiological development that were different between the varieties (Figure 17A). Concentrations of *E*-2-hexenal declined in Cabernet Sauvignon after veraison whereas in Riesling *E*-2-hexenal levels increased after veraison resulting in significantly ($p<0.05$) higher *E*-2-hexenal concentrations in Riesling berries compared to Cabernet Sauvignon berries at 15wpf (Figure 17A). Despite this significant difference after veraison, no significant differences ($p>0.05$) in concentrations of *E*-2-hexenal were observed between Riesling and Cabernet Sauvignon grapes pre-veraison (Figure 17A). From these data it can be hypothesized that alcohol dehydrogenase (ADH) activity (Figure 12) is higher in Cabernet Sauvignon than Riesling grapes after veraison, thus consuming *Z*-3-hexenal before it isomerises to *E*-2-hexenal or converting *E*-2-hexanal into *E*-2-hexan-1-ol. Alternatively, it can be hypothesised that enal isomerase (Figure 12) is less active in Cabernet Sauvignon than Riesling after veraison.

Hexanal concentrations were lower than *E*-2-hexenal, but significantly increased as the grapes of both cultivars began to ripen (Figure 17B) followed by slight but significant increases through to harvest. The low hexanal concentrations pre-veraison may indicate that HPL activity is low at this stage of development, or that ADH activity was high pre-veraison and so most of the hexanal formed was converted to hexan-1-ol (Figure 12).

Figure 17. Concentration changes during berry development of C_6-aldehydes from the lipoxygenase pathway depicting differences between Riesling and Cabernet Sauvignon grapes. Different letters in a graph represent significantly ($p<0.05$) different concentrations. Standard errors (SE) for three independent field samples (n = 3) were used for the error bars.



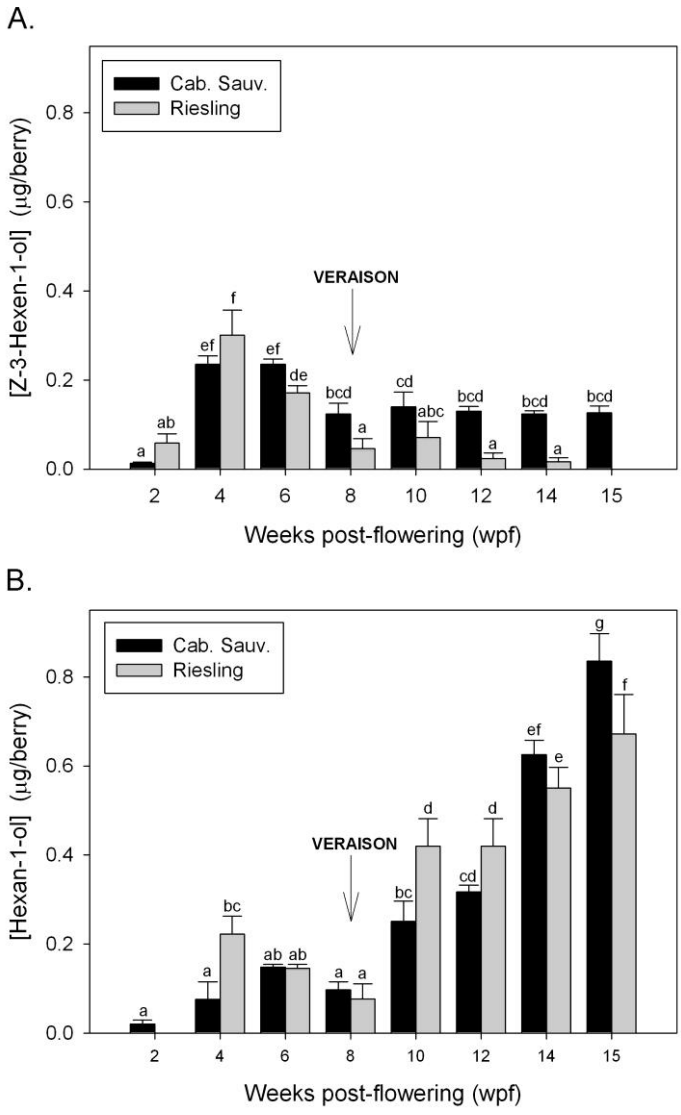
This significant increase in concentrations of the C_6 aldehydes hexanal and *E*-2-hexenal after veraison, in both Riesling and Cabernet Sauvignon grapes (Figure 17), suggests an increase in HPL activity post-veraison (Figure 12). By harvest (15wpf), the concentrations of both hexanal and *E*-2-hexenal were higher in Riesling than Cabernet Sauvignon (Figure 17) indicating a probable higher HPL activity in Riesling than Cabernet Sauvignon. In an earlier study (Garcia et al. 2003), the concentrations of these two aldehydes increased during grape ripening followed by stabilisation of concentrations and then even decreased, which is consistent with our findings.

There was a low abundance of *Z*-3-hexenal which is consistent with earlier studies

(Garcia et al. 2003; Yang et al. 2009), where *Z*-3-hexenal was not detected in grape material. This lack of detection of *Z*-3-hexenal was attributed to its rapid isomerization to *E*-2-hexenal or its reduction to *Z*-3-hexen-1-ol (Figure 12; Garcia et al. 2003). The reduction of the aldehydes, produced from the catalytic cleavage of hydroperoxides by the HPL enzyme (Figure 12), is further explored below.

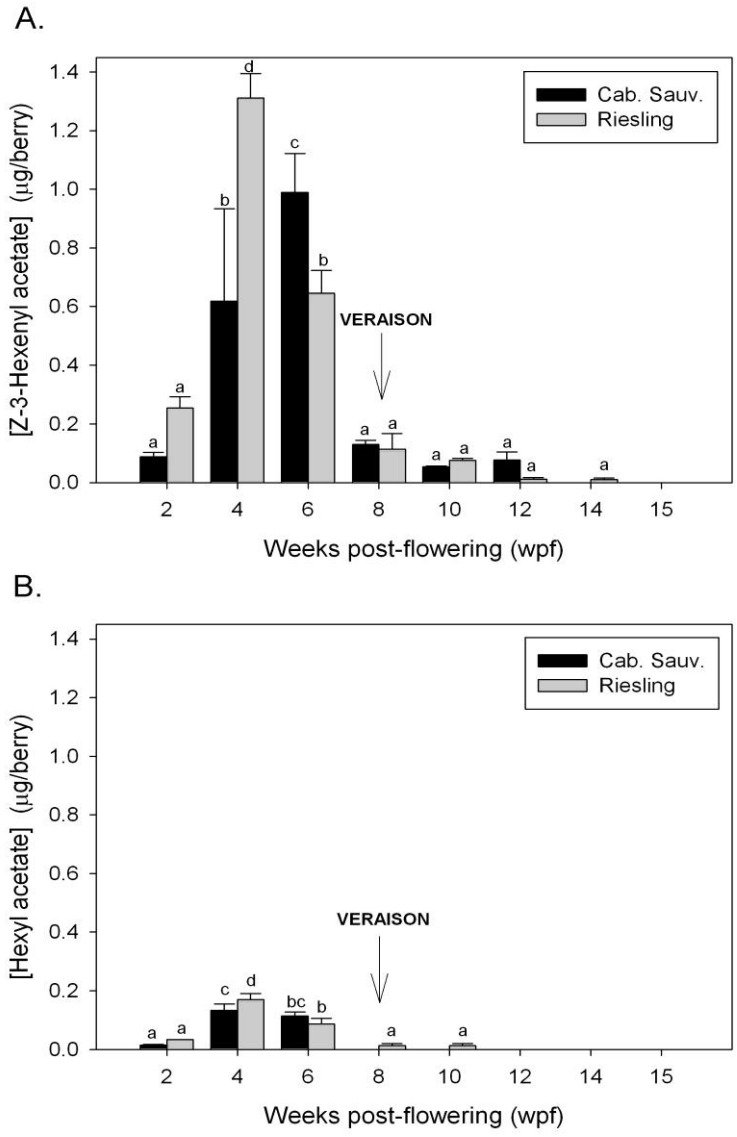
In both cultivars, there appeared to be little activity leading to C_6-alcohol production from *E*-2-hexenal, resulting in less *E*-2-hexen-1-ol being produced by the berries, compared to hexan-1-ol and *Z*-3-hexen-1-ol. Hence these two alcohols were the focus for the analysis of C_6 alcohol production (Figure 18).

Figure 18. Concentration changes during berry development of C_6-alcohols from the lipoxygenase pathway depicting differences between Riesling and Cabernet Sauvignon grapes. Different letters in a graph represent significantly ($p<0.05$) different concentrations. Standard errors (SE) for three independent field samples ($n = 3$) were used for the error bars.



Similar concentrations of hexan-1-ol and *Z*-3-hexen-1-ol were observed prior to veraison (Figure 18). For both alcohols and in both cultivars, the concentrations were lowest 2wpf then showed an increase at 4wpf before a slight reduction immediately before veraison. However, at veraison the concentrations of hexan-1-ol increased significantly in both cultivars, consistent with previous literature (Salinas et al. 2004). In contrast, the concentrations of *Z*-3-hexen-1-ol decreased at veraison in both cultivars, and then showed no significant change during the rest of berry development (Figure 18). The concentrations of *Z*-3-hexen-1-ol were generally significantly higher in Cabernet Sauvignon than Riesling after veraison (Figure 18A). *Z*-3-Hexen-1-ol concentrations in berries have been previously reported to be dependent on cultivar (Ferreira et al. 2000; Yang et al. 2009), which is consistent with our study.

Figure 19. Concentration changes during berry development of esters from the lipoxygenase pathway depicting differences between Riesling and Cabernet Sauvignon grapes. Different letters in a graph represent significantly ($p<0.05$) different concentrations. Standard errors (SE) for three independent field samples (n = 3) were used for the error bars.



The maximum concentration of *Z*-3-hexen-1-ol was observed pre-veraison in both Riesling and Cabernet Sauvignon berries (Figure 18A) and this coincided with maximum concentration for the corresponding ester, *Z*-3-hexenyl acetate (Figure 19A). These observations suggest there is a plentiful supply of unsaturated fatty acids for the lipoxygenase pathway (Figure 12) during early berry development followed by a decline in concentration of linolenic acid (C18:3) with grape maturity (Roufet et al. 1987). The alcohol/ester relationship was also similar pre-veraison for hexan-1-ol (Figure 18B) and hexyl acetate (Figure 19B), which originate from C18:2 (Figure 12). Despite the presence of high concentrations of hexan-1-ol (Figure 18B), a precursor to an ester (hexyl acetate); concentrations of hexyl acetate were negligible in post-veraison berries of both Riesling and Cabernet Sauvignon (Figure 19B). This suggests that, after veraison, there is little AAT activity in the berries.

Both Riesling and Cabernet Sauvignon had a more complex volatile compound composition preveraison than postveraison as illustrated with compounds that significantly discriminated these two cultivars throughout physiological development (Table 25). Observations made in this study suggest that there are varietal differences in the preveraison secondary metabolism of berries. More extensive studies are required to confirm these results under different growth conditions and to determine if the compounds produced during this period are lost or sequestered. Results described here suggest that previous conclusions that secondary metabolism in grapes is more active from veraison onwards (Coombe 1992; Garcia et al. 2003; Fang and Qian 2006) may not necessarily be correct. Furthermore, the proposition that volatile compounds reach their highest concentrations during ripening (Garcia et al. 2003; Yang et al. 2009) is arguable as it was observed in this study that not all volatile compounds show maximum concentrations at ripening. Grape composition at harvest is obviously an important determinant of wine flavour and aroma. However, secondary metabolite production preveraison could contribute to the pool of compounds that become contributors to flavour and aroma after vinification if they persist in the berries through the ripening period. It is also possible that the production of volatile compounds preveraison may act as an indicator of the potential for these grapes to synthesise flavour and aroma compounds postveraison. This opens an opportunity for researchers to explore other stages of berry development for the management of grape composition and its contribution to wine sensory attributes.

Subproject B2: Harvest-timing and the effects on wine chemistry and sensory

Harvest timing is problematic for the wine industry. Due to the desire for full-bodied, rich, fruit flavoured wines without ‘green’ characters, winemakers have increasingly utilised fruit that has been allowed to remain longer on the vine and accumulate more sugar. Thus, there has been a steady increase in the alcoholic content of Australian wines due to the fermentation of grapes with higher sugar levels. This is particularly true for Cabernet Sauvignon. Higher sugar content may be related not only to management practices but also environmental influences arising from climate change (higher temperatures, reduced water input) causing grapes to accumulate sugar more rapidly and earlier than previously. In this subproject, we sought to study the changes

that occur in wine chemistry and sensory attributes during the ripening period in a hot climate vineyard. The aim was to identify volatile compounds that change during this period and to assess the impact of harvest timing on wine sensory. The ultimate goal is to devise strategies to better predict optimum harvest times for flavour outcomes or to intervene in the process to obtain desired flavours at lower sugar levels.

The project has been conducted across three vintages (2008-2010) to ensure that the observations made are consistent and represent a strong relationship between berry ripening and wine composition or sensory attributes. Table 29 below shows the basic grape and wine data for three vintages.

Table 29. Berry and wine parameters for the harvest timing experiment in the 2008-2010 vintages.

Harvest Date	Juice ^{o}Brix	Juice pH	Juice titratable acidity (g/L)	Wine pH	Wine titratable acidity (g/L)	Wine alcohol (%)
			2008			
06/02/2008	20.5	3.34	7.9	3.42	8.04	11.4
13/02/2008	21.9	3.43	7.2	3.47	7.77	12.3
20/02/2008	23.7	3.52	5.9	3.43	7.35	13.8
27/02/2008	25.1	3.61	5.3	3.45	7.42	14.3
05/03/2008	25.6	3.65	5.2	3.40	7.28	14.9
			2009			
06/02/2009	17.3	3.28	7.3	3.23	7.60	10.3
16/02/2009	19.6	3.47	5.5	3.43	6.57	11.6
27/02/2009	21.7	3.61	4.1	3.45	6.85	12.8
10/03/2009	22.7	3.65	3.9	3.42	7.07	13.5
24/03/2009	24.0	3.70	3.5	3.40	7.28	14.6
			2010			
04/02/2010	19.4	3.29	6.5	3.30	7.63	10.8
11/02/2010	21.5	3.51	5.4	3.32	7.37	12.3
18/02/2010	23.1	3.35	4.9	3.42	7.50	13.3
25/02/2010	23.9	3.66	4.1	3.44	7.17	13.8
03/03/2010	24.6	3.83	3.9	3.44	7.49	14.4

Descriptive sensory analyses were conducted on the wines from each vintage approximately 3 months after bottling. In 2008, 32 sensory attributes were scored, whereas 35 were used in 2009 and 2010.

The sensory results for the unadjusted wines were reflective of what we might hypothesise the results to be from such an experiment. The main trends observed consistently across all three vintages were a decrease in “green” characters and an increase in “ripe/dark fruit” characters as harvest dates became later.

SPME-GCMS was used to analyse the volatile composition of these wines in a bid to identify compounds that are associated with certain sensory characteristics and also to follow trends in the changes in volatile profiles during ripening. There were many volatile compounds that showed changes in the wine samples analysed, and so a screening method was required to determine those most relevant to the sensory results. The chosen method involved k-means clustering to determine those compounds that changed over time in the same manner as the relevant sensory scores.

The challenge is now to identify compounds in the grapes that influence the

production of these compounds during winemaking. The data suggests that this should be possible as the concentration of these compounds in the wine was dependent of the harvest date of the berries, and hence the composition of the fruit used to produce these wines. Methods developed in our laboratory and described in Section A4 will help us achieve this goal.

To conclude, these findings should be applicable to the warm climate regions growing Cabernet Sauvignon. The broader application of the findings from this study to other regions or varieties would need to be examined further, but given the robust nature of the results across three years, we believe they should be able to be applied to other situations. In the future it will be important to conduct spiked wine studies to explore the sensorial impact of those compounds found to show similar trends across the three years of the study. The work will also need to focus on grape precursor identification, targeting only those compounds that have sensory impact. This in turn leads to the possibility of developing grape measures for these wine attributes and strategies to alter the accumulation or degradation of these compounds in the vineyard.

(Unpublished data to further support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Subproject B3: The impact of vine vigour on wine chemistry and sensory attributes

The work presented in this section has been published in the manuscript Bramley et al. (2011).

Vineyards are spatially variable and this can often be demonstrated in terms of grape yield, vine vigour or fruit attributes. This variation is associated with variation in soil and topographical attributes of the land underlying the vineyard and, as a result, different wines may derive from different parts of the same vineyard even when it is under uniform management (Bramley and Hamilton, 2007).

In a previous study, Bramley and Hamilton (2007) used a vineyard from the Sunraysia region to show that sensory differences existed between wines produced from areas of lower and higher vine vigour within the same vineyard. In this study, we conducted a detailed descriptive sensory and volatile headspace analysis of wines made from the high- or low-vigour regions of the vineyard across three vintages to assess the robustness of the relationship between vine vigour and wine composition.

The measurement of vine and fruit attributes often used to assess fruit for product designation showed that there was a strong seasonal effect and some inconsistency in between-zone differences over the 3 years of the study (Table 33). Nevertheless, there were significant differences in all these measures except berry weight and pH between the low and high vigour zones of the vineyard (Table 33).

Table 33. Fruit and vine attributes across three vintages for high and low-vigour vines (data supplied by Rob Bramley).

	2005			2006			2007			Zone		
	Low	**High**	**Sig[A]**	**Low**	**High**	**Sig[A]**	**Low**	**High**	**Sig[A]**	**Low**	**High**	**Sig[A]**
Bunch wt (g)	68.4	74.1	ns	79.7	112.3	***				74.0	93.2	***
Mean berry wt (g)	0.89	0.90	ns	1.03	1.07	ns	0.89	0.94	ns	0.94	0.97	ns
Berries/Bunch	76.9	82.5	ns	77.3	104.9	***				77.1	93.7	***
Brix (°)	25.0	24.1	**	24.5	24.6	ns	24.2	22.6	**	24.6	23.8	***
pH	3.47	3.57	**	3.53	3.52	ns	3.31	3.22	***	3.44	3.44	ns
TA (g L^{-1})	6.71	7.98	***	6.39	6.94	*	8.05	8.09	ns	7.05	7.62	**
Colour (mg g^{-1})	1.35	1.23	ns	1.28	0.88	***	1.40	1.26	ns	1.34	1.12	***
Phenolics (au g^{-1})	1.24	1.15	*	1.40	1.07	***	1.56	1.42	**	1.40	1.21	***
Pruning weight (g)	27.7	37.3	*	18.0	22.0	ns	14.5	28.9	**	19.9	29.4	**

[A]*Significance of difference where ***, **, * and ns denote $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant.*

The fact that there were no significant differences ($p > 0.05$) in berry weight between the zones suggests that skin surface areas have not differed between the zones, although skin thickness was not measured. As the major source of colour and phenolics are the skin, the significant between zone differences in the concentrations of these compounds is probably reflective of a difference in the biophysical characteristics of the two zones rather than berry size. However, °Brix was also significantly different ($p < 0.001$) between the zones in 2005 and 2007 (Table 33), although the between zone difference in 2005 was less than 1° and in 2007, the difference was 1.6°. This was in spite of careful monitoring of soluble solids in berries from the two zones in the lead up to vintage, and shows the difficulty wineries have in harvesting to particular targets. Chemical measures of the wines produced from the parcels of grapes are presented in Table 34. While similar pH, TA and VA means were recorded, the wines made from low vigour vines were more alcoholic than those from high vigour vines (Table 34).

Table 34. Attributes of basic wine chemistry provided by the winemaker at bottling. Data are means of triplicate ferments. All wines were adjusted to 80 ppm SO_2. TA denotes titratable acidity; VA denotes volatile acidity.

	2005		2006		2007	
	Low	**High**	**Low**	**High**	**Low**	**High**
pH	3.25	3.24	3.42	3.40	3.34	3.33
TA (g L^{-1} tartaric)	7.7	7.6	7.1	7.4	7.7	7.6
Alcohol (%)	15.1	14.8	14.8	13.8	14.6	13.6
VA (g L^{-1} acetic)	0.23	0.18	0.25	0.19	0.23	0.17

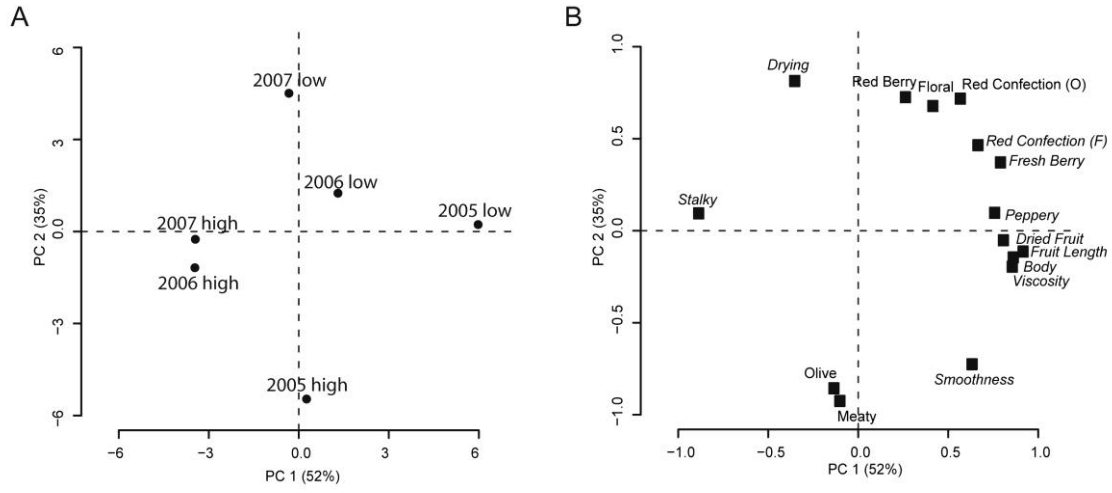
Duo-trio testing showed that the wines derived from the low and high zones could be distinguished from each other sensorially in each year of the study ($p < 0.01$ for 2005 and 2007, $p < 0.05$ for 2006). Descriptive analyses of the wines was conducted using 16 aroma and 15 flavour attributes which assessors used to describe the wines. Significant differences were identified between the wines for 5 aroma and 10 flavour attributes (Table 35). The wines from the low vigour zone wines had higher scores for 'red berry', 'red confection' and 'floral' aromas, and 'red confection', fresh berry' and 'dried fruit' flavours (Figure 27). Furthermore, the spectrum of fruit characters moved from 'red berry' and 'red confection' odour in the most recent vintage to 'dried fruit'

odour and ‘dark berry’ flavour in the older wines (Figure 27). The general trend was that the wines made from grapes from the low vigour vines were more fruity than those produced from the high vigour vines (Table 35 and Figure 27). The wines produced from the high vigour vines wines were characterised by the green attributes ‘stalky’ flavour and ‘olive’ aroma as well as meaty aromas (Table 35 and Figure 27). The ‘drying’ flavour attribute was negatively correlated to ‘smoothness’ (Figure 27) but these descriptors were indicative of the vintage and probably the age of the wines, not the vigour of the vines used to produce the wines.

Table 35. Sensory attributes found to be significantly different amongst the wines through descriptive analysis. Numbers followed by different letters are significantly different at p<0.05. High and low are denoted by H and L.

Attribute	2005H	2005L	2006H	2006L	2007H	2007L	Zone		Vintage	
							F value	P value	F value	P value
Aroma										
Red Berry	3.26c	3.94ab	3.81ab	3.61abc	3.53bc	3.98a	9.21	0.01	0.28	0.986
Red Confection	2.32c	2.85ab	2.55abc	2.72abc	2.41bc	2.94a	11.73	0.001	0.19	0.829
Floral	1.15c	1.70a	1.18bc	1.57ab	1.57ab	1.70a	10.70	0.001	2.09	0.127
Olive	3.59a	2.62b	3.07ab	2.53b	2.49b	2.27b	6.95	0.009	3.68	0.027
Meaty	2.69a	2.13bc	2.25b	2.04bc	2.20b	1.74c	11.93	0.001	4.50	0.013
Flavour										
Fresh Berry	5.21ab	5.64a	4.86b	5.68a	5.02b	5.36ab	10.77	0.001	0.70	0.50
Dried Fruit	3.65b	4.37a	3.47b	3.64b	3.15b	3.38b	5.29	0.023	7.17	0.001
Stalky	4.68a	3.77b	4.72a	4.49a	4.83a	4.93a	3.42	0.066	4.15	0.018
Red Confection	2.29bc	3.29a	1.73c	2.78ab	2.37bc	2.81ab	17.99	<0.001	2.54	0.082
Pepper	2.54ab	2.75a	2.10bc	2.38abc	1.90c	2.58ab	5.91	0.016	2.88	0.059
Fruit length	6.42a	6.93a	5.73b	6.37a	5.88ab	6.12ab	10.42	0.002	9.10	<0.001
Body	7.44ab	7.74a	6.90c	7.27bc	6.80c	7.20bc	7.69	0.006	7.99	<0.001
Viscosity	6.81ab	7.10a	6.32bc	6.71ab	6.17c	6.58ab	6.68	0.011	6.23	0.002
Smoothness	7.90a	7.79ab	6.91c	7.00c	7.11bc	6.51c	2.64	0.11	9.19	<0.001
Drying	7.83c	8.03bc	8.40bc	8.50c	8.14bc	9.33a	8.37	0.004	7.52	0.001

Figure 27. Discrimination of the wine samples, produced from the high and low vineyard zones, by sensory attributes and illustrated by the score (A) and loading (B) plots from principal component analysis. Aroma attributes are in italics and flavour attributes in regular typeface.



The results of the sensory analysis of these wines shows that wines produced from zones within the same uniformly managed vineyard block, in this case identified by their vigour and yield, can be sensorially different.

Table 36 lists 56 volatile compounds which were shown by SPME-GC-MS analysis to occur at significantly different concentrations in the wine headspace. All but four showed significant ($p < 0.05$) between-vintage differences, a result which emphasises the complexity of understanding the between-zone wine differences due to confounding effects of vintage. These vintage effects could be due to environment differences experienced by the fruit across the three years of the study or may reflect changes in wine chemistry during wine aging, as all of the wines were analysed in 2007. However, 21 compounds were measured at significantly different ($p < 0.05$) concentrations in the headspace of the wines from either vineyard zone (Table 36). Ten of these 21 compounds had higher means in wines from one zone compared to the other across the three vintages (italicised in Table 36). PLS analysis was conducted using all significant volatile components to see which compounds could predict the sensory attributes of the wines, and this is shown in Figure 28.

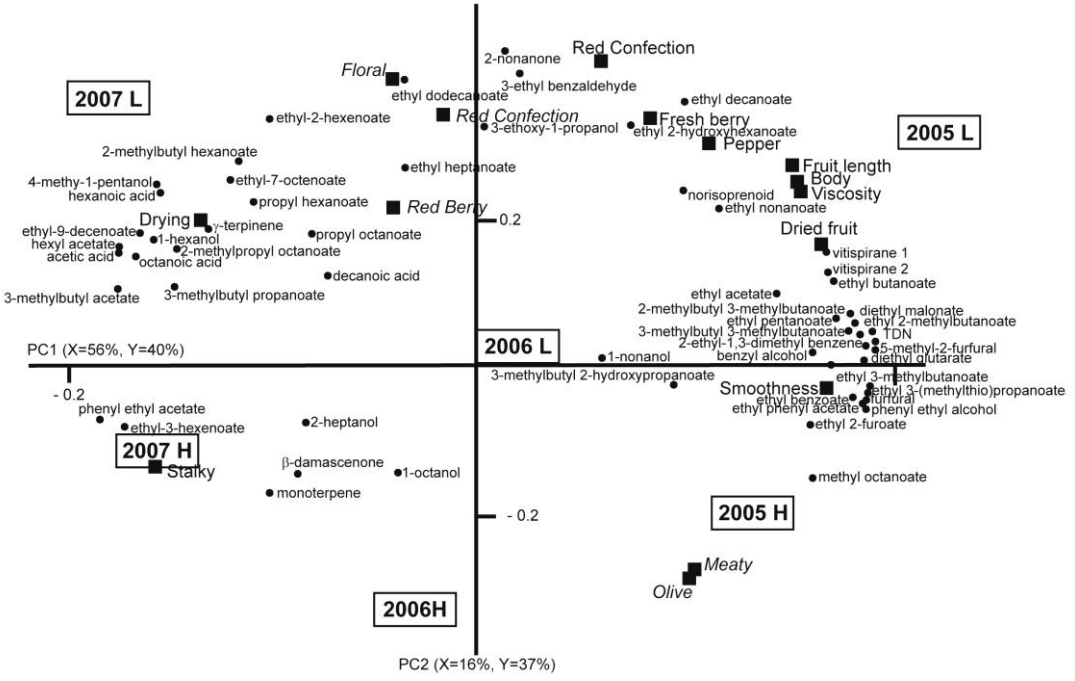
Table 36. Volatile compounds found to be significantly different in the headspace of the wines.

Compound	**Unique ion**[A]	**LRI**[B]	**Compound ID**[C]	**Zone P value**	**Vintage P value**
Ethyl acetate	61	887	A	0.082	0.014
Ethyl butanoate[D]	88	1018	A	0.005	<0.001
Ethyl 2-methylbutanoate	102	1028	A	<0.001	<0.001
Ethyl 3-methylbutanoate	88	1043	A	0.624	<0.001
3-Methylbutyl acetate	87	1089	A	0.014	<0.001
Ethyl pentanoate	88	1099	A	0.068	<0.001
3-Methylbutyl propanoate	70	1149	A	0.668	0.040
Gamma-terpinene	121	1228	A	0.675	<0.001
Hexyl acetate	84	1237	A	0.014	<0.001
2-Methylbutyl 3-methylbutanoate	85	1240	B	0.406	0.002
3-Methylbutyl 3-methylbutanoate	70	1256	B	0.327	<0.001
Ethyl 3-hexenoate	88	1272	A	0.004	<0.001
Propyl hexanoate	117	1280	A	0.929	<0.001
Ethyl heptanoate	88	1297	A	0.592	<0.001
4-Methyl-1-pentanol	56	1301	A	0.325	0.028
2-Heptanol	83	1304	A	<0.001	<0.001
Ethyl 2-hexenoate	97	1313	A	0.004	<0.001
1-Hexanol	69	1338	A	<0.001	<0.001
Methyl octanoate	87	1356	A	0.114	0.004
2-Nonanone	58	1358	A	0.007	0.202
3-Ethoxy-1-propanol	59	1373	A	0.158	<0.001
3-Methylbutyl hexanoate	99	1435	A	0.400	0.003
Furfural	96	1451	A	0.349	<0.001
2-Ethyl-1,3-dimethyl benzene	119	1455	B	0.020	<0.001
Ethyl 7-octenoate	88	1462	B	0.321	0.004
Acetic acid	60	1468	A	0.016	0.355
Unknown monoterpene	121	1486	C	0.005	0.055
Propyl octanoate	127	1499	A	0.326	<0.001
Vitispirane 1	192	1500	B	<0.001	<0.001
Vitispirane 2	192	1503	B	0.003	<0.001
Ethyl nonanoate	88	1517	A	0.314	<0.001
Ethyl 2-hydroxyhexanoate	87	1529	B	0.095	0.001
2-Methylpropyl octanoate	127	1538	B	0.745	0.001
1-Octanol	84	1540	A	0.001	0.028
Ethyl 3-(methylthio)propanoate	148	1548	A	0.519	<0.001
3-Methylbutyl 2-hydroxypropanoate	70	1554	B	0.038	0.003
5-Methyl-2-furfural	110	1559	B	0.386	<0.001

Compound	Unique ion[A]	LRI[B]	Compound ID[C]	Zone P value	Vintage P value
Diethyl malonate	115	1562	A	0.188	<0.001
Ethyl 2-furoate	95	1605	A	0.783	0.020
Ethyl decanoate	88	1625	A	0.002	0.148
1-Nonanol	70	1641	A	0.047	0.003
Ethyl benzoate	105	1648	A	0.644	0.002
Ethyl 9-decenoate	88	1675	B	0.465	0.005
3-Ethyl benzaldehyde	134	1691	B	0.558	0.004
Unknown norisoprenoid	192	1701	C	0.365	0.001
TDN	157	1725	B	0.030	<0.001
Diethyl glutarate	143	1767	A	0.896	<0.001
Ethyl phenyl acetate	91	1770	A	0.445	<0.001
Phenyl ethyl acetate	104	1798	A	0.474	<0.001
β-Damascenone	121	1801	A	0.001	0.004
Ethyl dodecanoate	88	1828	A	0.001	0.001
Hexanoic acid	60	1852	A	0.283	<0.001
Benzyl alcohol	107	1865	A	0.075	<0.001
Phenyl ethyl alcohol	122	1901	A	0.507	0.004
Octanoic acid	101	2065	A	0.905	0.003
Decanoic acid	129	2280	A	0.459	0.013

[A] *Unique ion (m/z): used for peak area determination.*
[B] *LRI calculated from retention relative to the retention of a series of n-alkanes (C_8-C_{26}).*
[C] *A: Identity confirmed by matching mass spectra and LRI with that of authentic standards; B: Tentative assignment based upon comparison with NIST05 and Wiley Registry (7th edition) mass spectral libraries, and published retention times; C: Tentative identification of compound class suggested by comparison of mass spectra with those of related compounds in compound libraries.*
[D] *Compounds in italics had higher means in wines from one zone compared to the other across the three vintages.*

Figure 28. Partial Least Squares analysis of the wines. Small black circles represent the volatile composition loadings (X matrix), the black squares represent the sensory attribute loadings (Y matrix), and the large rectangles represent the sample scores for PC1 and PC2. Aroma attributes are in italics and flavour attributes in regular typeface.



The first two latent vectors accounted for 72% and 77% of the variance for the X (volatile compounds) and Y (sensory attributes) variables respectively (Figure 28). The first latent vector differentiated the wines due to vintage and they were differentiated due to vineyard zone in the second latent vector. Of the ten compounds that showed consistent high/low zone differences, 2-nonanone positively correlated with 'red confection' flavour' (Figure 28). 1-Octanol, 2-heptanol and an unknown monoterpene were negatively correlated with the 'fresh berry' and 'red confection' flavour descriptors, whereas ethyl decanoate and ethyl dodecanoate were positively correlated with 'fresh berry' flavour and 'floral' odour respectively. The abundance of ethyl butanoate and vitispirane 1 were positively associated with 'dried fruit flavour' and ethyl 3-hexenoate was correlated with 'stalky' flavour. The concentration of 2-ethyl-1,3-dimethyl benzene showed a strong vintage effect (Table 36) and so did not co-localise with sensory attributes in the PLS analysis. Overall, the PLS model supports the principle components analysis (Figure 27) which showed that the fruity characters are associated with the wines from the low vigour vineyard zone, but it also shows that there are more compounds associated with the latent vector differentiating these wines from those made from the high zone. Therefore, in general, the low zone wines have higher concentrations of a range of volatile components in their headspace, which may subsequently lead to higher fruit-driven sensory attributes.

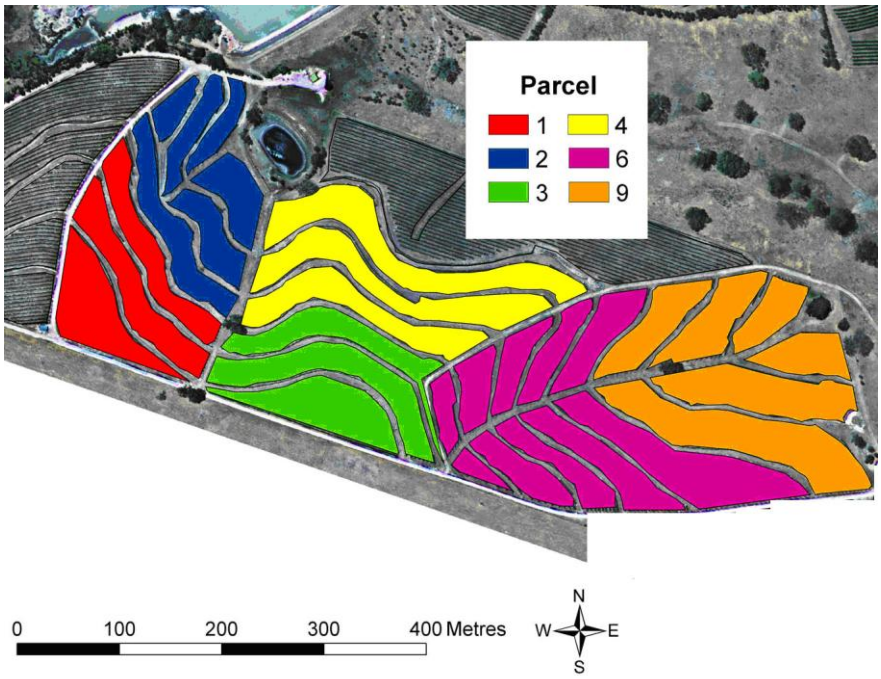
This work has shown that zonal differences identified in vineyard blocks can translate through into wines with different sensory characteristics in a robust manner across vintages. In this case, the more vigorous zone, from which the wines with less fruity characterises were derived, was also characterised by larger bunches, higher titratable acidity and lower concentrations of colour and phenolics. This example supports industry beliefs regarding yield-quality interactions, if higher fruit characters can be regarded as an indicator of quality. Nevertheless, these experiments do highlight that a company can produce two distinct wine products from one block. Depending on which one better suits their portfolio (assuming the company doesn't want both products), management interventions aimed at either increasing vigour and yield in the low zone, or reducing them in the high zone such that the zones were no longer identifiable may assist a company in producing grapes fit for purpose.

The strategy suggested above obviously assumes the vigour/yield difference drove the sensory differences and that management practices could be used to "normalise" these vine properties across the block. However, neither the PCA (Figure 27) nor correlation analysis provides robust evidence of cause and effect. Nevertheless, this work has shown that the differences in vine, fruit and soil attributes of the zones identified in this vineyard can produce robust sensory differences in the wines made from fruit obtained from each zone. Further investigation of cause and effect links between the biophysical environments in the vineyard and the attributes of wines derived from them is necessary as without this understanding the prospects for controlling wine sensory attributes through viticultural interventions will be limited. This work has also shown that the tools of precision viticulture, coupled to the analytical chemistry and sensory methods used here, can be used to identify useful vineyards upon which to base future studies. This will allow the biochemistry behind the changes in berry composition to be understood and lead to targeted measures to alter the biochemical pathways to achieve desired outcomes.

Subproject B4: Spatial variation in the composition of parcels of Riesling grapes and wines from a single vineyard

Subproject B3 demonstrated that spatial variation in vineyards can generate grape parcels which produce wines of differing chemical and sensory properties. To further explore this phenomenon in a white wine variety, we conducted analyses of Riesling juice and wines from different parcels of a single vineyard from Eden Valley. The sections of the vineyard used to create each parcel had been selected based on historical knowledge by the winemakers and viticulturalists from the company that owned the vineyard (Figure 29). The grapes from sections 1, 2, 3, and 4 were generally used for a cheaper price-point wine than the grapes harvested from sections 6 and 9 (Figure 29).

Figure 29. Representation of the location of the vines selectively harvested as discrete parcels in a Riesling vineyard (Image supplied by Rob Bramley).



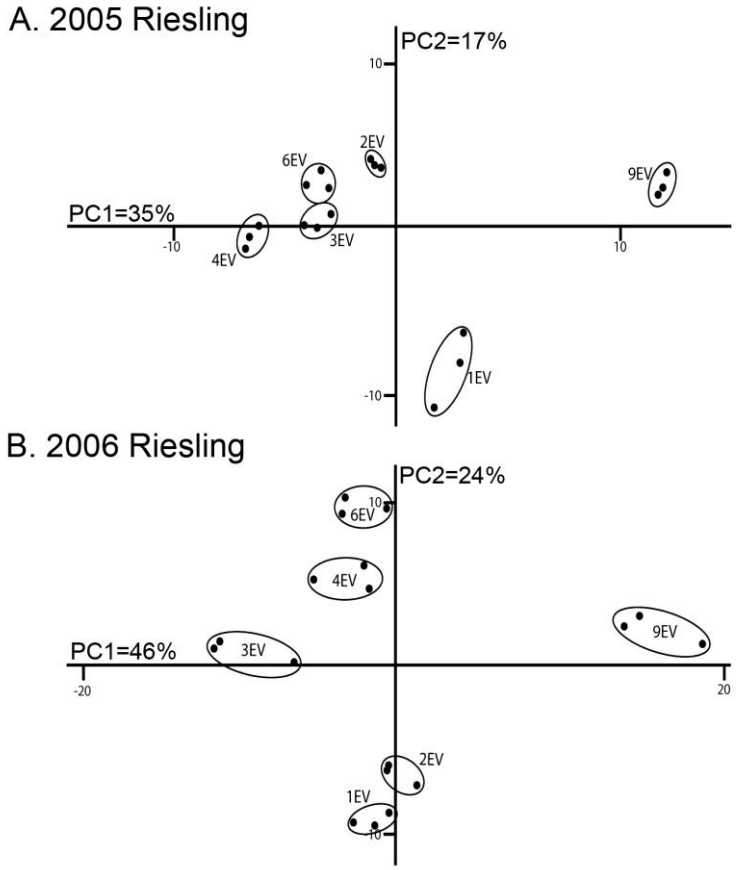
This project sought to explore the sensory and chemical differences between wines made from six sections of the vineyard (namely sections 1, 2, 3, 4, 6 and 9 in Figure 15) in the 2005 and 2006 vintages. These data could then be compared to measures made on the volatile composition of the juice samples obtained from each section. This enables multiple comparisons to be made across the datasets to test for correlative relationships between vineyard parameters, grape volatile composition, wine volatile composition and wine sensory attributes.

Descriptive sensory analyses were conducted on the wines using a trained panel. Panel training conducted as part of the descriptive analysis of the wine identified 12 aroma and 13 flavour attributes which assessors used to describe the wines. Of these,

significant differences were identified between the wines for one aroma and four flavour attributes in both years, although these attributes were not the same in both vintages. This suggests that these parcels of grapes could certainly produce wines of different sensory characteristics, although the differences were subtle

A total of 135 volatile compounds were quantified in the headspace of these wines using HS-SPME-GCMS. The profiles were then used to conduct a principal components analysis (PCA) to see if the parcels of grapes can be discriminated by their volatile composition (Figure 31). Similar results were seen for the 2005 and 2006 vintages. In both years the wine made from section 9 was discriminated from the other wines by the first principal component (i.e. separated on the horizontal axis). In 2005, wines from sections 3, 4 and 6 were grouped together by the PCA, and although they were discriminated along the second principle component in 2006, they appear to be more similar to each other than the other three wines in this vintage. The wines from section 1 were also discriminated by the second principle component in both years, but in the second year the wine from section 2 co-localised with section 1 (Figure 31).

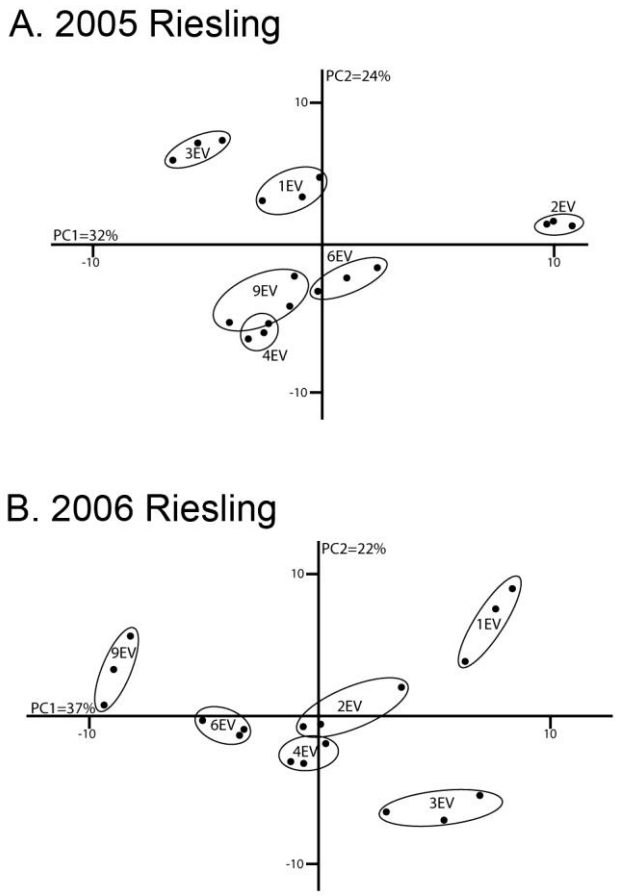
Figure 31. Discrimination of the 2005(A) and 2006(B) Riesling wine samples, produced from six sections of one vineyard, by volatile composition and illustrated by the score plots from principal component analysis.



As well as the 135 volatiles analysed in the wine samples, 130 volatiles have also been identified and quantified in the juice samples across the two vintages. PCA was

conducted on those compounds that were significantly different in the juice samples from both vintages (Figure 32). In 2005, section 9 is not modelled well by the data and is not discriminated from sections 4 and 6. Whereas, section 2, in this same vintage, is quite distinct with regards to its juice volatile composition (Figure 32A), while it is not modelled well by the sensory or wine volatile analysis in this vintage (Figure 31A).

Figure 32. Discrimination of the 2005(A) and 2006(B) Riesling juice samples, produced from six sections of one vineyard, by volatile composition and illustrated by the score plots from principal component analysis.



The juice volatile profiles from 2006 (Figure 32B) show a better agreement with the sensory and wine chemical profiles (Figure 31B) from this vintage than the 2005 data. However, this is mainly due to the discrimination of section 9 from the others, whereas the grouping of the samples from sections 1 and 2 is not so evident. This suggests that there is still work to be done in identifying and modelling grape predictors of wine chemistry and sensory outcomes. The work described in section A of this report has developed the techniques required for the discovery of these grape precursors and predictors in the future.

The results from these analyses raise a number of possibilities with regards to the

management and harvesting strategy employed in this vineyard. In blending the wines from sections 6 and 9 together, are the winemakers looking for complexity or do they believe both parcels will make a similar style of wine? The second possibility would not appear to be the case from the two vintages of this study where they produce wines of distinct sensory and chemical composition (Figure 31). Therefore, if section 9 was harvested alone it may produce a wine capable of being sold at a higher price point than when it is blended with section 6. Furthermore, could the grapes from section 1 also be used to make a wine of a different style, rather than grouping it with sections 2-4? The wine volatile analysis would suggest this (Figure 31), although it was not considerably different from many of the other sections sensorially in both vintages studied.

The data obtained from these Riesling wines could be used to look for associations between juice composition and wine composition in a variety other than Cabernet Sauvignon. The completion of this study in a variety other than Cabernet Sauvignon will enable us to determine if associations between grape and wine metabolites and sensory attributes are robust across varieties. This is important for the development of indicators of wine style or management strategies as some may need to be variety specific and others may be generic. Time and resources did not allow for this to be conducted in this project, but it should be a focus of work in future years.

In conclusion, this study produced some exciting results whereby 6 sections from a single vineyard were shown to produce wines able to be separated into at least 3 groups based on wine composition and which have distinct sensory characteristics. However, this variability in wine characteristics was different from the robust differences seen in the Cabernet Sauvignon study described in subproject B3. This may be reflective of the underlying causes of the vineyard variability. For example, the differences in the high and low vigour regions of the Sunraysia Cabernet Sauvignon vineyard may be due to long standing and robust differences in soil composition. However, the different sections of the Eden Valley Riesling vineyard appear to be aligned more with topographical features. Thus, although these sections produce subtly different wine styles each vintage, this may be due to the interaction of topography with the climate, which will play a role in influencing wine sensory attributes across vintages. Indeed the variation in vine and berry properties measured across the samples in both vintages (Table 37) suggests the environment had a major impact on the vines and grapes in both years. While this confounds our understanding of how grape composition affects wine chemistry, it can also be used to understand the effects of climactic variables which will play an important role in wine industry strategic planning in the future given issues associated with changes in our climate.

(Unpublished data to further support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Subproject B5: The effects of irrigation strategies on wine composition and sensory attributes.

The Australian winegrape industry is operating in an environment where water use is under scrutiny and this has resulted in the need for better information about vine water requirements so that water use efficiency can be improved. There is also a growing

realisation that water management can be used as a tool to manipulate vine vigour, fruit yield and berry composition. This project made use of irrigation studies being conducted in other CSIRO projects to study the effects of irrigations strategies on wine sensory attributes.

Two sites were used for this work. The first was a commercial vineyard in Sunraysia with a one hectare experimental plot within a larger block of own-rooted Cabernet Sauvignon vines. Three drip irrigation treatments have been imposed since 2002: a well-watered control, regulated deficit irrigation (RDI) and a prolonged pre-veraison deficit (PD). The PD treatment represents an irrigation strategy where a standard regulated deficit irrigation regime (RDI) was extended in both time and severity, with a two to three week period of no irrigation immediately following the end of the RDI period. Vine attributes for the 2007 and 2008 vintages are listed in Table 40.

Table 40. Vine attributes for the 2007 and 2008 vintages for the irrigation strategy trial from Sunraysia (data supplied by Everard Edwards). Values are means ± standard error.

	2007			2008		
	Control	**RDI**	**PD**	**Control**	**RDI**	**PD**
Irrigation (ML/ha)	10.6	5.3	4.3	11.3	5.7	4.9
Yield (t/ha)	34.8 ± 1.3	27.2 ± 1.9	23.0 ± 1.5	31.6 ± 1.8	27.5 ± 1.4	24.5 ± 1.6
Bunch weight (g)	124.7 ± 5.7	111.4 ± 8	101.7 ± 4.3	103.4 ± 4.9	93.9 ± 4.5	90.7 ± 4.8
Berry weight (g)	1.07 ± 0.02	0.95 ± 0.03	0.94 ± 0.03	1.11 ± 0.02	0.98 ± 0.02	0.93 ± 0.02

The RDI and PD treatments involve a significant reduction in total irrigation, and there is some effect on yield, mainly by a reduction in berry weight (Table 40). Parcels of grapes were obtained from these treatments in 2007 and 2008 and small scale wines made from them. These have been subject to difference testing to see if these wines could be distinguished on the basis of sensory properties. For both vintages, differences could be detected between at least two of the treatments (Table 41).

Table 41. Duo-trio difference test results for the irrigation strategy Cabernet Sauvignon wines.

Wine 1	**Wine 2**	**Correct IDs**	**% Correct IDs**	**P-value**
2007				
Control	RDI	19/27	70	0.010
Control	PD	16/27	59	0.124
RDI	PD	14/27	52	0.351
2008				
Control	RDI	20/30	67	0.021
Control	PD	21/30	70	0.008
RDI	PD	17/30	57	0.181

Given that there were at least two wines that could be distinguished from each other in each year, a full descriptive sensory analysis was conducted on each series of wines. For the 2007 samples, 14 aroma and 15 flavour/mouthfeel descriptors were quantified

and in 2008, 14 aromas and 14 flavour/mouthfeel descriptors were used.

The results of the sensory analyses suggested that a detailed study of the volatile profiles of these wines was not warranted. In the 2007 vintage the differences seen were minimal and were only present in mouthfeel properties of the wine. As such, any volatile differences were not impacting on the sensory properties of the wine.

This study shows that water management can be used to produced wines with different flavour characteristics, but not consistently. Nevertheless, if the treatments result in greater water use efficiency, it could also be argued that in years where no differences were observed in the sensory properties, the reduced inputs would result in more sustainable grape growing with no reduction in berry quality. Nevertheless, the irrigation strategies certainly have the potential to alter wine sensory attributes, and in the vineyard studied here, to increase fruit characters of wine. If this is a style a wine company seeks, then altering irrigation regimes may enable them to achieve the desired results. However, the lack of consistency between vintages needs to be explored to identify confounding variables that alter the final response of the fruit to the treatments. One possibility is that the effect is cumulative, and so future vintages may show a more robust response, but 2007 was too early into the treatment for any great effect to be seen.

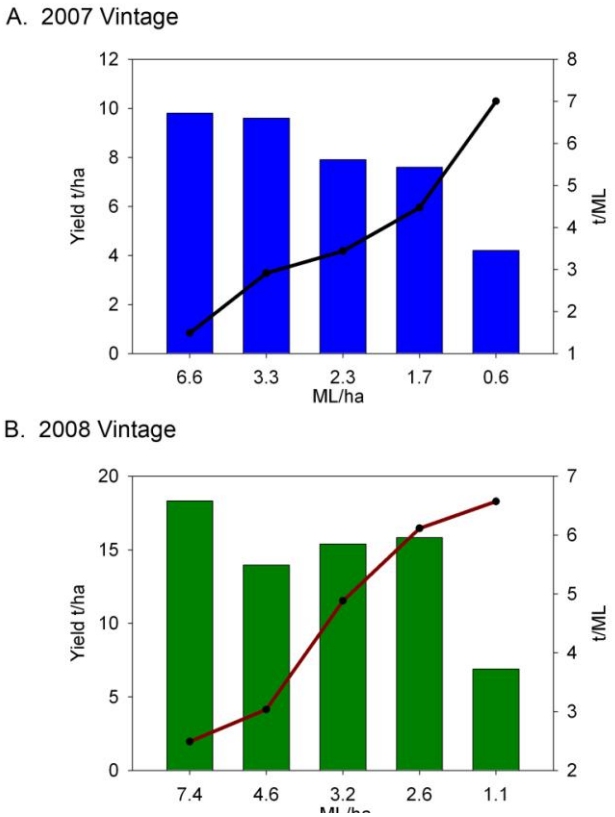
The second irrigation project site is in the Riverland and was a collaborative project with an industry partner and another CSIRO team. For this experiment, five different irrigation levels were imposed on a number of rows in a single Cabernet Sauvignon block over three vintages (2007-2009). For the 2007 and 2008 vintages, the amount of water used on the rows has an effect on the yield per ML of water, but only markedly reduces the yield in the vines watered least (Figure 33).

Wines were made from these treatments for three vintages (2007-2009). Unfortunately, the treatment which was given the least water defoliated during the 2009 vintage and the crop was lost and the lowest irrigation treatment from 2007 was mislabelled and lost in the winery. Nevertheless, the other wines from all three vintages underwent sensory analysis at the same time. This allowed vintage effects to be observed as well as wine sensory effects due to irrigation levels. In total, 18 aroma and 18 flavour/mouthfeel attributes were scored in the wines.

This study provides an insight into the effects of decreasing total irrigation on wine sensory characteristics. In general, the wines produced from vines irrigated less produce fruitier characteristics with less green characters than the wines from grapes grown with more irrigation. The capability of a vineyard to withstand a reduction in irrigation will require a broader study across various climates and soil conditions. Nevertheless, with Australian irrigated vineyards facing water limits and long term drought conditions, an understanding of how irrigation levels may affect wine chemistry and sensory characteristics may help winemakers plan for an increased intake of such fruit and to adapt their practices to still produce wine of a specified style their market expects.

(Unpublished data to further support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Figure 33. Yield per hectare (histogram) and yield per megalitre (line graph) for five sections of a Cabernet Sauvignon block in the 2007 (A) and 2008 (B) vintages.



Subproject B6: Yield effects on wine sensory and chemistry.

Many wine industry practices and tenets are based on observations by growers and winemakers with little or no scientific basis. One such belief is the concept that lower yielding vines produce wines of better quality. However, the underlying cause for a reduction in yield, be it reduced irrigation, altered soil conditions, reduced bud number or rootstock effects, may be changing berry biochemistry itself and the yield effect is a consequence of these actions. This subproject was established to test our ability to produce wines with different sensory attributes by changing vine characteristics in a single vineyard and to investigate yield effects on wine composition. The work was conducted in a commercial vineyard in Willunga, South Australia.

To manipulate yield we drastically altered bunch numbers on Cabernet Sauvignon vines by the removal of inflorescences at approximately stage 15 of vine development (as defined by the modified E-L system of Coombe 1995). The result of the inflorescence removal was the reduction of the bunch number on the treated vines to approximately 50% and 25% of the control bunch number (Table 34) in the 2005 vintage, with less of a reduction in 2006. This flowed through to a significant effect on yield in both vintages (Table 34).

Table 43. Vine yield and bunch parameters from the 2005 and 2006 yield experiments. Values sharing the same letter are not significantly different at P<0.05.

	Treatment			P value
	Low	**Medium**	**High (control)**	
2005				
Bunch number	35 c	63 b	127 a	<0.001
Bunch weight (g)	120 a	114 a	85 b	<0.001
Berry weight (g)	1.19 a	1.09 b	0.87 c	<0.001
Vine yield (kg)	4.13 c	7.12 b	10.80 a	<0.001
2006				
Bunch number	42 c	78 b	105 a	<0.001
Bunch weight (g)	139 a	113 b	93 c	<0.001
Berry weight (g)	1.09 a	0.93 b	0.86 c	<0.001
Vine yield (kg)	5.91 b	8.89 a	9.81 a	<0.001

Small scale (50L) ferments of the grapes from the each of the treatments were carried out and the wine analysed by descriptive sensory analysis. The main sensory differences between wines of different yield were found in both the odour and flavour with only slight differences in mouthfeel and no differences in aftertaste-afterfeel. The chemical composition of the wines were analysed by headspace SPME-GCMS.

This experiment shows that yield reduction by inflorescence removal, and thus in the absence of other viticultural variables, can change the sensory properties of wines made from these grapes. These results support the findings of the experiments conducted in subproject B3 where yield effects were driven by the variable nature of the vineyard, and the lower yield wines tended to have more fruit characters. The chemical analyses conducted on these wines are inconclusive. Nevertheless, the findings from 2005 indicate again that fruit composition can alter the production of esters during fermentation. To be able to advise growers on cropping conditions to produce grapes with distinct wine outcomes we need to conduct more research. Ultimately an understanding of the biochemistry behind these yield effects on fruity wine characters will enable Australian growers to optimise vineyard conditions to produce grapes with greater and targeted flavour potential more consistently and efficiently.

(Unpublished data to further support the conclusions of this section exists and interested persons should contact GWRDC for more information)

Outcome/Conclusion

A major determinant of consumer purchasing decisions, when choosing wine, is the flavour and aroma. There is an increasing body of knowledge regarding the compounds which contribute to aroma and flavour in the finished wine. However, there is a large gap in our knowledge about how compounds in the grape berries contribute to the final flavour and aroma characteristics of the wine. There is no technology for the objective measurement of grape flavour attributes that growers and wineries can easily use to assess their product. There are also no scientifically validated methods of flavour management in the vineyard that provide producers with the ability to better manage the flavour potential of their grapes. The project described in this report aimed to address these issues and has made considerable progress towards achieving these goals. The outputs of this project to date have the potential to greatly benefit the Australian wine industry and these are listed below. These outputs also provide a solid basis for future work to determine the link between berry and wine composition and indicate areas where future research will be beneficial.

First, we have identified a number of grape and wine target compounds that are associated with particular wine sensory attributes. Some of the associations detected in this study are consistant with data from previous studies. Others demonstrate novel correlations between particular sensory attributes and defined secondary metabolites. The grape target compounds may not necessarily be precursors to wine volatile compounds, but may act as markers that indicate altered berry metabolism and therefore composition. This would arise from the up- or down-regulation of certain biochemical pathways within the grape in response to growth conditions. These grape biochemical markers of wine sensory outcomes would be useful in streaming or grading fruit once suitable protocols for their measurement can be developed and verified. Such objective measures of berry flavour potential would be of much benefit to the industry.

Second, we have developed new methods for investigating the relationship between berry metabolism and wine composition. The novel experiments conducted by the fermentation of model musts supplemented with grape juice or pure compounds will encourage other researchers to reassess the input grapes have into wine volatile composition. The major discovery of this work was that wine ester production is greatly influenced by the grape component of the must. Previously these compounds have been considered yeast-derived and few studies have investigated how grape composition may alter their production. Australian wine is known for its fruit-driven style, much of which is due to wine esters. An understanding of how grape composition influences ester production during fermentation will help Australia maintain this market position through the ability to stream fruit (when a measure of ‘ester potential’ is developed) or alter growth practices to enhance ester levels in the resulting wine. The separation chemistry approach to this question we have developed during this project will potentially identify target compounds for wine ester production, be they precursors utilised by the yeast or indirect enhancers of yeast ester production.

Third, our studies into the changes in berry composition during development show that the timing of grape flavour compound accumulation postveraison is different

depending on the class of compound. This has implications for harvest timing. Furthermore, some flavour and aroma components, such as methoxypyrazines, are synthesised preveraison. Grape composition at harvest is obviously an important determinant of wine flavour and aroma. However, secondary metabolite production preveraison will contribute to the pool of compounds that become contributors to flavour and aroma after vinification if they persist in the berries until harvest. Preveraison metabolite levels may also act as indicators of flavour potential in grapes and enable intervention in the field to alter berry composition postveraison. Therefore, the findings of this project should stimulate researchers to explore stages of berry development other than the ripening period for the management of grape composition and its contribution to wine sensory attributes.

Fourth, in collaboration with CSIRO Sustainable Ecosystems we have continued to show the potential benefits that could be gained by vineyard mapping and selective harvesting of parcels of grapes. This work demonstrates that wine sensory outcomes can be managed by precision viticulture techniques. Due to the extensive biophysical profiling of these vineyards and the metabolic and sensory profiling of the wines, we can now test cause and effect links between vineyard variables and berry biochemistry. The industry would then benefit from a clear understanding of how soil properties or topographical aspects will affect berry composition and thus the properties of wine produced from these grapes.

Fifth, we have demonstrated that irrigation strategies and yield manipulation have the potential to alter the sensory properties of wines produced from grapes within a single vineyard block. Further work is required to develop robust knowledge of the relationships between such variables and wine sensory attributes. We believe that this knowledge can only be gained through an understanding of the biochemistry behind the changes in grape metabolism that underpins these different wine outcomes. The information we have gained about the changes in wine chemistry that accompany the wine sensory differences provides the starting point for future studies that will provide guidelines for growing grapes to winery flavour specifications.

Finally, this project has enhanced the Australian wine industry's capability to conduct research into grape and wine flavour and aroma. Our novel approach is grape centric and adds to the existing research into wine flavour and aroma. This has resulted in the development of several new methodologies that will improve our ability to discover and measure grape metabolites responsible for wine style and to develop means to alter the levels of these compounds in the field. We have trained several postdoctoral fellows and research assistants in the field of flavour and aroma research and CSIRO now has an established research team with the knowledge required to undertake such work. Furthermore, the CSIRO Urrbrae laboratory now has a suite of analytical instruments for grape and wine metabolite studies and staff highly skilled in their use and maintenance. Collaborations developed with Australian and international researchers continue to allow the latest knowledge and techniques to be rapidly applied to issues facing the Australian wine industry to improve grape growing practices and improve flavour and aroma outcomes.

Recommendations

1. Continue scientific research

Our current research has identified a number of grape and wine compounds that are potential markers for wine sensory attributes (e.g. trans-geraniol, octanal and (*Z*)-3-hexenol). However, future work should aim to identify those grape compounds that influence the production of fermentation-derived wine volatiles. The research described in this report has shown that must composition, as determined by the amount of grape juice present or due to viticultural treatments, can greatly alter the production of wine esters and other volatiles by yeast. The natural products chemistry approaches developed in this project will be vital in the discovery of important wine volatile precursors by iteratively fractionating grapes and identify components that can alter wine chemistry. The challenge will then be to examine the pathways that lead to the production of these grape metabolites important for wine flavour and aroma, and to develop tools to study the impact of management and environment on their production in the vineyard. This will enable the prediction and development of strategies for manipulating grape composition to achieve specific wine sensory outcomes. It will also result in the development of perfect grape markers for wine volatile compounds which in turn should lead to better means of predicting wine sensory characteristics from grape composition.

2. Explore practical applications

The profound influence that grape composition has on wine style requires effective grading and streaming of fruit, but also provides the potential to manage wine flavour and aroma more effectively in the vineyard. Objective measures of fruit flavour potential and a means of predicting wine sensory attributes from grape composition could provide a step change in improving our ability to efficiently grow grapes to suit desired wine styles. It will help to promote improved decisions about harvest timing as well as batching and streaming of fruit to consistently produce desired wine styles. More importantly, it will provide tools to optimise grape flavour potential in the vineyard and deliver the means of producing grapes with a desired chemical profile that can be used to make wines of a specified flavour profile. While this project has identified target compounds and has shown that management of the vineyard can alter wine sensory attributes, further practical trials are needed to extend these results to enable generic recommendations to be developed and to test the applicability of methods of volatile measurements on grapes under the hectic conditions at vintage.

3. Communication with industry

Australian grape growers, viticulturists and winemakers are the target audiences for the outcomes of this research. The knowledge and techniques developed should be of value to both cool and hot climate producers. The outcomes from this project that will impact on the industry concern the understanding of the role grape composition has on wine chemistry and how this information can be used to inform vineyard management decisions to manipulate wine style, predict harvest timing and improve the streaming

of grape parcels. The research outcomes will continue to be disseminated through scientific journals, industry journals, conferences (for example the Australian Wine Industry Technical Conference), industry organised meetings and if suitable through the public media via factsheets, news releases and podcasts. It is also proposed that a GWRDC Innovator Network Module about grape influences on wine flavour and aroma is developed in the near future. As some of the research was conducted in commercial vineyards this has already aided in the progression of further trialling and uptake which will have to be undertaken with industry support and input. It is expected that this will result in downstream application and evaluation in the commercial world with rapid feedback expected from industry collaborators on the results and application of the research.

Appendix 1: Communication

Journal articles, conference proceedings, book chapters

Kalua CM, Boss PK (2011) Formation of volatile compounds during alcoholic fermentation of Cabernet Sauvignon and Shiraz grapes (*Vitis vinifera* L.): Linking grape composition to wine aroma. *Food Chemistry* (submitted)

Bramley RGV, Ouzman J, Boss PK (2011) Variation in vine vigour, grape yield and vineyard soils and topography as an indicator of variation in the chemical composition of grapes, wine and wine sensory attributes. *Australian Journal of Grape and Wine Research* **17:** 217-229.

Forde C, Cox A, Williams E, Boss PK (2011) Correlations between the sensory attributes and volatile profiles of Cabernet Sauvignon wine and the volatile composition of the grapes used for wine production. Journal of Agricultural and Food Chemistry **59:** 2573–2583.

Kalua CM, Boss PK (2010) Comparison of major volatile compounds for Riesling and Cabernet Sauvignon grapes (*Vitis vinifera* L.) from fruit-set to harvest. *The Australian Journal of Grape and Wine Research* **16:** 337-348.

Keyzers RA, Boss PK (2010) Changes in volatile production in fermentations made from musts with increasing grape content. *Journal of Agricultural and Food Chemistry* **58:** 1153-1164.

Dunlevy JD, Kalua CM, Keyzers RA, Boss PK (2009) The production of flavour and aroma compounds in grape berries. In 'Molecular Biology and Biotechnology of Grapevine, 2nd Edition', K.A. Roubelakis-Angelakis ed. Springer, Berlin, Germany, pp 293-340.

Kalua CM, Boss PK (2009) Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry* **57:** 3818-3830.

Kalua CM, Boss PK (2008) Sample preparation optimization in wine and grapes Dilution and sample/headspace volume equilibrium theory for headspace solid-phase microextraction. *Journal of Chromatography A* **1192:** 25-35.

Boss PK, Dunlevy J, Cox A, Tomas A, Nicholson E, Krake L, Davies C (2008) The pathways to a greater understanding of grape flavour development. Proceedings of the 13th Australian Wine Industry Technical Conference. Eds: Blair, R., Williams, P. and Pretorius, S. pp 47-51.

Industry communications and extension activities

Online podcast produced (http://www.csiro.au/multimedia/Wine-Aroma-Research.html), 12 January 2011.

Oral presentation (Paul Boss) at Treasury Wine Estates annual winemakers meeting, Magill, 5 November 2010.

Oral presentation (Paul Boss) at Yalumba Wines, Angaston, 23 September 2010.

Oral presentation (Paul Boss) to McLaren Vale Grape, Wine and Tourism Association, Willunga, 22 September 2010.

Oral presentation (Paul Boss) at CCW Co-operative Ltd, Glossop, 17 August 2010.

Boss PK, Dennis EG (2010) Grapes, the essential raw material determining wine volatile composition. It's not just about varietal characters. The Australian and New Zealand Grapegrower and Winemaker 560: 78-82.

Invited oral presentation (Paul Boss) at the 14th AWITC, Adelaide, July 2010.

Poster presentation at the 14th AWITC 2010: Dennis EG, Kalua C, Keyzers RA, Boss PK What do Grapes Contribute to Wine Aroma? Highlighting Grape-Dependent Aroma Compounds with Simple Fermentation Experiments.

R&D@Work Article: Linking Grape Composition to Wine Volatiles, February, 2010.

Oral presentation (Paul Boss) at the 1st WIC Research Day, November 2009.

Kalua C, Boss PK (2009) Changes in volatile composition during Cabernet Sauvignon berry development – implications for flavour management in the vineyard. The Australian and New Zealand Grapegrower and Winemaker Annual Technical Issue.

Invited oral presentation (Paul Boss) at the 13th AWITC, Adelaide, July 2007 (see above)

Poster presentation at the 13th AWITC 2007: Cox, A., Tomas, A., Nicholson, E., Loveys, B., and Boss, P.K Comparing the levels of secondary metabolites in grapes to wine volatile composition.

Oral presentation (Agnieszka Cox) at the 39th annual Australian Institute of Food Science and Technology Convention, Adelaide 2006: Cox A ,Boss PK, O'Riordan P, Forde C Green or plummy? Comparing grape composition to wine flavour characteristics.

Oral presentation (Paul Boss) to Pernod Ricard (then Orlando) grapegrowers at their annual meeting in Adelaide, August 2006.

Boss PK (2004) Food futures: From vineyard to palate. The Australian and New Zealand Grapegrower and Winemaker 485a: 74-76.

Oral presentation (Paul Boss) to Fosters Wine Group (then SouthCorp) winemakers at their annual meeting in Adelaide November 2004.

Publications arising from collaborations associated with this project

Robinson AL, Adams DO, Boss PK, Heymann, H, Solomon PS, Trengove RD (2011) The relationship between sensory attributes and wine composition for Australian Cabernet Sauvignon wines. *Australian Journal of Grape and Wine Research* (In press).

Robinson AL, Boss PK, Heymann, H, Solomon PS, Trengove RD (2011) Influence of yeast strain, canopy management, and site on the volatile composition and sensory attributes of Cabernet Sauvignon wines from Western Australia. *Journal of Agricultural and Food Chemistry* **59:** 3273–3284.

Robinson AL, Boss PK, Heymann, H, Solomon PS, Trengove RD (2011) Development of a sensitive non-targeted method for characterizing the wine volatile profile using HS-SPME/GC×GC-TOFMS. *Journal of Chromatography A* **1218:** 504-517.

Dunlevy JD, Soole KL, Perkins MV, Keyzers RA, Kalua CM, Boss PK (2010) Two *O*-methyltransferases involved in the biosynthesis of methoxypyrazines, key grape derived aroma compounds important to wine flavour. *Plant Molecular Biology* 74: 77-89.

Davies C, Bottcher C, Harvey K, Forde C, Boss PK (2010) Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. *Australian Journal of Grape and Wine Research* **17:** 1-8.

Robinson AL, Mueller M, Ebeler SE, Heymann H, Boss PK, Solomon PS, Trengove RD (2010) The effect of simulated shipping conditions on the sensory attributes and volatile composition of commercial white and red wines. *American Journal of Enology and Viticulture* **61:** 337-347.

Robinson AL, Ebeler SE, Heymann H, Boss PK, Solomon PS, Trengove RD (2009) Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry* **57:** 10313-10322

D'Onofrio C, Cox, A, Davies C, Boss PK (2009) Induction of secondary metabolism in grape cell cultures by jasmonates. *Functional Plant Biology* **36:** 323-338.

Berna AZ, Clifford D, Boss PK, Trowell S (2009) Selection of optimal sensor/temperature conditions for winegrape analysis using generalized additive modeling of thermally cycled metal oxide sensors. *Proceedings of the 8th IEEE Conference on Sensors, Christchurch, New Zealand.* pp 1117-1120.

Appendix 2: Intellectual property

Intellectual property arising from this project is being communicated by various means to both the industry and scientific communities through various channels. This is outlined in Appendix 1. The knowledge gained regarding the impact of viticultural treatments on wine sensory attributes is useful to the industry in that it better informs them of means to alter wine flavour in the vineyard. This will assist in decision making and catalyse discussion and innovation to further modify vineyard practices to achieve positive changes in grape composition. Dissemination of knowledge has been through various media including; publications in peer-reviewed grape and wine scientific journals, industry journals, oral and poster presentations to growers through national and regional meetings, presentations to regional groups and individual companies, press releases, web pages and podcasts.

Other intellectual property involves the identification of potential grape measures of wine sensory outcomes that was achieved in this project. At present, we believe that this knowledge would need to be confirmed in a broader industry study to assess robustness across regions, varieties and vintages. Proof of strong correlation between grape metabolites and wine sensory would then provide the impetus to develop rapid and cheap measures of these target compounds to assist in fruit streaming and decision making in the vineyard.

Appendix 3: References

Allen M. S. (2008) Green and mean aromas. Proceedings of the 13th Australian Wine Industry Technical Conference. Eds: Blair, R., Williams, P. and Pretorius, S. pp 56-64.

Aznar, M.; Lopez, R.; Cacho, J. F.; Ferreira, V. Identification and quantification of impact odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem.* **2001,** *49*, 2924-2929.

Bartowsky, E. J. *Oenococcus oeni* and malolactic fermentation - moving into the molecular arena. *Aust. J. Grape Wine Res.* **2005,** *11*, 174-187.

Beekwilder, J.; Alvarez-Huerta, M.; Neef, E.; Verstappen, F. W. A.; Bouwmeester, H. J.; Aharoni, A., Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol.* **2004,** *135*, 1865-1878.

Bely, M.; Sablayrolles, J. M.; Barre, P. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. *J. Ferment. Bioeng.* **1990,** *70*, 246-252.

Binder, S.; Knill, T.; Schuster, J. Branched-chain amino acid metabolism in higher plants. *Physiol. Plant.* **2007,** *129*, 68-78.

Bohlscheid, J. C.; Fellman, J. K.; Wang, X. D.; Ansen, D.; Edwards, C. G. The influence of nitrogen and biotin interactions on the performance of *Saccharomyces* in alcoholic fermentations. *J. Appl. Microbiol.* **2007,** *102*, 390-400.

Boss, P. K.; Dunlevy, J.; Cox, A.; Tomas, A.; Nicholson, E.; Krake, L.; Davies, C. The pathways to a greater understanding of grape flavour development. Proceedings of the 13th Australian Wine Industry Technical Conference. Eds: Blair, R., Williams, P. and Pretorius, S. **2008** pp 47-51.

Bramley, R. G. V. and Hamilton, R. P. Hitting the zone – Making viticulture more precise. *In:* Blair, R. J., Williams, P. J. and Hoj, P. B. (*Eds*) Proceedings of the 12th Australian Wine Industry Technical Conference. Winetitles, Adelaide **2005**.

Bramley, R. G. V.; Hamilton, R. P. Terroir and precision viticulture: Are they compatible? *J. Int. Sci. Vigne Vin* **2007,** *41*, 1-8.

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969,** *17*, 1322–1327.

Chisholm, M. G.; Guiher, L. A.; Vonah, T. M.; Beaumont, J. L. Comparison of some French-American hybrid wines with white Riesling using gas-chromatography olfactometry. *Amer. J. Enol. Vitic*. **1994,** *45*, 201-212.

Coelho, E.; Coimbra, M. A.; Nogueira, J. M. F.; Rocha, S. M. Quantification approach for assessment of sparkling wine volatiles from different soils, ripening

stages, and varieties by stir bar sorptive extraction with liquid desorption. *Anal. Chim. Acta* **2009,** *635*, 214-221.

Coelho, E.; Rocha, S. M.; Barros, A. S.; Delgadillo, I.; Coimbra, M. A., Screening of variety- and pre-fermentation-related volatile compounds during ripening of white grapes to define their evolution profile. *Anal. Chim. Acta* **2007,** *597*, 257-264

Coelho, E.; Rocha, S. M.; Delgadillo, I.; Coimbra, M. A., Headspace-SPME applied to varietal volatile components evolution during *Vitis vinifera* L. cv. 'Baga' ripening. *Anal. Chim. Acta* **2006,** *563*, 204-214.

Coombe, B. G. Research on development and ripening of the grape berry. *Amer. J. Enol. Vitic.* **1992,** *43* 101-110.

Coombe, B. Grapevine growth stages - The modified E-L system. *Aust. J. Grape Wine Res.* **1995,** *1*, 100-110.

Cullere, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004,** *52*, 1653-1660.

Daniel, M. A.; Puglisi, C. J.; Capone, D. L.; Elsey, G. M.; Sefton, M. A. Rationalizing the formation of damascenone: Synthesis and hydrolysis of damascenone precursors and their analogues, in both aglycone and glycoconjugate forms. *J. Agric. Food Chem.* **2008,** *56*, 9183-9189.

Delphia, C. M.; Mescher, M. C.; De Moraes, C. M. Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. *Journal of Chemical Ecology* **2007,** *33*, 997-1012.

Dixon, J.; Hewett, E. W. Exposure to hypoxia conditions alters volatile concentrations of apple cultivars. *J. Sci. Food Agric.* **2001,** *81*, 22-29.

Dunlevy, J. D.; Kalua, C. M.; Keyzers, R. A.; Boss, P. K. The production of flavour and aroma compounds in grape berries. In *Grapevine Molecular Physiology and Biotechnology*, 2nd ed.; Roubelakis-Angelakis, K. A., Ed. Springer Science+Business Media: Berlin, **2009**; pp 293-340.

Ebeler, S. E.; Thorngate, J. H. Wine chemistry and flavor: Looking into the crystal glass. *J. Agric. Food Chem.* **2009,** *57*, 8098-8108.

Edwards, C. G.; Beelman, R. B.; Bartley, C. E.; McConnell, A. L. (). Production of Decanoic Acid and Other Volatile Compounds and the Growth of Yeast and Malolactic Bacteria During Vinification. *Amer. J. Enol. Vitic*, **1990,** *41*, 48-56.

Fang, Y.; Qian, M. C. Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *J. Agric. Food Chem.* **2006,** *54*, 8567-8573.

Farina, L.; Boido, E.; Carrau, F.; Versini, G.; Dellacassa, E. Terpene compounds as possible precursors of 1,8-cineole in red grapes and wines. *Journal of Agricultural and Food Chemistry* **2005,** *53*, 1633-1636.

Ferreira, A. C. S.; Monteiro, J.; Oliveira, C.; de Pinho, P. G. Study of major aromatic compounds in Port wines from carotenoid degradation. *Food Chem.* **2008,** *110*, 83-87.

Ferreira, V.; Lopez, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000,** *80*, 1659-1667.

Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rose wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002,** *50*, 4048-4054.

Forde, C.G.; Cox, A.; Williams, E.; Boss, P.K. Correlations between the sensory attributes and volatile profiles of Cabernet Sauvignon wine and the volatile composition of the grapes used for wine production. *J. Agric. Food Chem.* **2011,** *59*: 2573–2583.

Frankel, E. N. Chemistry of autoxidation: mechanism, products and flavor significance. In *Flavor chemistry of fats and oils*, Min, D. B.; Smouse, T. H., Eds. AOCS: Champaign, IL, **1985**; pp 1-37.

Franco, M.; Peinado, R. A.; Medina, M.; Moreno, J. Off-vine grape drying effect on volatile compounds and aromatic series in must from Pedro Ximenez grape variety. *J. Agric. Food Chem.* **2004,** *52*, 3905-3910.

Garcia, E.; Chacon, J. L.; Martinez, J.; Izquierdo, P. M., Changes in volatile compounds during ripening in grapes of Airen, Macabeo and Chardonnay white varieties grown in La Mancha Region (Spain). *Food Sci. Tech. Int.* **2003,** *9*, 33-41.

Gnekow, B.; Ough, C. S. Methanol in wines and musts - source and amounts. *Amer. J. Enol. Vitic.*, **1976,** *27*, 1-6.

Gomez, E.; Martinez, A.; Laencina, J. Changes in volatile compounds during maturation of some grape varieties. *J. Sci. Food Agric.* **1995,** *67*, 229-233.

Gomez-Miguez, M. J.; Cacho, J. F.; Ferreira, V.; Vicario, I. M.; Heredia, F. J. Volatile components of Zalema white wines. *Food Chem.* **2007,** *100*, 1464-1473.

Gurbuz, O.; Rouseff, J. M.; Rouseff, R. L. Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography - Olfactometry and gas chromatography - Mass spectrometry. *J. Agric. Food Chem.* **2006,** *54*, 3990-3996.

Hashizume, K., Samuta, T. Green odorants of grape cluster stem and their ability to cause a wine stemmy flavor. *J. Agric. Food Chem.* **1997,** *45*, 1333-1337.

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

Henschke, P. A.; Jiranek, V. Yeasts - Metabolism of nitrogen compounds. In *Wine Microbiology and Biotechnology,* Fleet, G. H., Ed. Harwood Academic Publishers: Chur, Switzerland, **1993**; pp 77-164.

Herraiz, T.; Martinalvarez, P. J.; Reglero, G.; Herraiz, M.; Cabezudo, M. D. Effects of the presence of skins during alcoholic fermentation on the composition of wine volatiles. *Vitis* **1990,** *29*, 239-249.

Herraiz, T.; Ough, C. S. Formation of ethyl-esters of amino-acids by yeasts during the alcoholic fermentation of grape juice. *Amer. J. Enol. Vitic.*, **1993,** *44*, 41-48.

Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987,** *38*, 41-44.

Jackson, R. *Wine Tasting: A professional handbook*. Elsevier Academic Press: San Diego, **2002**.

Janusz, A.; Capone, D.L.; Puglisi, C.J.; Perkins, M.V.; Elsey, G.M.; Sefton, M.A. (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene: A potent grape-derived odorant in wine. *J. Agric. Food Chem.* **2003,** *51,* 7759-7763.

Kalua, C. M.; Allen, M. S.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D.; Robards, K. Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chem.*, **2007,** *100*, 273-286.

Kalua, C. M.; Boss, P.K. Sample preparation optimization in wine and grapes: Dilution and sample/headspace volume equilibrium theory for headspace solid-phase microextraction. *J. Chromat. A* **2008,** *1192,* 25-35.

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

Keyzers, R. A.; Boss, P. K. Changes in the volatile compound production of fermentations made from musts with increasing grape content. *J. Agric. Food Chem.* **2010**.

Killian, E.; Ough, C. S. Fermentation esters - Formation and retention as affected by fermentation temperature. *Am. J. Enol. Vitic.* **1979,** *30*, 301-305.

Kliewer, W. M.; Dokoozlian, N. K. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* **2005,** *56*, 170-181.

Lamikanra, O.; Grimm, C. C.; Inyang, I. D. Formation and occurrence of flavor components in Noble muscadine wine. *Food Chem.* **1996,** *56*, 373-376.

Lilly, M.; Bauer, F. F.; Lambrechts, M. G.; Swiegers, J. H.; Cozzolino, D.; Pretorius, I. S. The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. *Yeast* **2006,** *23*, 641-659.

Liu, S. Q.; Holland, R.; Crow, V. L. Esters and their biosynthesis in fermented dairy products: a review. *Int. Dairy J.* **2004,** *14*, 923-945.

Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007,** *55*, 6674-6684.

Lucker, J.; Bowen, P.; Bohlmann, J. *Vitis vinifera* terpenoid cyclases: functional identification of two sesquiterpene synthase cDNAs encoding (+)-valencene synthase and (-)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. *Phytochem.* **2004,** *65*, 2649-2659.

Lund, C. M.; Thompson, M. K.; Benkwitz, F.; Wohler, M. W.; Triggs, C. M.; Gardner, R.; Heymann, H.; Ncolau, L. New Zealand Sauvignon blanc distinct flavor characteristics: Sensory, chemical, and consumer aspects. *Am. J. Enol. Vitic.* **2009,** *60*, 1-12.

Luning, P. A.; Ebbenhorstseller, T.; Derijk, T.; Roozen, J. P. Effect of hot-air drying on flavor compounds of bell peppers (*Capsicum annuum*). *J. Sci. Food Agric.* **1995,** *68*, 355-365.

Maga, J.A. Sensory and stability properties of added methoxypyrazines to model and authentic. In: Charalambous, G. (ed.) *Proceedings of 6th international flavour conference*. Amsterdam: Elsevier. **1989**.

Martin, D. M., Bohlmann, J. Identification of *Vitis vinifera* (-)-alpha-terpineol synthase by *in silico* screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. *Phytochem.*, **2004,** *65*, 1223-1229.

Mathieu, S.; Terrier, N.; Procureur, J.; Bigey, F.; Gunata, Z. A Carotenoid Cleavage Dioxygenase from *Vitis vinifera* L.: functional characterization and expression during grape berry development in relation to C_{13}-norisoprenoid accumulation. *J. Exp. Bot.* **2005,** *56*, 2721-2731.

Mauricio, J. C.; Moreno, J.; Zea, L.; Ortega, J. M.; Medina, M. (1997). The effects of grape must fermentation conditions on volatile alcohols and esters formed by Saccharomyces cerevisiae. *J. Sci. Food Agric.* **1997,** *75*, 155-160.

Meilgaard, M., Civille, G., Carr, B. *Sensory Evaluation Techniques, 2nd edn.* CRC Press, Boca Raton, FL. **1991**.

Molina, A. M.; Swiegers, J. H.; Varela, C.; Pretorius, I. S.; Agosin, E. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *App. Microbiol. Biotechnol.* **2007,** *77*, 675-687.

Morata, A.; Calderon, F.; Gonzalez, M. C.; Gomez-Cordoves, M. C.; Suarez, J. A. Formation of the highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde. *Food Chem.* **2007,** *100,* 1144-1152.

Moshonas, M. G.; Shaw, P. E., Quantitation of volatile constituents in mandarin juices and its use for comparison with orange juices by multivariate analysis. *J. Agric. Food Chem.* **1997,** *45,* 3968-3972.

Murray, K. E., Whitfield, F. B. Occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food Agric.* **1975,** 26: 973-986.

Nykanen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Amer. J. Enol. Vitic.* **1986,** *37,* 84-96.

Palomo, E. S.; Diaz-Maroto, M. C.; et al. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. *Food Control* **2007,** *18,* 398-403.

Park, S. K.; Morrison, J. C.; Adams, D. O.; Noble, A. C. Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *J. Agric. Food Chem.* **1991,** *39,* 514-518.

Park, Y. C.; Shaffer, C. E. H.; Bennett, G. N. Microbial formation of esters. *App. Microbiol. Biotechnol.* **2009,** *85,* 13-25

Perez-Serradilla, J. A.; de Castro, M. D. L. Role of lees in wine production: A review. *Food Chem.* **2008,** *111,* 447-456.

Pineau, B.; Barbe, J. C.; Van Leeuwen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007,** *55,* 4103-4108.

Pineau, B.; Barbe, J.-C.; van Leeuwen, C.; Dubourdieu, D. Examples of perceptive interactions involved in specific "Red-" and "Black-berry" aromas in red wines. *J. Agric. Food Chem.* **2009,** *57,* 3702-3708.

Puglisi, C. J.; Daniel, M. A.; Capone, D. L.; Elsey, G. M.; Prager, R. H.; Sefton, M. A. Precursors to damascenone: Synthesis and hydrolysis of isomeric 3,9-dihydroxymegastigma-4,6,7-trienes. *J. Agric. Food Chem.* **2005,** *53,* 4895-4900.

Puglisi, C. J.; Elsey, G. M.; Prager, R. H.; Skouroumounis, G. K.; Sefton, M. A. Identification of a precursor to naturally occurring *β*-damascenone. *Tetrahedron Lett.* **2001,** *42,* 6937-6939.

Rapp, A. Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung-Food* **1998,** *42,* 351-363.

Rapp, A.; Mandery, H., Wine Aroma. *Experientia* **1986,** *42,* 873-884.

Roufet, M.; Bayonove, C. L.; Cordonnier, R. E. Lipid-composition of grapevine berries, *Vitis vinifera* L. - changes during maturation and localization in the berry.

Vitis **1987,** *26*, 85-97.

Saerens, S. M. G.; Delvaux, F.; Verstrepen, K. J.; Van Dijck, P.; Thevelein, J. M.; Delvaux, F. R. Parameters affecting ethyl ester production by Saccharomyces cerevisiae during fermentation. *App. Environ. Microbiol.* **2008,** *74*, 454-461.

Salinas, M.; Zalacain, A.; Pardo, F.; Alonso, G. L. Stir bar sorptive extraction applied to volatile constituents evolution during *Vitis vinifera* ripening. *J. Agric. Food Chem.* **2004,** *52*, 4821-4827.

Schreier, P. Flavor composition of wines - review. *CRC Crit. Rev. Food Sci. Nutrit.* **1979,** *12*, 59-111.

Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavor compounds. *Plant J.* **2008,** *54*, 712-732

Sefton, M. A. Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis vinifera* grape cvs Merlot and Cabernet Sauvignon. *Aust. J. Grape Wine Res.* **1998,** *4*, 30-38

Siebert, T. E.; Smyth, H. E.; Capone, D. L.; Neuwohner, C.; Pardon, K. H.; Skouroumounis, G. K.; Herderich, M.; Sefton, M. A.; Pollnitz, A. P. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal. Bioanal. Chem.* **2005,** *381*, 937-947.

Skinkis, P. A.; Bordelon, B. P. et al. Comparison of monoterpene constituents in Traminette, Gewurztraminer, and Riesling winegrapes. *Amer. J. Enol. Vitic.* **2008,** *59*, 440-445.

Stahl-Biskup, E.; Intert, F.; Holthuijzen, J.; Stengele, M.; Schulz, G. Glycosidically bound volatiles - A review 1986-1991. *Flavour Fragrance J.* **1993,** *8*, 61-80.

Strauss, C. R.: Wilson, B.; Gooley, P. R.; Williams, P. J. Role of Monoterpenes in grape and wine flavor. *ACS Symposium Series*, **1986,** *317*, 222-242.

Suomalainen, H. Yeast and its effect on flavour of alcoholic beverages. *J. I. Brewing*, **1971,** *77*, 164-177.

Suomalainen, H.; Ronkainen, P. Mechanism of diacetyl formation in yeast fermentation. *Nature*, **1968,** *220*, 792-793.

Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005,** *11*, 139-173.

Torija, M. J.; Beltran, G.; Novo, M.; Poblet, M.; Guillamon, J. M.; Mas, A.; Rozes, N. Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. *Int. J. Food Microbiol.* **2003,** *85*, 127-136.

Torrens, J.; Urpi, P.; Riu-Aurnatell, M.; Vichi, S.; Lopez-Tamames, E.; Buxaderas, S.

Different commercial yeast strains affecting the volatile and sensory profile of cava base wine. *Int. J. Food Microbiol.* **2008,** *124*, 48-57.

Trought, M Fruitset – possible implications on wine quality. In: de Garis, K., Dundon, C., Johnstone, R. and Partridge, S. (Eds.). *Transforming flowers to fruit. Proceedings of an ASVO seminar, Mildura, 29 July, 2005*. Australian Society of Viticulture and Oenology, Adelaide. **2005**.

Van Belle, D.; Andre, B. A genomic view of yeast membrane transporters. *Curr. Opin. Cell Biol.* **2001,** *13*, 389-398.

Varela, C.; Pizarro, F.; Agosin, E. Biomass content governs fermentation rate in nitrogen-deficient wine musts. *Appl. Environ. Microbiol.* **2004,** *70*, 3392-3400.

Vianna, E.; Ebeler, S. E. Monitoring ester formation in grape juice fermentations using solid phase microextraction coupled with gas chromatography-mass spectrometry. *J. Agric. Food Chem*. **2001,** *49*, 589-595.

Vilanova, M.; Cortes, S.; Santiago, J. L.; Martinez, C.; Fernandez, E. Aromatic compounds in wines produced during fermentation: Effect of three red cultivars. *Int. J. Food Properties* **2007,** *10*, 867-875.

Verstrepen, K. J.; Derdelinckx, G.; Dufour, J. P.; Winderickx, J.; Thevelein, J. M.; Pretorius, I. S.; Delvaux, F. R. Flavor-active esters: Adding fruitiness to beer. *J. Biosci. Bioeng.* **2003,** *96*, 110-118

Vogel, J. T.; Tan, B. C.; McCarty, D. R.; Klee, H. J. The carotenoid cleavage dioxygenase 1 enzyme has broad substrate specificity, cleaving multiple carotenoids at two different bond positions. *J. Biol. Chem.* **2008,** *283*, 11364-11373.

Wang, X. D.; Bohlscheid, J. C.; Edwards, C. G. Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *J. Appl. Microbiol.* **2003,** *94*, 349-359

Webb, A. D. Some aroma compounds produced by vinous fermentation. *Biotech. Bioeng.* **1967,** *9*, 305-319.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massywestropp, R. A. Use of C18 reversed-phase liquid-chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J. Chromat.* **1982,** *235*, 471-480.

Winter, E.; Rousseau, J.; Whiting, J. R. *Winegrape berry sensory assessment in Australia*. Winetitles: Adelaide, South Australia, **2004**.

Winterhalter, P.; Sefton, M. A.; Williams, P. J. Volatile C-13-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am. J. Enol. Vitic.* **1990,** *41*, 277-283.

Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vossing, T.; Widder, S.; Krammer, G. Sefton, M. A.; Herderich, M. J. From wine to pepper: Rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008,** *56*; 3738-3744.

Yang, C. X.; Wang, Y. J.; et al. Volatiles of grape berries evaluated at the germplasm level by headspace-SPME with GC-MS. *Food Chem.* **2009,** *114*, 1106-1114.

Yunoki, K.; Tanji, M.; Murakami, Y.; Yasui, Y.; Hirose, S.; Ohnishi, M. Fatty acid compositions of commercial red wines. *Biosci. Biotech. Biochem.* **2004,** *68*, 2623-2626.

Yunoki, K.; Yasui, Y.; Hirose, S.; Ohnishi, M. Fatty acids in must prepared from 11 grapes grown in Japan: Comparison with wine and effect on fatty acid ethyl ester formation. *Lipids* **2005,** *40*, 361-367.

Appendix 4: Staff

All staff below spent periods of time on the project and were members of both the CSIRO Food Futures Flagship and Plant Industry:

Dr Paul K. Boss, Project Supervisor & Chief Investigator.
Dr Peter Clingeleffer, Co-investigator
Dr Agnieszka Cox, Postdoctoral Fellow.
Dr Eric Dennis, Postdoctoral Fellow.
Ms Alexandra Downie, Research Officer.
Dr Curtis Kalua, Postdoctoral Fellow.
Dr Robert Keyzers, Postdoctoral Fellow.
Mr Les Krake, Research Officer.
Dr Brian Loveys, Project Supervisor & Co-investigator.
Mrs Sue Maffei, Research Officer.
Ms Emily Nicholson, Research Officer.
Dr Simon Robinson, Co-investigator.
Dr Jim Speirs, Co-investigator.
Ms Caroline Tarr, Research Officer.
Mr Anthony Tomas, Research Officer.

Appendix 5: Other relevant material

Collaborating researchers

Researchers involved in this project interact with a number of other researchers in CSIRO, Australia and the rest of the world. The benefit of such interactions to the Australian wine industry is felt through the wealth of knowledge and expertise that can be brought to bear on issues of industry concern. A list of our major collaborators is included below:

1. International

Dr Claudio D'Onofrio (University of Pisa, Italy) Secondary metabolite production in cell cultures.
Dr Stella Grando (IASMA, Trento, Italy) Grapevine genetics
Dr Juri Batillana (IASMA, Trento, Italy) Terpene production in grapes
Professor Hildegarde Heymann (UC Davis, USA) Sensory analysis of wines
Professor Susan Ebeler (UC Davis, USA) Chemical analysis of wines
Mr Sol Green (Plant and Food, NZ) Terpene synthase enzymes

2. Australian academic and scientific

Dr Emlyn Williams (ANU) Statistical analyses
Associate Professor Robert Trengove (Murdoch University) Separations chemistry
Mr Tony Robinson (Murdoch University) PhD student
Associate Professor Kathy Soole (Flinders University) Biochemistry
Associate Professor Mike Perkins (Flinders University) Synthetic chemistry
Mr Jake Dunlevy (Flinders University/CSIRO) PhD student
Dr Kerry Wilkinson (Adelaide University) Flavour chemistry
Ms Anthea Fudge (Adelaide University) PhD student
Dr Chris Soar (SARDI) Vine physiology
Dr Mike McCarthy (SARDI) Vineyard management
Mr Gary Trist (University of Melbourne) Masters student
Ms Chi Zhang (University of Adelaide) Honours student

3. Australian wine industry

Yalumba (especially Louisa Rose who chaired our winemaker taste panel)
Wingara Wine Group
Chalk Hill Wines
Fosters Wine Group
Pernod-Ricard
Constellation Wines
Koltz Wines
Tin Shed Wines
Provisor (winemaking and sensory analysis)

4. CSIRO

Dr Rob Bramley & Mrs Jackie Ouzman (Ecosystem Sciences) Precision viticulture
Dr Ciaran Forde and Dr Patrick O'Riordan (Food and Nutritional Sciences) Sensory analysis of grapes and wine
Dr Amalia Berna (Ecosystem Sciences) E-nose, grape and wine assessment
Dr Stephen Trowell (Ecosystem Sciences) Cybernose
Dr Christopher Davies (Plant Industry) Berry ripening
Dr Everard Edwards (Plant Industry) Irrigation and physiology