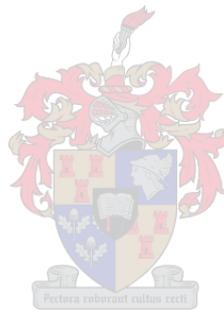


**Interaction of water deficit, canopy
modification and ripening: Effect on
the phenolic and colour
composition of Shiraz grapes &
subsequent wine**

by

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Declaration

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Summary

Phenolic compounds are important quality indicators of a red wine, as they can contribute to the colour of a young red wine, colour stability during ageing as well as astringency, bitterness, body and overall mouthfeel properties. Wine composition is commonly influenced by winemaking and viticultural practices.

In South Africa it often happens that vines are excessively vigorous, resulting in canopies that are too dense, which in turn could have a negative effect on the quantity and quality of the grapes produced. Viticultural practices such as judicious canopy management and irrigation are designed to control vine vigour and yield, thus improving fruit ripening and colour development.

Artificial shading and water deficit have been reported to have an influence on the sensory properties of red wine as well as on the flavonoid composition. These effects are dependent on a number of factors, however, including the season, cultivar, light intensity, and the extent and timing of water deficit.

There is limited research on the possible interactive effects of grapevine water deficits and canopy manipulation on grape and wine flavonoid composition in Shiraz, as well as the relationships between berry and wine composition. We thus investigated the effect of canopy reduction in combination with water deficit on the phenolic and colour composition of Shiraz grapes at different levels of ripeness, and in their corresponding wines after alcoholic and malolactic fermentation as well as after six months' ageing. This study found that it is possible to improve the phenolic composition of grapes and wine by shoot removal, and some of the tendencies in the wines were also observed after the ageing period. If the shoot removal is not performed at a very early stage, sunburn damage can occur and this will result in berries with a lower mass and volume at harvest due to excessive exposure without the berry having adapted to the imposed conditions.

Harvesting at different ripeness levels also affected the chemical and phenolic composition of the grapes and resulting wines. The water deficit effect on most phenolic parameters measured in the grapes and wine was not as prominent as that of the canopy manipulation treatment.

This study improved our understanding of how an improvement in the canopy microclimate of Shiraz could be reflected in the phenolic composition of wines, along with a potentially important effect of harvesting date. On this basis it may be possible to attain a specific wine style. Harvesting at a ripe stage, for example, could result in the production of wines with higher colour density and astringency, while unripe grapes could result in wines with higher levels of perceivable fresh berry attributes. In particular, canopy reduction could increase the astringency and body of wines made from grapes subjected to water deficit.

Opsomming

Fenoliese verbindings is belangrike kwaliteitsparameters van rooiwyn, aangesien dit kan bydra tot die kleur van 'n rooiwyn, kleurstabiliteit tydens veroudering sowel as frankheid, bitterigheid en mondgevoel. 'n Wyn se fenoliese samestelling word algemeen bepaal deur wynmaak- en wingerdkundige praktyke.

In Suid-Afrika gebeur dit gereeld dat wingerde uitermatig groeikragtig is, wat lei tot te digte lower wat sodoende 'n negatiewe effek op kwantiteit en kwaliteit van die druiwe wat geproduseer word, het. Wingerdkundige praktyke soos oordeelkundige lowerbestuur en besproeiing is ontwerp om wingerdstokke se groeikrag en opbrengs te beheer, en sodoende vrugrypwording en kleurontwikkeling te verbeter.

Kunsmatige beskaduwing en waterstres is gerapporteer om 'n invloed te hê op die sensoriese eienskappe van rooiwyn sowel as op die flavonoïedsamestelling. Hierdie effekte is egter afhanklik van 'n verskeidenheid faktore, insluitende die seisoen, kultivar, ligintensiteit en die mate en tyd van waterstres toegepas.

Daar is beperkte navorsing op die moontlike interaktiewe effekte van waterstres en lowermanipulasie op die druif en wyn flavonoïedsamestelling in Shiraz, sowel as die verhoudings tussen druif en wyn samestelling. Ons het dus die effekte van lowerbestuur in kombinasie met waterstres op die fenoliese en kleursamestelling van Shiraz druiwe by verskillende rypheidsvlakke ondersoek, asook in hul ooreenstemmende wyne na alkoholiese- en appelmelksuurfermentasie sowel as na ses maande veroudering. Hierdie studie het gevind dat dit moontlik is om die fenoliese samestelling van druiwe en wyn deur lootverwydering te verbeter, en sommige van die tendense is ook waargeneem in die wyn na die verouderingsperiode. Indien lootverwydering nie toegepas word by 'n baie vroeë stadium nie, kan sonbrand voorkom en dit kan lei tot korrels met 'n laer massa en volume by oes as gevolg van oormatige blootstelling sonder dat die korrel aangepas het by die spesifieke kondisies.

Oes by verskillende rypheidsvlakke affekteer ook die chemiese en fenoliese samestelling van die druiwe en ooreenstemmende wyne. Die waterstreseffek op meeste van die fenoliese parameters gemeet in druiwe en wyn was nie so prominent soos dié van die lowermanipulasie behandeling nie.

Hierdie studie het ons begrip verbeter van hoe 'n verbetering van die lower mikroklimaat van Shiraz gereflekteer kan word op die fenoliese samestelling van die wyn, saam met 'n potensiële belangrike effek van oesdatum. Op grond van hierdie basis is dit dus moontlik om 'n spesifieke wynstyl te verkry. Oes by 'n ryp stadium, byvoorbeeld, kan die produksie van wyn met 'n hoër kleurdigtheid en frankheid tot gevolg hê, terwyl onryp druiwe wyne met hoër vlakke van waarneembare vars bessiekenmerke tot gevolg kan hê. Verlaging van lowerdigtheid kan veral die frankheid en mondgevoel van wyne gemaak van druiwe blootgestel aan waterstres, verbeter.

This thesis is dedicated to my heavenly Father and all those who supported and encouraged me during the process of this study.

Biographical sketch

Marelize van Noordwyk was born on 20 November 1986 in Cape Town and matriculated at Parel Vallei High School in Somerset West in 2005. Marelize obtained a BSc-degree in Agricultural Science (Viticulture and Oenology) in 2009 at Stellenbosch University. In 2010 she enrolled for a MScAgric-degree in Oenology at the same University.

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Preface

This thesis is presented as a compilation of 5 chapters. Each chapter is introduced separately and Chapter 3 and 4 are written according to the style of the *South African Journal of Oenology and Viticulture*.

Chapter 1 **Introduction and Project aims**

Chapter 2 **Literature Review**

The impact of viticultural practices and the environment on the flavonoid composition of grapes and wine.

Chapter 3 **Research Results**

Interactive effects of growth manipulation and water deficit in grapevine (*Vitis vinifera* L.) cv. Shiraz: Impact on grape chemical and phenolic composition.

Chapter 4 **Research Results**

Interactive effects of growth manipulations and water deficit in grapevine (*Vitis vinifera* L.) cv. Shiraz: Impact on wine phenolic and sensory characteristics.

Chapter 5 **General Discussion & Conclusion**

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Chapter 1

Introduction and project aims

1. General introduction and project aims

1.1 INTRODUCTION

Winemaking in South Africa dates back to the 17th century, when the Dutch began setting up refreshment points for passing ships at the Cape of Good Hope. Products such as fabric and wine (alcohol) were traded in exchange for land and meat. During the 1650s, the first vineyard was planted and the first wine was produced in the region now known as the Western Cape Province, with wine exports starting in 1788. South Africa has the advantage of being able to supply foreign markets with a range of wine styles that reflect the great diversity of this vibrant and vivacious country. Over time, as winemaking skills improved, a more scientific approach among wine producers, with the objective to produce specific styles of high-quality red wines, started to gain popularity.

Grape flavonoid compounds are important contributors to wine quality, as they influence the colour, colour stability and sensory properties of a red wine (Glories, 1988). The colour of a young red wine is mainly due to monomeric anthocyanins or copigments (Boulton, 2001). Anthocyanins can form interactions with themselves (self-association) or complexes with other phenolic compounds (flavonol glycosides and cinnamic acids) (Brouillard & Mazza, 1989; Boulton, 2001). Direct and indirect condensation of anthocyanins and flavanols also occur during the winemaking process, leading to more polymeric pigments in wine. As wine ages, a larger fraction of wine colour is thus due to these stable polymeric pigments, the result of polymerisation reactions and copigmentation associations that are more resistant to pH fluctuations and sulphur dioxide bleaching (Boulton, 2001).

The concentration and composition of anthocyanin and phenolic compounds may be influenced by vineyard management and climatic conditions (Matthews *et al.*, 1990; Kennedy *et al.*, 2002; Downey *et al.*, 2006). Fruit flavonoid concentration, as well as the rate of extraction of these compounds during fermentation, is an important parameter that determines wine flavonoid concentration (Romero-Cascales *et al.*, 2005; Ristic *et al.*, 2007; Río Segade *et al.*, 2008). Fruit ripeness, ethanol content and berry size have been reported to influence the extraction of flavonoids (Canals *et al.*, 2005).

Excessive vigour, which may reduce the photosynthetic activity of leaves and increase humidity, has been found to negatively affect canopy microclimate, source:sink relationships in grapevines, yield, grape composition and wine quality. It can also promote bunch rot and prevent effective pest and disease control (Hunter *et al.*, 1995). In South Africa, a favourable climate, which may contribute to vigorous growth, highlights the need for judicious canopy management and irrigation to control vine vigour and yield and to improve fruit ripening and colour development (Hunter *et al.*, 2004).

Although several studies have found that light exposure has a positive effect on grape flavonoid concentration (Rojas-Lara & Morrison, 1989; Morrison & Noble, 1990; Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Jeong *et al.*, 2004; Chorti *et al.*, 2010), another study showed no effect of sunlight on anthocyanin biosynthesis (Downey *et al.*, 2004). Differences in the results found between studies regarding the effect of shading on flavonoid concentration could be ascribed to differences in the experimental lay out, application of the studies as well as climatic differences. Bunch exposure does not only influence flavonoid concentration in grapes, but could also induce changes in the composition within proanthocyanidins and anthocyanins (Price *et al.*, 1995; Downey *et al.*, 2004; Cortell & Kennedy, 2006; Ristic *et al.*, 2007; Koyama & Goto-Yamamoto, 2008). According to the literature, extensive shading could result in wine with decreased concentrations of colour and phenolic compounds, which could be maintained during ageing (Smart *et al.*, 1985; Price *et al.*, 1995; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007, 2010). It could also influence the sensory characteristics of a red wine (Price *et al.*, 1995; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007, 2010). The effects of light on fruit composition are also dependent upon the extent to which berry temperature is elevated as a result of increased sunlight exposure, as high berry temperature may inhibit colour development (Kliewer, 1970; Downey *et al.*, 2006).

Water deficits may affect the concentration of phenolic compounds due to berry size reduction or a direct action on biosynthesis, which can be positive or negative, depending on the type of phenolic compound, period of application, and severity of water deficit (Kennedy *et al.*, 2002; Ojeda *et al.*, 2002). The application of water deficit could also affect wine sensory properties positively (Matthews *et al.*, 1990; Escalona *et al.*, 1999; Koundouras *et al.*, 2006). Studies that reported a positive effect of water deficit on the phenolic composition of wine have been done on a variety of cultivars (Matthews *et al.*, 1990; Chapman *et al.*, 2005; Peterlunger *et al.*, 2005; Koundouras *et al.*, 2006; Bindon *et al.*, 2008, 2011; Chalmers *et al.*, 2010).

Limited literature is available on shoot removal in very dense canopies, as authors have mainly studied the effects of leaf removal and artificial shading on wine composition, applying it at different stages during ripening. The effects of different water deficit levels have also been studied and were also applied at different growth stages. These effects seemed to have been dependent on season, cultivar and the intensity and timing of the treatment applied. The general effects of bunch exposure and water deficits on red grape quality have therefore been studied well, but our main aim with this study was to evaluate the interactive effect of a reduction in canopy density, by shoot removal, and water deficit on the colour and phenolic composition of Shiraz grapes at harvest, as well as in the resulting red wines. By means of shoot removal, considered as a more extreme option to limit vigour compared to leaf removal, optimal source:sink relationships could be maintained in grapevines. Approximately 50% of the shoots were removed during flowering, and the effects of this were determined on the colour and phenolic composition of the grapes, as well as on the resulting wines after alcoholic

fermentation, malolactic fermentation and six months ageing. Since malolactic fermentation is a process that normally takes place in most commercial red wines, it was included as a winemaking process in order for the results to be more representative of the industry.

Harvesting at different ratios of total soluble solids to titratable acidity have been reported to yield different wine styles for Shiraz (Hunter *et al.*, 2007). As the flavonoid composition of grapes is greatly influenced by the degree of ripeness, a part of this study therefore focussed on how the colour and phenolic composition of a red wine is influenced by different ripeness levels. This research forms part of a larger research programme that is being conducted on the interactive effect of a reduction in canopy density, by shoot removal, and water deficits by the Department of Viticulture and Oenology, Stellenbosch University.

1.2 PROJECT AIMS

The main aims of the study were as follows:

- a.) to evaluate the interactive effects of reduction in canopy density, water deficit and different stages of maturation on the phenolic composition and ease of extractability of *Vitis vinifera* cv. Shiraz grapes in the Stellenbosch region of South Africa, under extremely vigorous growing conditions;
- b.) to assess how these changes in the grapes affect the colour and phenolic composition of the resulting wine at different stages during the winemaking process; and
- c.) to determine how the viticultural treatments affect the sensory characteristics of the resulting wine.

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Chapter 2

Literature review

The impact of viticultural practices and the environment on the flavonoid composition of grapes and wine

2. Literature review

2.1 INTRODUCTION

Flavonoids are a large and diverse group of compounds that can contribute greatly to red wine quality. Considerable research has been done to examine the viticultural and oenological factors that influence their biosynthesis, and how this knowledge might be used to manipulate the berry and resulting wine flavonoid composition (Downey *et al.*, 2006). This review examines the contribution of flavonoids to wine quality and recent research done on the impacts of sunlight and water deficit on the flavonoid content and composition of grape berries and wine.

2.2 FLAVONOIDS IN GRAPES AND WINE

In wine production, knowledge of the characteristics, content and extractability of grape anthocyanins allows for the management of red wine fermentation and the prediction of wine colour. Phenolic compounds originating from the grapes are classified as nonflavonoids (phenolic acids and stilbenes) and flavonoids (anthocyanins, flavan-3-ols and flavonols) (Ojeda *et al.*, 2002).

Anthocyanins and flavan-3-ols are based on a common C6-C3-C6 skeleton (Figures 1 and 2) with two phenolic rings (A and B) linked via a heterocyclic pyran ring (C) (Ollé *et al.*, 2011). Anthocyanins in grapes (*Vitis vinifera* L.) consist of cyanidin, peonidin, delphinidin, petunidin, and malvidin (each having different combinations of -H, OH and OCH₃ groups on the B ring). Each of these is glucosylated at the 3 position of the C ring, and the glucoside can be further substituted with acetyl and coumaroyl moieties. In all there are 15 anthocyanins that are commonly detected in Shiraz grapes (Mazza, 1995).

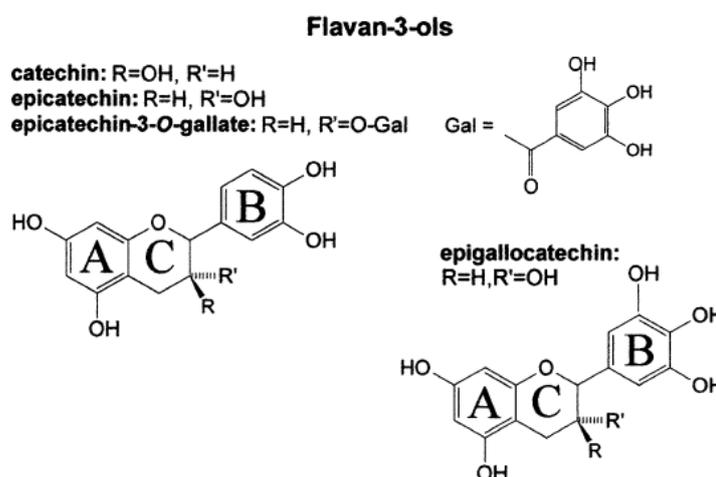


Figure 1 Chemical structures of flavan-3-ols (catechin, epicatechin, epicatechin-3-O-gallate and epigallocatechin) (Ollé *et al.*, 2011).

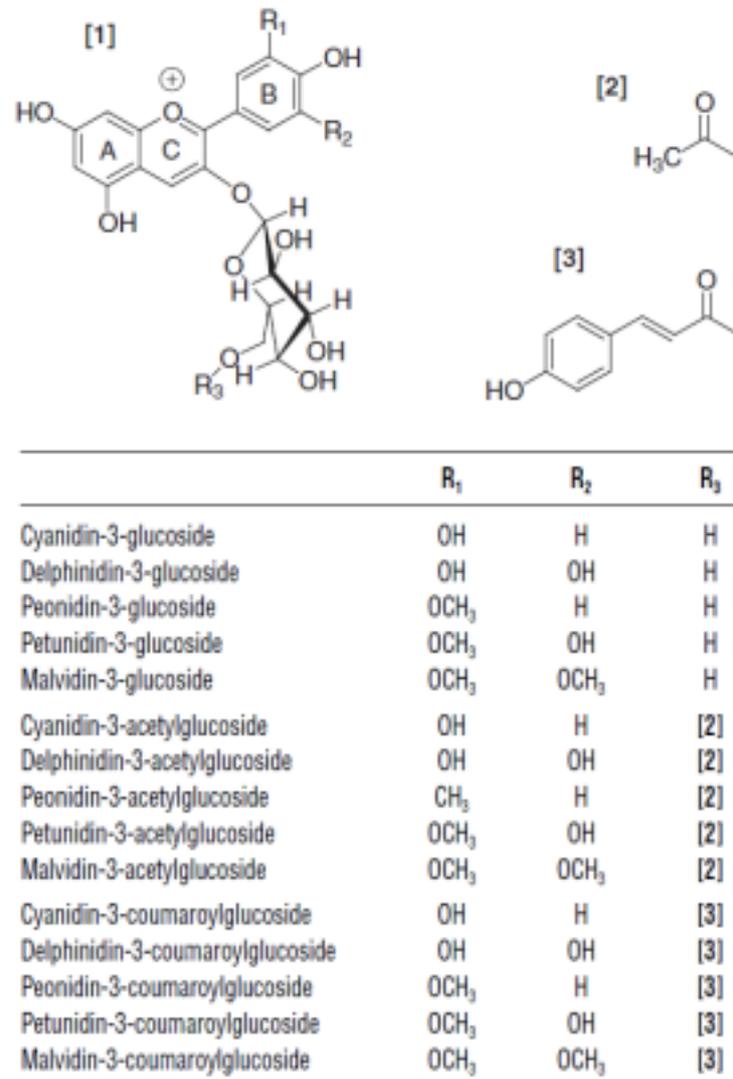


Figure 2 Anthocyanins generally present in grape berries, variously substituted at positions R₁, R₂ and R₃. Substitutions at R₃ include acetyl [2] and coumaroyl [3] moieties (Downey *et al.*, 2004).

Two types of tannins are normally found in red wine, namely condensed (derived from grapes) and hydrolysable (originating from oak wood) tannins. Hydrolysable tannins, however, are not classified as flavonoid compounds (Downey *et al.*, 2006). Grape-derived tannins normally include a range of polyphenolic compounds, ranging from small oligomeric forms to large proanthocyanidin polymers, also known as condensed tannins. These oligomers and polymers are composed of flavan-3-ol subunits, which are linked by C4-C8 and, to a lesser extent, by C4-C6 interflavan bonds (Haslam, 1998) (Figure 3).

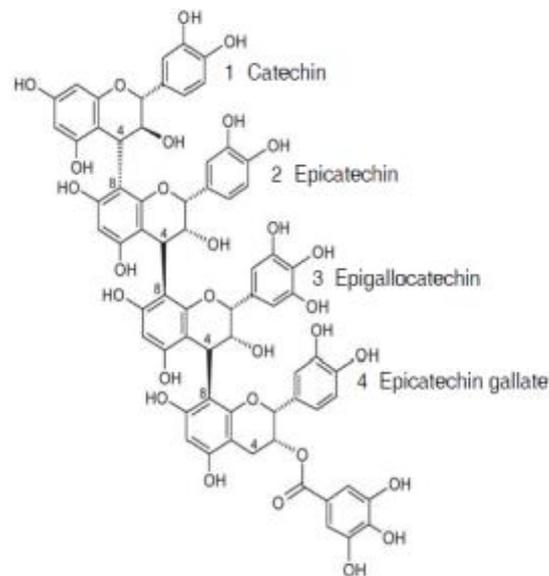


Figure 3 A hypothetical condensed tannin, made up of four subunits. The first three subunits are referred to as "extension" subunits, and only the epicatechin gallate, with its free 4 position, is referred to as a "terminal" unit. Catechin is bound to epicatechin by an interflavan bond between carbon 4 of catechin and carbon 8 of epicatechin (Adams, 2006).

The flavonols kaempferol, quercetin, myricetin and isorhamnetin are present in wine, but in grapes they normally occur as the corresponding glucosides, galactosides and gluconorides, where R3 is occupied by glucose, galactose or glucuronic acid respectively (Figure 4) (Adams, 2006). Flavonols are present at relatively low levels in grapes (Souquet *et al.*, 1996).



Figure 4 Flavonols in wine. In grapes they are present as the corresponding glucosides, galactosides and gluconorides, where R3 is glucose, galactose and glucuronic acid respectively (Adams, 2006).

2.3 DISTRIBUTION AND FUNCTION OF FLAVONOIDS

An important role of flavonoid compounds is to protect plants from ultraviolet (UV) radiation (Koes *et al.*, 1994) by absorbing light in both the ultraviolet and visible spectra, which is a result of its chromophoric nature (Markham, 1982).

Anthocyanins are responsible for the colour of red wine and grapes (Glories, 1984). Flavonols, which are known to act as protective agents against UV and free-radical scavengers (Flint *et al.*, 1985), also contribute to the colour of young red wine by acting as copigments (Asen *et al.*, 1972; Boulton, 2001), as well as to bitterness (Gawel, 1998). Condensed tannins are thought to prevent herbivorous animals and insects from feeding on grapes due to their bitterness and astringency (Feeny, 1976). In red wine, however, flavan-3-ols and

proanthocyanidins contribute to the body, mouthfeel (Glories, 1988) and colour stability of wine by forming polymeric complexes with anthocyanins (Timberlake & Bridle, 1976).

Anthocyanins are found only in berry skins (Ribéreau-Gayon, 1964), except in teinturier varieties, in which they are also found in the flesh. Flavan-3-ols are found in the skins, seeds and pulp. Epicatechin and catechin units are present in all compartments. Epigallocatechin units are only found in grape skins (Souquet *et al.*, 1996) and pulp (Mane *et al.*, 2007), while epicatechin-3-O-gallate units are primarily found in the seeds (Prieur *et al.*, 1994). Unlike proanthocyanidins from seeds, proanthocyanidins from skins contain prodelfinidins and have a higher degree of polymerisation and a lower proportion of galloylated subunits (González-Manzano *et al.*, 2004). Proanthocyanidin polymers of seeds consist of similar amounts of catechin and epicatechin subunits (Downey *et al.*, 2003a). In the skin, proanthocyanidin polymers are comprised mainly of epicatechin subunits (Cheynier *et al.*, 1997; Kennedy *et al.*, 2001; Downey *et al.*, 2003a). Total tannin content is reported to be significantly higher in seeds than in skins (Downey *et al.*, 2003a). Flavonols are found mainly in the pulp, as well as in the skin (Adams, 2006).

During vinification, anthocyanins and tannins are partly extracted from grape skins and can undergo structural transformations through many reactions, with significant effects on wine sensory characteristics (Vidal *et al.*, 2002). The concentration of anthocyanins in the grapes and their ease of extraction are the main factors affecting their concentration in wine (Romero-Cascales *et al.*, 2005).

2.4 FLAVONOID BIOSYNTHESIS

Berry growth follows a double sigmoid habit that can be divided into two growth phases (Stage I and III), separated by a lag phase (Stage II) (Coombe, 1976). The transition from Stage II to Stage III is named véraison and is considered to be the onset of ripening.

The phenylpropanoid pathway is generally considered to produce hydroxycinnamates, stilbenes, lignin, lignan, aurones, flavones, isoflavonoids, as well as the flavonoids (flavonols, tannins and anthocyanins) (Harborne, 1967; Downey *et al.*, 2006), which share several early steps in the pathway. Tannins are synthesised before véraison, while anthocyanins are synthesised after véraison.

The phenylpropanoid pathway synthesises flavonoids from carboxylated acetyl-CoA (malonyl-CoA) and the amino acid phenylalanine, which is produced via the shikimate pathway (Dewick & Haslam, 1969; Downey *et al.*, 2006) (Figure 5A). Phenylalanine ammonia-lyase (PAL) is usually the key enzyme in the shikimate pathway, which channels phenylalanine away from protein synthesis toward that of flavonoid and anthocyanin compounds (Roubelakis-Angelakis & Kliewer, 1986). During the chalcone synthase reaction, either p-coumaroyl-CoA (Figure 5B) or caffeoyl-CoA (Figure 5C) combines with three malonyl-CoAs to give a chalcone and CO₂ (Adams, 2006). The flavan-3,4-diol produced from the flavanonol by dihydroflavanol

reductase (DFR) already has the 2,3-trans configuration of catechin and can be converted to catechin directly by a leucoanthocyanidin reductase (LAR) (Figure 5D). Epicatechin is produced from cyanidin by the action of the enzyme anthocyanidin reductase (ANR) (Xie *et al.*, 2003). Anthocyanin reductase converts cyanidin and delphinidin to epicatechin and epigallocatechin respectively. The ANR gene is thought to be expressed in the skin and seeds of grape berries until the onset of ripening, and the two LAR genes show different patterns of expression in skins and seeds (Bogs *et al.*, 2005). The timing and expression of these genes have been proven to be consistent with the accumulation of proanthocyanidins. The accumulation of anthocyanins in the skin of red grapes coincides with the expression of the gene encoding the final step in anthocyanin biosynthesis, UDP-glucose: flavonoid 3-O-glucosyl transferase (UFGT) (Boss *et al.*, 1996), which catalyses the glycosylation of unstable anthocyanidin aglycones into pigmented anthocyanins. Two primary anthocyanins (cyanidin and delphinidin) are synthesised by UFGT in the cytosol of berry epidermal cells. Cyanidin has a B-ring dihydroxylated at the 3' and 4' positions, whereas delphinidin has a tri-hydroxylated B-ring due to an additional hydroxyl group at the 5' position. Cyanidin and delphinidin are derived from parallel pathways that originate downstream of flavonoid 3'-hydroxylases and flavonoid 3' 5'-hydroxylases (Bogs *et al.*, 2006; Castellarin *et al.*, 2006). The 3' position of cyanidin and delphinidin and sequentially the 5' position of delphinidin can be methoxylated by OMT that generate peonidin, petunidin and malvidin respectively. One or more members of the glutathione S-transferase (GST) protein family participate in the export of anthocyanins from the cytoplasm to the vacuoles (Marrs *et al.*, 1995; Mueller *et al.*, 2000).

Kobayashi *et al.* (2002) showed that *myb*-related regulatory genes, *VlmybAs*, were involved in the regulation of anthocyanin biosynthesis via *UFGT* gene expression, as well as the enhanced expression of other enzyme genes of the biosynthetic pathway. A homologue of *VlmybAs*, *VvmybA1*, is expressed in the berry skins of coloured cultivars of *V. vinifera*, but not in white ones (Kobayashi *et al.*, 2004). Abscisic acid (ABA), a plant hormone, is known to enhance the expression of *VvmybA1*, which coincides with the enhanced expression of anthocyanin synthetic enzyme genes such as *PAL* (phenylalanine ammonia-lyase), *CHS* (chalcone synthase), *CHI* (chalcone isomerase), *DFR* (dihydroflavonol 4-reductase), *LDOX* (leucoanthocyanidin dioxygenase) and *UFGT* (UDP-glucose:flavonoid 3-O-glucosyltransferase) (Ban *et al.*, 2003), and anthocyanin accumulation in the berry skins (Jeong *et al.*, 2004). Flavonol synthases (FLS) lead to the production of flavonols (Figure 6).

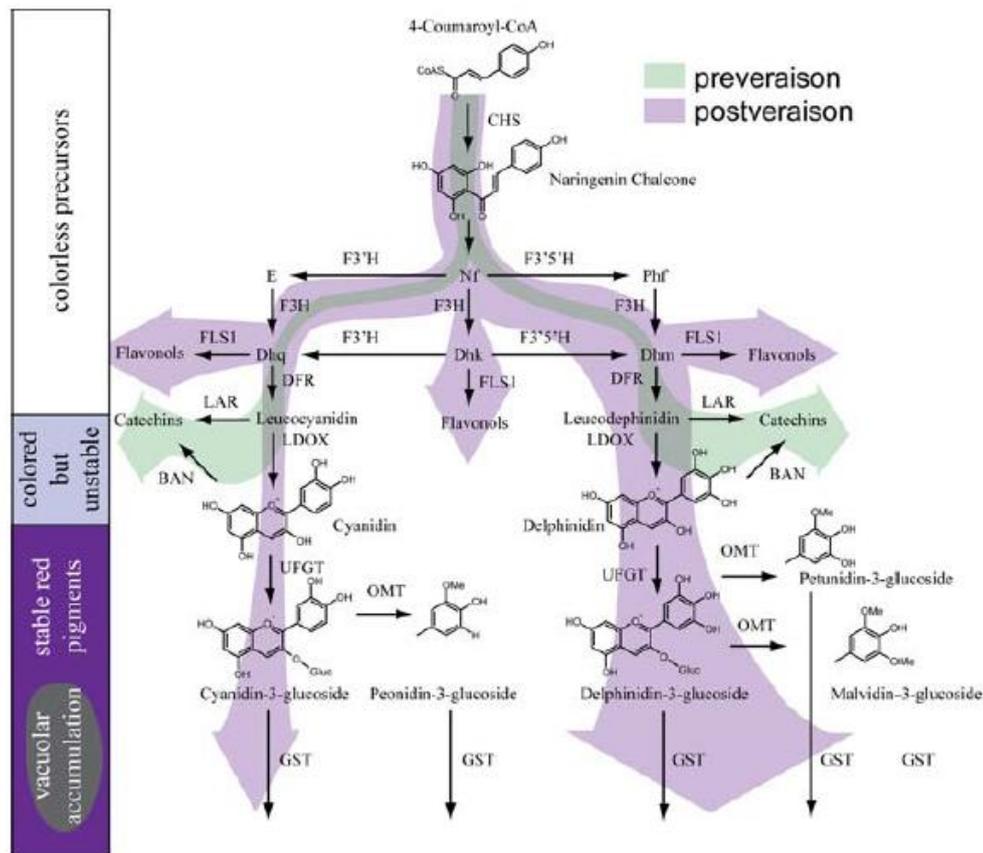


Figure 6 Summary of changes in the flavonoid pathway during berry development (Castellarin et al., 2007a).

2.5 FACTORS AFFECTING FLAVONOID BIOSYNTHESIS

Downey *et al.* (2006) discuss the effects of many factors known to affect flavonoid biosynthesis in plants, including light, temperature, altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators and various developmental processes. The greatest effects, however, are thought to be site and season (Ryan & Revilla, 2003; Downey *et al.*, 2006). Not only does the genetic variation between cultivars result in an enormous diversity of flavonoid content and composition, but many of the above factors are closely interrelated, making it difficult to study these effects individually.

2.6 EFFECT OF DIFFERENT RIPENING STAGES ON THE PHENOLIC AND COLOUR COMPOSITION OF GRAPES AND THE RESULTING WINE

2.6.1 Effect on anthocyanin accumulation

The accumulation of anthocyanins in skins starts at véraison, and the concentrations of anthocyanins are known to reach a maximum at a certain point, after which they decline just before harvest and/or during over-ripening (Somers, 1976; Roggero *et al.*, 1986; Fournand *et al.*, 2006). Whether the anthocyanins are degraded or incorporated into other molecules is not

known. Keller and Hrazdina (1998) suggest that the breakdown of anthocyanins may be caused by glycosidase and peroxidase activity in the grape skin vacuoles. Malvidin-3-glucoside is the dominant anthocyanin in *Vitis vinifera* skins (Roggero *et al.*, 1986). Malvidin-3-glucoside and peonidin-3-glucoside levels usually increase, while the other anthocyanidin monoglucosides tend to decrease at the end of ripening, which could be due to the fact that malvidin-3-glucoside and peonidin-3-glucoside are the final products of the anthocyanin biosynthesis pathway (Roggero *et al.*, 1986; Canals *et al.*, 2005). Ryan and Revilla (2003) found that the relative content of malvidin-3-O-acetylglucoside was quite stable during ripening, but that malvidin-3-O-*p*-coumaroylglucoside usually increased in the first stages of ripening and then decreased at the end (Ryan & Revilla, 2003). The increase of total *p*-coumaroyl anthocyanins was found to be only significant in the presence of ethanol in the extraction medium (Canals *et al.*, 2005). Acylated anthocyanins increase throughout ripening, although in some cases they decrease at the end of the process (González-SanJosé *et al.*, 1990; Fernández-López *et al.*, 1992; Canals *et al.*, 2005).

2.6.2 Effect on flavonol biosynthesis

Flavonol biosynthesis in the grape occurs only in the skin of the berry (Kennedy *et al.*, 2002). However, unlike in anthocyanin biosynthesis, there are two distinct periods of flavonol synthesis in grape berries, the first around flowering and the second beginning one to two weeks after véraison and continuing throughout ripening (Downey *et al.*, 2003b).

2.6.3 Effect on hydroxycinnamate biosynthesis

Previous studies have reported a peak in total hydroxycinnamates on a per berry basis prior to véraison, and then a decline leading to a constant amount (per berry) as the fruit ripened (Romeyer *et al.*, 1983). This decline on a per berry basis is typical of several phenolic compounds (for example flavan-3-ols from the skin) and could be due to the catabolism of the compounds or their utilisation in the biosynthesis of other phenolic compounds, or both (Adams, 2006). The most abundant hydroxycinnamate, found predominantly in the pulp but also in the skins in low concentrations, is caftaric acid, followed by coumaric acid.

2.6.4 Tannins and monomeric flavan-3-ols

2.6.4.1 Skins

A previous study has suggested that skin tannins are produced very early in berry development and change very little from véraison to harvest on a per berry basis, but that the concentration (mg/g fresh weight or mg/L) declines during ripening in proportion to berry growth (Harbertson *et al.*, 2002). This is consistent with some previous studies (Fournand *et al.*, 2006), but not with others that showed a decrease (Downey *et al.*, 2003a) or increase (Kennedy *et al.*, 2002) (mg/berry). However, it must be borne in mind that the analytical methods used in these studies were not always the same.

2.6.4.2 Seeds

A previous study reported a decline in seed tannin concentration in Cabernet Sauvignon grapes during ripening (Harbertson *et al.*, 2002). However, concentrations remained constant during the four weeks before harvest (mg CE/berry) and was accompanied by colour changes in the seeds (Ristic & Iland, 2005). In certain cases, the decrease could occur at an earlier stage, before colour change, with the concentration remaining relatively constant throughout the ripening period (Oberholster, 2003). Catechin and epicatechin levels are normally at a maximum just after véraison, followed by a sharp decrease as the fruit continue to ripen (Romeyer *et al.*, 1983). However, the mean degree of polymerisation of seed tannins increases during ripening (Kennedy *et al.*, 2000a, 2000b).

2.6.5 Influence on phenolic extractability

A few studies have examined the impact of different ripening stages on the extractability of phenolic compounds into the wine (Pérez-Magariño & González-San José, 2004; Canals *et al.*, 2005; Fournand *et al.*, 2006; Hanlin *et al.*, 2010). Studies claim that the increase in phenol extractability throughout grape ripeness is a result of the degradation of the skin cell walls by pectolytic enzymes (Ribéreau-Gayon *et al.*, 2000). Differences in galactose- and arabinose-based polysaccharides, the cellulose content, degree of methylation of the pectins, density of the cell walls (Ortega-Regules *et al.*, 2006), skin thickness as well as skin hardness could also be responsible for differences in extractability (Rio Segade *et al.*, 2008). Torchio *et al.* (2010) determined that the thickness of the berry skin was most affected by the different levels of sugars in the pulp, while the hardness of the skin, evaluated by the break skin force (F_{sk}), was related to the cultivation site. The greatest effects on all phenolic composition, extractability indices and mechanical parameters measured were found to vary according to the interactions between the local climate, soil and site location (termed the 'terroir' by the French) (Torchio *et al.*, 2010).

In general terms, the extraction of anthocyanins (from skins) and proanthocyanidins (from skins and seeds) increases significantly during ripening (Canals *et al.*, 2005; Hunter *et al.*, 2007). A lower extraction yield for coumaroylated anthocyanins and for tannins with a high degree of polymerisation has also been observed in comparison with other phenolic classes (Fournand *et al.*, 2006). A reduced tannin concentration could also occur from véraison towards harvest, which could be attributed to a reduction in tannin extraction as a result of tannins binding with other components in the grape berry, such as proteins and polysaccharides (Cheynier *et al.*, 1997; Kennedy *et al.*, 2000a; Downey *et al.*, 2003a; Hanlin & Downey, 2009). The work by Hanlin *et al.* (2010) suggests that these tannin–cell wall interactions are formed by hydrogen bonding and hydrophobic interactions, with the binding capacity of the cell walls being influenced by tannin and polysaccharide structure and composition. Cell wall changes during berry development may increase the tannin-binding capacity of cell walls, while tannin structure may also influence the tannin's affinity for cell wall material. Canals *et al.* (2005), however, reported that proanthocyanidins are more easily extracted throughout ripening and that the combination of polysaccharides with proanthocyanidins in riper grapes may diminish their capacity to bind proteins (Escot *et al.*, 2001), resulting in less astringent wines.

During vinification, the extraction of total phenolic compounds from seeds is slower and more progressive in comparison with the skins (González-Manzano *et al.*, 2004; Canals *et al.*, 2005). Therefore, increased extraction of flavan-3-ols from the seeds of overripe grapes could result in increased tannin values in the wine, although phenol extraction from the skins measured with the Glories method (Ribéreau-Gayon *et al.*, 2000) seems limited in overripe grapes (Hunter *et al.*, 2007). The presence of ethanol is known to facilitate anthocyanin and especially proanthocyanidin extraction, but also decreases copigmentation phenomena, which can decrease the colour intensity of wine (Canals *et al.*, 2005). Several authors have found good correlations between anthocyanin concentrations in the grapes and the corresponding wines (Cagnasso *et al.*, 2008; Jensen *et al.*, 2008; Du Toit, 2011).

2.7 EVOLUTION OF FLAVONOIDS DURING VINIFICATION

2.7.1 Reactions involving anthocyanins

Given the normal pH range of red wines (pH 3 to 4), only 25% of the total anthocyanins are normally in the red form in a young wine (Brouillard & Delaporte, 1977; Glories, 1984). Five different anthocyanin forms are normally found in wine: the flavylium ion (red), carbinol base (colourless), chalcones (yellow), quinoidal base (violet) and flavene sulphone form (colourless). The anthocyanin equilibrium can be shifted depending on various factors, primarily the pH, SO₂ concentration and age of the wine (Figure 7). Anthocyanins in the flavylium form have a positive (+) charge on the C-ring, which is responsible for the colour reactivity of the pigment, leading to a red form that can be measured at 520 nm.

During vinification, different factors play a role in the evolution of phenolic compounds, including skin contact, fermentation time and ageing of the wines. The colour of a young red wine is attributed mainly to monomeric anthocyanins, which are extracted during maceration prior to fermentation and/or the beginning of alcoholic fermentation (Figure 8) (Ribéreau-Gayon *et al.*, 2006). At the stage when the extraction of anthocyanins from the grape skins is almost completed and the alcohol content reaches a certain level, several reactions may lead to a decrease in anthocyanin concentrations. These include adsorption of anthocyanins on solids (yeast, pomace) (Morata *et al.*, 2003), modifications in their structures (formation of tannin-anthocyanin complexes) and, possibly, oxidative breakdown reactions (Moreno-Arribas *et al.*, 2008). Tannin extraction continues for a longer period due to the location of the tannins in the skins and seeds. Tannins from the seeds are solubilised when the cuticle is dissolved by ethanol (Singleton & Esau, 1969). Copigmentation is the term used to describe associations between pigments and other, usually non-coloured, phenolic molecules in solution, known as copigments or cofactors. The latter include phenolic acids, flavan-3-ols and, in particular, flavonols (Brouillard & Mazza, 1989). Copigmentation accounts for between 30 and 50% of the colour of a young red wine and results in a shift of 5 to 20 nm in the maximum absorbance (hyperchromic shift), causing a blue-purple tone (Scheffeldt & Hrazdina, 1978; Boulton, 2001). Alcohol breaks down these copigments, but colour intensity may increase again due to the formation of new tannin-anthocyanin complexes, as well as new anthocyanin-tannin copigments, if these substances are present in large enough quantities (Ribéreau-Gayon *et al.*, 2006).

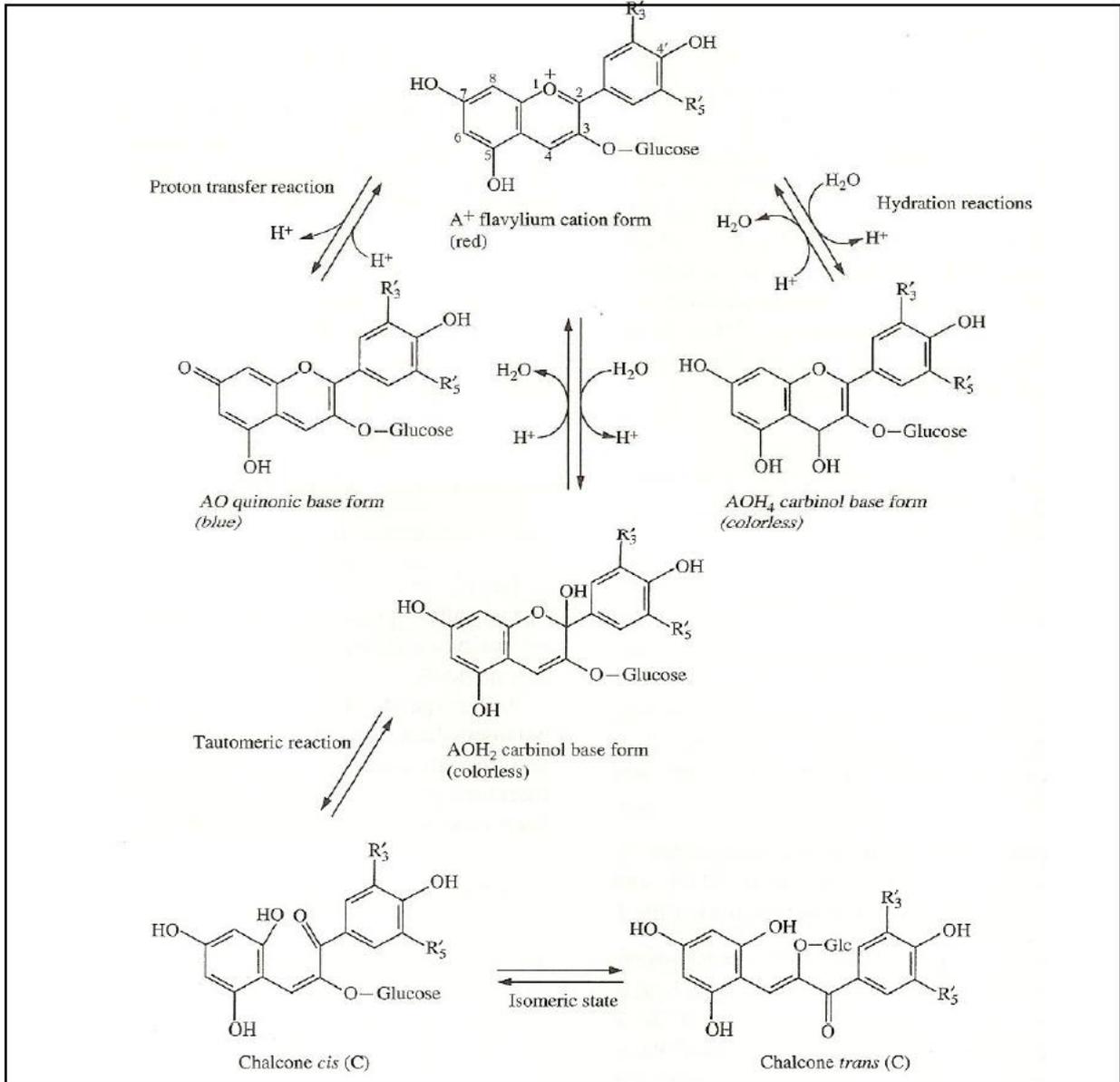


Figure 7 Anthocyanin equilibria illustrating the different forms in wine as affected by pH (Brouillard *et al.*, 1978).

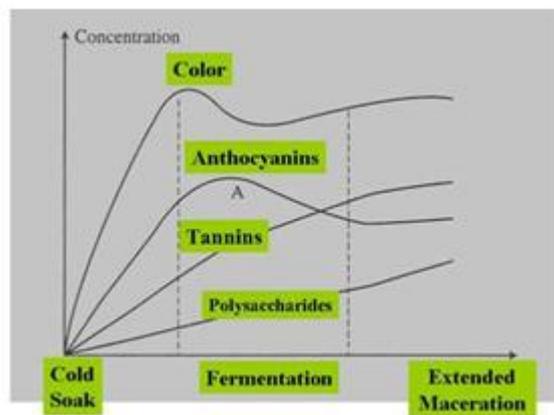


Figure 8 Evolution of flavonoids during vinification (Ribéreau-Gayon *et al.*, 2006).

The concentration of free anthocyanin decreases regularly during and after malolactic fermentation because of possible absorption on bacteria, breakdown and stabilisation reactions (Du Toit *et al.*, 2006; Du Toit, 2011). As wine ages, the greater degree of colour is due to stable polymeric pigments, the result of polymerisation reactions (Fell *et al.*, 2007) and copigmentation associations that are more resistant to pH fluctuations, sulphur dioxide bleaching and increases in alcohol concentrations (Somers, 1971; Jurd, 1972; Somers & Evans, 1977; Boulton, 2001). Several mechanisms lead to the formation of tannin-anthocyanin combinations, depending on the conditions in the medium (temperature, oxidation), as well as the type of tannins and the tannin/anthocyanin ratio. The colour of the new pigments ranges from mauve to orange (Ribéreau-Gayon *et al.*, 2006). An increase in colour intensity is observed in well-balanced, properly aged wines (Jurd, 1972; Ribéreau-Gayon *et al.*, 2006). During ageing, when oxidation occurs very slowly, electrophilic carbocations, formed from procyanidins in a low pH medium such as wine, can react with nucleophilic C6 or C8 carbons of the anthocyanin in its hydrated hemiacetal form (T-A product). When the temperature is relatively high (> 20°C), ageing might be accelerated, promoting polymerisation and condensation reactions and causing yellow tints in the wine, degrading malvidin and producing red pigments. During bottle ageing, which is characterised by the absence of oxidation, the colour evolves fairly rapidly towards brick red and orange. Pigments must be stabilised by oxidation mechanisms during ageing to avoid colour loss (Ribéreau-Gayon *et al.*, 2006).

2.7.2 Reactions involving tannins

During vinification, procyanidin molecules from the grapes tend to polymerise, condense with anthocyanins and combine with plant polymers such as proteins and polysaccharides. Direct C4-C8 and C4-C6 polymerisation reactions between procyanidin molecules produce products that are more reactive with proteins. This development continues up to a limit of 8 or 10 flavan units. On the contrary, molecules from ethanol-mediated polymerisation are less reactive than procyanidins, although they have the same quantity of flavanols and, when combined with other components such as anthocyanins, neutral polysaccharides and proteins, their reactivity could decrease (Ribéreau-Gayon *et al.*, 2006).

Controlled oxidation during vinification eliminates reduction odours, enhances the fruity aroma during fermentation, intensifies and stabilises the colour and softens the flavour during ageing. An excess, however, can lead to: a) oxidative breakdown of the anthocyanins, b) partial stabilisation of the anthocyanins by the formation of mauve complexes with an ethyl cross-bond (Escribano-Bailon *et al.*, 2001), c) the development of orange-coloured ethanol addition compounds, and d) the oxidation of tartaric acid to form yellow xanthylum salts (Mirabel *et al.*, 1999). Favourable tannin to anthocyanin ratio is also required, namely in the order of 4:1. Too low a ratio may lead to anthocyanin breakdown reactions and a too high ratio to over-polymerisation and precipitation (Ribéreau-Gayon *et al.*, 2006).

2.8 EFFECT OF WATER DEFICITS ON THE PHENOLIC AND COLOUR COMPOSITION OF GRAPES AND THE RESULTING WINE

According to the literature, the influence of a grapevine water deficit on the concentration of phenolics in grape berries and the resulting wine has produced variable results. Many possible explanations have been suggested for the variation in results, including differences in cultivar (Chalmers *et al.*, 2010; Bindon *et al.*, 2011), site, season (Bindon *et al.*, 2011), sampling, the quality of light, analytical technique (Bindon *et al.*, 2008; Chalmers *et al.*, 2010), as well as irrigation scheduling approaches, which could have resulted in various levels of water stress (Koundouras *et al.*, 2006).

Studying the effect of water deficit also proved to be difficult, as it influences a wide range of plant processes apart from flavonoid biosynthesis, such as stomatal closure, reducing photosynthesis and thereby all metabolite accumulation (Downey *et al.*, 2006). Water deficit may influence berry weight and canopy density, decrease root and shoot growth (Jones, 1992) and result in the senescence of some tissues, altering source-sink relationships (Coombe, 1989) and the extent of bunch exposure within the plant. Therefore, it is clear that it is not possible to investigate the influence of irrigation treatments on the phenolic compositional changes of grapes in isolation.

2.8.1 Effect on grape flavonoid composition

Ojeda *et al.* (2002) confirmed two types of berry responses to water deficit: an indirect and always positive effect on the concentration of phenolic compounds due to berry size reduction, and a direct action on biosynthesis that can be positive or negative, depending on the type of phenolic compound, period of application, and severity of the water deficit (Kennedy *et al.*, 2002; Ojeda *et al.*, 2002). Other than a direct stimulation of biosynthesis, water deficits could also increase the concentrations of skin tannins and anthocyanins due to the differential growth responses of the skin and inner mesocarp tissue to water deficits, resulting in greater skin mass and relative skin mass per berry, and therefore greater amounts of skin-localised solutes (Roby *et al.*, 2004).

The timing and intensity of water deficit during the green berry stage are known to play major roles in berry development (Ollé *et al.*, 2011). It is possible that water deficit applied from anthesis until véraison does not result in a recovery of berry size (Ginestar *et al.*, 1998; Ojeda *et al.*, 2001, 2002) and that, by lowering fruit cell turgor, it may cause an increase in ABA levels, which in turn could activate anthocyanin pathway genes (Castellarin *et al.*, 2007a). Conversely, water deficit applied from the end of fruit set to véraison was found not to permanently affect Shiraz berry mass, since the application of 100% evapotranspiration after véraison allowed the berries to recover to the same masses as the control berries throughout ripening (Ollé *et al.*, 2011). Water deficit after véraison is known to have only a minor effect on berry weight at

maturity and, during the month before harvest, berries are insensitive to water deficit (McCarthy, 1999).

Both pre- and post-véraison water deficits were found to differentially affect the anthocyanin composition. Moderate water deficits (pre-dawn leaf water potentials (PDLWP) between -0.5 and -0.8 MPa) between anthesis and véraison or strong water deficit between véraison and harvest maturity (PDLWP close to -1.0 MPa) compared to control grapes (PDLWP between -0.2 and -0.4 MPa), or grapes subjected to high water deficits between anthesis and véraison (PDLWP between -0.6 and -1 MPa), were found to enhance the biosynthesis of flavonols. Water stress after véraison could improve the biosynthesis of proanthocyanins and anthocyanins (Ojeda *et al.*, 2002) and specifically enhance the overall anthocyanin accumulation, particularly malvidin and *p*-coumaroylated derivatives throughout ripening in Shiraz skins (Ollé *et al.*, 2011) and Cabernet Sauvignon (Castellarin *et al.*, 2007a). This could be attributed to the expression of UFGT (UDP-glucose: flavonoid 3-O-glucosyl transferase) and the production of anthocyanins, which only begins at véraison (Boss *et al.*, 1996; Ollé *et al.*, 2011). In another study, water stress before véraison (a PDLWP of between -0.70 and -0.46 MPa) increased accumulation of all anthocyanins except malvidin and *p*-coumaroylated derivatives after véraison (Ollé *et al.*, 2011). Water deficits are also known to increase the degree of tannin polymerisation (Ojeda *et al.*, 2002), but seem to have limited influences on the accumulation of proanthocyanidins (Roby *et al.*, 2004; Castellarin *et al.*, 2007a) and flavonols (Castellarin *et al.*, 2007a) and on the expression of the genes involved in their biosynthesis.

The activation of anthocyanin pathway genes in grapes subjected to water deficit could be attributed primarily to increased solar radiation in the bunch zone due to reduced leaf turgor and leaf senescence, as well as increases in abscisic acid (ABA) (Castellarin *et al.*, 2007a, 2007b). Water deficits induce the up-regulation of most flavonoid biosynthetic genes and, in particular, of UFGT (Castellarin *et al.*, 2007a, 2007b), CHS2, CHS3 (Castellarin *et al.*, 2007b), DFR, LDOX and GST (Castellarin *et al.*, 2007a). They could also increase B-ring trihydroxylated anthocyanins in Cabernet Sauvignon through the differential regulation of flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H). Correlations were confirmed in water-stressed Merlot (stem water potential of between -1.2 and -1.4 MPa) between increased anthocyanin accumulation and the up-regulation of genes coding for F3'5'H and O-methyltransferase (OMT), leading to higher contents of particularly peonidin and malvidin derivatives (Castellarin *et al.*, 2007b).

2.8.2 Effect on wine flavonoid composition

Barbagallo *et al.* (2011) concluded that the relationship between grape phenolic composition and wine phenolic concentration and composition is not simple (De Beer *et al.*, 2006; Cortell *et al.*, 2007; Jensen *et al.*, 2008; Barbagallo *et al.*, 2011; Bindon *et al.*, 2011), while strong relationships were found under some conditions (Kennedy *et al.*, 2002; Cortell *et al.*, 2005;

Peterlunger *et al.*, 2005; Koundouras *et al.*, 2006; Bindon *et al.*, 2008; Jensen *et al.*, 2008; Du Toit, 2011). A poor relationship between the phenolic composition of grapes produced by deficit irrigation and that of the finished wines could be due to a restriction in the extractability of phenolics, particularly anthocyanins and tannins, which can occur in grapes produced under conditions of water deficit (Sivilotti *et al.*, 2005). This may be due to a tighter berry cell-wall structure in stressed plants, resulting from lower tissue hydration (Sivilotti *et al.*, 2005). Little or no relationship between grapes and wine could further be attributed to a) differences in extraction and stability among individual phenolics (Roggero *et al.*, 1984; Guidoni *et al.*, 2008; Bindon *et al.*, 2011), b) the complexity of the fermentation, winemaking and ageing processes, which strongly influence wine phenolic concentration, making comparison between experimental studies difficult, c) many compounds that are undetectable in berries but tend to develop during the fermentation process (Nagel & Wulf, 1979; Price *et al.*, 1995; Sacchi *et al.*, 2005), and d) the high variability in anthocyanin content in berries (Cortell *et al.*, 2007).

Studies that have reported a positive effect of deficit irrigation on wine phenolic composition were done on Cabernet Franc (Matthews *et al.*, 1990), Shiraz (Chalmers *et al.*, 2010) and Cabernet Sauvignon (Chapman *et al.*, 2005; Bindon *et al.*, 2008; Chalmers *et al.*, 2010), Merlot (Peterlunger *et al.*, 2005; Bindon *et al.*, 2011) and Agiorgitiko (Koundouras *et al.*, 2006). Analyses were conducted at bottling (Chalmers *et al.*, 2010), after six months (Bindon *et al.*, 2008, 2011; Chalmers *et al.*, 2010), after 18 months of ageing (Bindon *et al.*, 2011) and three months after harvest (Koundouras *et al.*, 2006).

It is suggested that a higher concentration of red pigments in wines made from grapes subjected to water deficit compared to wines made from irrigated grapes, after six months of ageing, could be caused by a change in anthocyanin concentration or a change in the chemical properties of anthocyanins to more polymeric or co-pigmented forms (Levengood, 1996; Levengood & Boulton, 2004; Bindon *et al.*, 2008; Chalmers *et al.*, 2010). This could be attributed to the generally higher concentrations of flavan-3-ols, anthocyanins and co-factors, which are needed for the formation of these pigments, being available in wines made from grapes subjected to water deficit.

As for the grapes, early water deficit (water withheld before véraison) seems to produce wines with higher concentrations of total anthocyanins and phenolics than late deficit (water withheld after véraison) (Matthews *et al.*, 1990). The extraction of anthocyanins during fermentation is known to be greater from fruit subjected to pre-véraison water deficit, although the loss of anthocyanins at the end of fermentation is also greater, thereby cancelling out differences in the concentration of anthocyanins attributable to the irrigation treatment (Sipiora & Gutierrez, 1998).

The application of water deficits can also positively affect wine composition with regard to wine sensory properties (Matthews *et al.*, 1990; Koundouras *et al.*, 2006). Chapman *et al.* (2005) have suggested that pre-véraison water deficits could result in wines with more fruity and

less vegetal aromas and flavours than those from grapevines subjected to lower water deficit stress levels (Chapman *et al.*, 2005). Post-véraison water deficit (maintaining grapevines at a stem water potential of -1.4 MPa), however, was shown to increase astringency, body and colour (Peterlunger *et al.*, 2005).

2.9 INFLUENCE OF LIGHT AND TEMPERATURE ON THE PHENOLIC AND COLOUR COMPOSITION OF GRAPES AND THE RESULTING WINE

2.9.1 Effect on grape flavonoid composition

Investigations into the effects of light on flavonoid biosynthesis in grapes and wine have taken a range of approaches, most involving the application of physical shade treatments, including opaque boxes (Downey *et al.*, 2004; Cortell & Kennedy, 2006), plastic sheeting (Kliewer *et al.*, 1967; Chorti *et al.*, 2010), shade cloth (Joscelyne *et al.*, 2007; Koyama & Goto-Yamamoto, 2008), bags (Kliewer & Antcliff, 1970), bird nets wrapped around the canopies (Smart *et al.*, 1985; Ristic *et al.*, 2010) and boxes made from white polypropylene sheeting painted black on the inside (Ristic *et al.*, 2007). Others have applied defoliation (Kliewer & Antcliff, 1970; Hunter *et al.*, 1991, 1995; Petrie *et al.*, 2003; Poni *et al.*, 2006; Chorti *et al.*, 2010) or tried to sample different sites (Rustioni *et al.*, 2011) or different parts of the canopy where the intensity of light was perceived to be different (Price *et al.*, 1995; Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). In addition, treatments have been applied at different developmental stages. These approaches resulted in a range of exposure levels of fruit and, in some cases, different levels of foliage exposure, with simultaneous impacts on photosynthesis.

Generally, it is known that berries that develop under open canopy conditions, in comparison to those that develop under shaded canopy conditions, have higher sugar concentration (TSS), lower juice pH and higher titratable acidity, and often an increased concentration of berry phenolics and less unripe herbaceous fruit characters (Gladstones, 1992). Altered light conditions could also modify the varietal character of berries (Hunter *et al.*, 1991). The combination of shading and leaf removal in the bunch zone between fruit set and véraison could substantially decrease the photosynthetic capacity of vines, resulting in decreased accumulation of all metabolites (Joscelyne *et al.*, 2007). Some studies reported no effect of bunch exposure on berry mass at harvest (Spayd *et al.*, 2002; Downey *et al.*, 2004; Chorti *et al.*, 2010), while others reported that sunlight exclusion during the initial stages of fruit growth could reduce berry weight and diameter compared to berries exposed to light during the same period (Dokoozlian & Kliewer, 1996), as long as fruit temperatures were not elevated beyond the optimum for development (Bergqvist *et al.*, 2001). This can probably be attributed to light-mediated effects on cell division and/or cell enlargement. Leaf removal seems to affect berry dry weight, sugar content and soluble solids more than berry volume or fresh weight (Petrie *et al.*, 2000).

Morrison and Noble (1990) examined the difference between the effects of leaf shading and cluster shading on grape composition. They found that shaded bunches caused a reduction in the phenol and anthocyanin concentrations, while shading of the leaves caused a delay in berry growth and sugar accumulation.

The response of anthocyanin accumulation to different light intensities is known to vary among cultivars (Haselgrove *et al.*, 2000). The effects of light on fruit composition are also heavily dependent on the extent to which berry temperature is elevated as a result of increased sunlight exposure, because high berry temperature (above 100 $\mu\text{mol}/\text{m}^2/\text{s}$ incident solar irradiation (Bergqvist *et al.*, 2001)) can inhibit colour development (Kliewer, 1970; Downey *et al.*, 2006). Studies by Pirie (1977) suggest that the temperature range for the activity of enzymes involved in the anthocyanin biosynthetic pathway is between 17 and 26°C. Temperatures outside this range are therefore likely to inhibit anthocyanin synthesis. This frequently occurs in Winkler viticultural regions IV and V (Winkler *et al.*, 1974) and other warm climate viticultural areas (Smart & Sinclair, 1976). Total phenolics generally follow a similar pattern.

The response of anthocyanin accumulation to different temperatures is also known to vary among cultivars. The most sensitive cultivars seem to be those with a high proportion of 3'-hydroxylated anthocyanins, as the enzyme catalysing 3'-hydroxylated anthocyanin biosynthesis (F3'H) has been suggested to be highly sensitive to temperature (Guidoni *et al.*, 2008; Chorti *et al.*, 2010). The effect of temperature can also vary greatly along developmental stages (Yamane *et al.*, 2006). One to three weeks after véraison, the concentration of abscisic acid (ABA) in berry skins was found to be higher at 20°C than at 30°C (Yamane *et al.*, 2006).

The actual degree of canopy openness and bunch exposure required for optimal anthocyanin accumulation is difficult to define. A desirable canopy for vines grown in hot climatic conditions is one where bunches are moderately exposed, conditions that are often described by the phrase 'dappled light within the canopy' (Haselgrove *et al.*, 2000).

The influence of light on skin proanthocyanidin concentration seems to vary according to cultivar (Downey *et al.*, 2004; Cortell & Kennedy, 2006; Koyama & Goto-Yamamoto, 2008). There was no observable effect of bunch exposure on either the proanthocyanidin content or composition of the seeds of ripe fruit (Downey *et al.*, 2004), although Ristic *et al.* (2007) found that shaded fruit had an increased seed tannin content (due to sunlight exclusion in clusters before flowering, resulting in increased seed weight), but decreased skin tannin levels.

Levels of quercetin-glucoside are also enhanced by bunch exposure (Price *et al.*, 1995; Haselgrove *et al.*, 2000) and may be an indicator of the degree of fruit exposure, but not of anthocyanin concentration. The metabolism of these compounds can respond differently to altered light and temperature regimes (Haselgrove *et al.*, 2000), as the regulation of the pathways of flavonols and anthocyanins occurs independently (Price, 1994).

Bunch exposure does not only influence the flavonoid concentration in grapes, but could also induce changes in the composition of proanthocyanidins and anthocyanins (Price *et al.*,

1995; Downey *et al.*, 2004; Cortell & Kennedy, 2006; Ristic *et al.*, 2007; Koyama & Goto-Yamamoto, 2008). Iacono *et al.* (1994) reported that shading lowered the percentages of delphinidin, cyanidin and petunidin monoglucosides while malvidin-3-glucoside was least affected (Keller & Hrazdina, 1998). The accumulation of the coumarate derivative of malvidin-3-glucoside has also been found to be enhanced under shaded conditions (Haselgrove *et al.*, 2000).

Artificial bunch shading reduces the accumulation of certain phenolics by reducing the transcription and expression of some genes of the biosynthetic pathways of several phenolics (Price *et al.*, 1995; Jeong *et al.*, 2004; Ristic *et al.*, 2007; Koyama & Goto-Yamamoto, 2008), as well as the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in secondary metabolism (Roubelakis-Angelakis & Kliewer, 1986). The stimulation of PAL is mediated by a co-action of UV-B light and phytochrome (Singh *et al.*, 1999). Dokoozlian and Kliewer (1996) have suggested that exposing fruit to light during stages I and II, before the onset of pigment production during stage III, may increase the initial concentration or activity of one or several anthocyanin biosynthetic enzymes (Takeda *et al.*, 1988), prevent delayed ripening and result in increased anthocyanin concentrations at harvest. However, light is needed during ripening to maintain the maximum activity of these enzymes (Dokoozlian & Kliewer, 1996).

Grapevines that are too vigorous could result in excessively dense canopies, which may have a negative effect on the quantity and quality of the grapes produced. Viticultural practices such as judicious canopy management and irrigation regimes are employed to control vine vigour and yield and to improve fruit ripening and colour development (Hunter *et al.*, 2004). Shoot removal could be considered as a more extreme option to limit vigour compared to leave removal and should be addressed early in the growing season while the shoots are only 5 to 10 cm long, and too much energy has not yet been expended in growing these shoots and potential berries (Davidson, 2002). By means of shoot removal, optimal source:sink relationships can be maintained in grapevines, improving berry colouration and accelerating ripening (Kliewer & Dokoozlian, 2000).

2.9.2 Effect on wine flavonoid composition

Recent investigations into the effects of light on the flavonoid composition of red wine have been performed on a variety of cultivars, including Cabernet Sauvignon (Joscelyne *et al.*, 2007), Shiraz (Smart *et al.*, 1985; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007, 2010) and Pinot noir (Price *et al.*, 1995). Phenolic analyses were conducted at various stages during the winemaking process, including after alcoholic fermentation (Price *et al.*, 1995; Ristic *et al.*, 2010), at bottling (Ristic *et al.*, 2007), and after four months (Price *et al.*, 1995), eight months (Ristic *et al.*, 2007), 12 months (Ristic *et al.*, 2010), two years (Joscelyne *et al.*, 2007) and three years (Ristic *et al.*, 2007) of ageing.

There is a limited amount of literature on the influence of light and temperature on flavonoid extractability. Cortell and Kennedy (2006) reported that shading could decrease the extractability of anthocyanins, skin tannins and flavonols and suggested that other factors, such as skin thickness, cell size and cell wall properties, could influence the extraction of flavonoids from the fruit during winemaking (Ristic *et al.*, 2007). A recent study found that bunch exposure resulted in delayed anthocyanin monomer extraction, which could be related to a different pigment profile as well as different skin tissue characteristics in these grapes, compared to grapes subjected to shading (Rustioni *et al.*, 2011).

Generally, extensive shading is known to result in wine with decreased colour and phenolic compounds that can be maintained during ageing. The influence on polymeric pigments, anthocyanin and total phenol levels seems to be inconsistent (Price *et al.*, 1995; Ristic *et al.*, 2007, 2010) and vary according to season (Ristic *et al.*, 2010) and cultivar (Price *et al.*, 1995; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007). Polymeric phenols, caffeic acid and quercetin aglycone were found to be lower, while catechin and caftaric acid were higher, in wines made from shaded fruit (Price *et al.*, 1995). The low levels of caftaric acid in wines from sun-exposed clusters appear to be related to the more rapid hydrolysis of the tartaric ester moiety, with wines from highly sun-exposed clusters having more caffeic acid than those from shaded clusters (Price *et al.*, 1995). In some situations, berries with similar concentrations of total anthocyanins may produce wines with a different colour intensity, as the coumarate form of malvidin-3-glucoside is lost during winemaking (Leone *et al.*, 1984) and is also less extractable than the other forms when the anthocyanins are extracted from berries with 10% v/v ethanol (Haselgrove *et al.*, 2000).

Wines made from shaded fruit are generally lower in proanthocyanidin concentration, and subtle changes in composition (Ricardo da Silva *et al.*, 1991; Bacon & Rhodes, 2000) are likely to influence wine colour stability and also wine sensory properties. This could result in wine with lower astringency (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007) and colour intensity, a lighter body and shorter length, and which are sourer (Joscelyne *et al.*, 2007). These wines have also been found to have lower overall fruit flavour and fruit flavour persistence (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007), and an intensified sensory detection of 'straw' and 'herbaceous' characters (Ristic *et al.*, 2010). Cabernet Sauvignon wines made from shaded fruit were perceived to be less bitter (Joscelyne *et al.*, 2007), although no difference in bitterness was observed in Shiraz (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007). The results found by Morrison & Noble (1990) were in contrast to the results of these studies as they reported no perceived differences in wine aroma or flavour between wines made from shaded and exposed fruit. In a recent study, site was shown to have the most significant influence on sensory scores and wine composition of Cabernet Sauvignon, followed by canopy management (Robinson *et al.*, 2011).

2.10 CONCLUSION

A lot of the data that has been obtained on the impact of the environment and cultural practices on grape and wine phenolic composition is limited to only a few cultivars. It can be concluded that, of the many factors that influence the flavonoid content and composition of a grape cultivar, climate may be the most important. The thresholds of both day and night temperatures also play a role (Hunter & Bonnardot, 2011) and, although it is known that the microclimate of a vineyard could be manipulated by canopy management as well as irrigation, the response of grapevines to these management practices proves to be cultivar dependent. Therefore, the solution lies in determining which cultivars show similar responses so that management strategies can be developed for groups of cultivars (Downey *et al.*, 2006). It is clear that the translation of analytical information from grapes to wine is complex, which highlights the need for a greater understanding of the factors affecting phenolic extractability under varying viticultural conditions. Major research objectives for the future are to develop management strategies for optimising grapevine flavonoid composition for vigorous vineyards and for different styles of red wine, as well as to develop prediction models, using grape parameters to determine certain phenolic qualities in aged red wine. Future work should also include developing these management strategies and prediction models for a variety of viticultural regions in South Africa. Such studies should also assess the development of phenolic compounds during different stages of vinification.

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Chapter 3

Research results

Interactive effects of growth manipulation and water deficit in grapevine (*Vitis vinifera* L.) cv. Shiraz: Impact on grape chemical and phenolic composition

3. Research results

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The aim of this study was to determine how the interaction of bunch exposure and water deficit influences the flavonoid composition of Shiraz grapes at different stages of maturation over two seasons under extremely vigorous conditions. The vines subjected to the reduced canopy treatment were pruned to two buds and suckered to one shoot per bearer at flowering, and the vines given the full canopy treatment were suckered to two shoots per bearer. The irrigation trial was set up according to measurements of predawn leaf water potential (Ψ_{PD}), with targets for the non-stress and stress treatment grapevines of less negative than -400 KPa and less negative than -1700 KPa respectively. Generally, canopy reduction by shoot removal led to an increase in the concentration of most flavonoids, with the effect being more prominent than that of the water deficit treatment in most cases. For some parameters, the effect of water deficit was intensified in the full canopy treatment and resulted in clearer treatment differences in this treatment compared to the reduced canopy treatment. Generally, grape titratable acidity levels were lower in “non-stressed” treatments compared to the water deficit treatments, while berry volume and fresh mass levels were higher in grapes not subjected to water deficit compared to grapes that were subject to water deficit. Harvesting at a ripe stage compared to an unripe stage resulted in grapes that generally were lower in berry volume, fresh mass and titratable acidity levels, with higher values of total soluble solids, pH and polymeric pigments. Harvesting at an overripe stage, however, generally resulted in increased levels of polymeric pigments, total soluble solids and pH, and lower levels of berry volume, fresh mass and monomeric anthocyanins.

3.1 INTRODUCTION

Phenolic compounds are important quality indicators of red wine (Glories, 1988). Anthocyanins, flavan-3-ols, proanthocyanidins, and flavonols form part of a large class of plant secondary metabolites known as flavonoids (Downey *et al.*, 2006). Flavonols, flavan-3-ol monomers and proanthocyanidins are biosynthesised during the first phase of berry growth, whereas anthocyanins are biosynthesised during fruit ripening (Boss *et al.*, 1996; Bogs *et al.*, 2005). The concentration of flavonoids in grapes may be affected by vineyard management and climatic conditions (Matthews *et al.*, 1990; Kennedy *et al.*, 2002; Downey *et al.*, 2006).

In South Africa, where extremely dense canopies are a prevalent problem, sound canopy management and irrigation could control vine vigour and improve fruit ripening and grape quality (Hunter *et al.*, 2004). The effect of fruit exposure has been investigated in a number of previous studies that have involved the shading or defoliation of the vines or the sampling of fruit from different parts of the canopy (Price *et al.*, 1995; Spayd *et al.*, 2002; Downey *et al.*, 2004, 2006; Cortell & Kennedy, 2006; Ristic *et al.*, 2007). Many possible explanations have been suggested for the range of results, including differences in cultivar, site, and season as well as sampling and analytical techniques (Downey *et al.*, 2006). Although anthocyanin content could increase in berries ripened under high light conditions (Kliewer & Antcliff, 1970; Kliewer & Torres, 1972; Morrison & Noble, 1990; Ristic *et al.*, 2010), there is a point at which high temperatures begin to have a negative impact (Downey *et al.*, 2006). High temperatures can limit anthocyanin accumulation under increased light incidence, potentially due to both decreased synthesis and increased degradation within the biosynthetic pathway (Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). A recent study in South Africa showed that colour and flavour levels in the berries would decrease above a maximum night and maximum day temperature range of 20°C and 30°C respectively (Hunter & Bonnardot, 2011).

Grapevine water deficit can affect the concentration of phenolic compounds due to a reduction in berry size, or it can affect the biosynthesis of these compounds (Kennedy *et al.*, 2002; Ojeda *et al.*, 2002). According to the literature, the influence of water deficit on the concentration of phenolics in grape berries and the resulting wine has produced variable results (Koundouras *et al.*, 2006; Bindon *et al.*, 2008, 2011; Chalmers *et al.*, 2010). This is mainly because of different irrigation dosages leading to various levels of water stress. Furthermore, grape response to moderate irrigation might also be cultivar-dependent as *Vitis Vinifera* varieties have been shown to respond differently to water stress (Schultz, 1996).

It is known that a lower extractability of phenolics, particularly anthocyanins and seed tannins, could occur in grapes produced under conditions of water deficit (Sivilotti *et al.*, 2005), and that altered anthocyanin composition in shaded fruit may influence its extractability and stability during winemaking (Ristic *et al.*, 2007). Cortell and Kennedy (2006) reported that the extractability of anthocyanins, skin tannins and flavonols could be decreased in shaded Pinot noir fruit and suggested that other factors, such as skin thickness, cell size and cell wall properties, could influence the extraction of flavonoids from the fruit during winemaking (Ristic *et al.*, 2007). The cell maturity index (%EA) represents the ease of anthocyanin extraction and can be measured using a method proposed by Glories (Ribéreau-Gayon *et al.*, 2000).

The aim of the current study was to evaluate the interactive effect of a reduction in canopy density after flowering (by shoot removal) and water deficits on the phenolic composition and extractability of *Vitis vinifera* cv. Shiraz fruit for two consecutive seasons under South African conditions in the Stellenbosch region. As grape flavonoid composition is influenced to a great extent by the degree of ripeness of the grapes, part of this study focussed on how the colour and

phenolic composition of red grapes is influenced by different stages of ripening. Harvesting at different ratios of total soluble solids to titratable acidity were found to each represent a different style of Shiraz (Hunter *et al.*, 2007). Therefore, these ratios were used as a guideline in determining specific harvest stages. Note that this study forms part of an integrated viticultural and oenological study on Shiraz, and that this thesis deals with the oenological investigations specifically. The results presented in Chapter 3 will also be published under joint first authorship with the principal viticultural investigator in a separate publication.

3.2 MATERIALS AND METHODS

3.2.1 Vineyard

Experiments were conducted in a *Vitis vinifera* L. cv. Shiraz (clone SH9C) vineyard grafted onto 101-14 Mgt (*Vitis riparia* x *Vitis rupestris*) rootstock. The vineyard was established in 2000 with a north-south row direction on a flat terrain at the Welgevallen Experimental Farm of the Department of Viticulture and Oenology, Stellenbosch University, South Africa (33°56'S, 18°52'E, 157 m mean height above sea level). The Stellenbosch winegrowing region is characterised by a Mediterranean climate. The vines are spaced at 2.7 x 1.5 m and the trellising consisted of a seven-wire hedge trellis system (vertically shoot positioned) with three sets of moveable canopy wires. Irrigation was applied using a pressure compensated drip system spaced at 40 cm, at a rate of 2.3 L/h. Spur pruning was applied. In this chapter, the 2010 season refers to the 2009/10 growing season and the 2011 season refers to the 2010/11 growing season.

3.2.2 Experimental layout

The experiment was designed according to a split-plot design incorporating six main plots with an irrigation treatment assigned to each, namely non-stressed (NS) and stressed (S), with two sub-plots of 12 grapevines each subjected to a different canopy manipulation treatment, namely full canopy (F) or reduced canopy (R) (Figures 1 and 2). For the 2011 season the trial layout was modified to represent the same main plots, but sub-plots were modified to 18 sub-plots of three grapevines each for the canopy manipulation treatments. The irrigation trial was set up according to measurements of predawn leaf water potential (Ψ_{PD}), with targets for NS and S grapevines of less negative than -400 KPa and less negative than -1700 KPa respectively. For the canopy reduction treatment, shoot removal was performed at 55 to 60 days after budburst (DAB) in the 2010 and 2011 seasons by removing the apical shoot on a two-bud spur, followed by suckering to a single shoot per bearer (Figure 3). In the 2011 season, secondary shoots were removed continuously from the lower 25 to 30 cm of the reduced canopy treatment (bunch zone) to study the effects on fruit composition when the compensatory secondary shoot growth was not present.

3.2.3 Climate measurements

Temperature data was obtained from a weather station approximately 1 500 m from the vineyard (Heritage Garden, Infruitec, Stellenbosch, Lat -33.92714; Long 18.87226, Alt 112 m, courtesy of the Agro-Climatology Division of the Institute of Soil Climate and Water of the Agricultural Research Council: Agro-Climatology, ARC – ISCW, Pretoria, ZA). Budburst was defined as the stage when 50% of the shoots were 2 cm long, their first leaves had unfolded and the leaves had reached a length of approximately 20 mm. This was also used to calculate DAB where applicable.

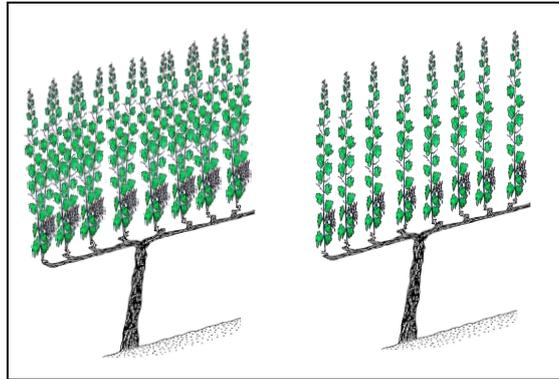


Figure 3 Illustration of the canopy reduction treatment imposed (secondary shoots not shown). Left: full canopy, right: reduced canopy suckered to a single shoot per spur position (illustration courtesy AE Strever, 2012).

3.2.4 Predawn leaf water potential measurements

For all the treatments, predawn leaf water potential (Ψ_{PD}) was determined using a pressure chamber on fully expanded leaves on primary shoots, according to Scholander *et al.* (1965).

3.2.5 Berry sampling and harvest

Berry chemical composition and development, as quantified by total soluble solids (TSS), berry mass, volume, pH and titratable acidity (TA), was assessed twice weekly, starting approximately at véraison. In 2010, grapes were harvested for winemaking at an average TSS value of 22.2°B (TSS/ TA 3.7), 26.0°B (TSS/ TA 5.7) and 28.9°B (TSS/ TA 6.4), which presented an unripe, ripe and overripe stage respectively. Grapes from three field repeats were kept separate. In 2011, grapes were harvested at an average TSS value of 21.8°B (TSS/ TA 4.6), 22.8°B (TSS/ TA 5.4) and 26.2°B (TSS/ TA 6.4), which presented an unripe, medium ripe and ripe stage respectively. In this study it was attempted to harvest grapes within a window of 1°B at each harvest stage, as these differences may affect the composition and characteristics of the grapes. Therefore, due to slower ripening observed in the full canopy treatments, these grapes were harvested slightly later. Table 1 shows how many days after budburst the harvesting of the different treatments took place as well as the TSS and TSS/ TA ratios aimed for at each harvest stage. In 2011, these targets were more difficult to reach due to logistical issues, resulting in both the full canopy treatments having higher TSS values at the unripe stage. The NSF treatment was also harvested at a higher TSS level for the medium ripe stage. Grapes were harvested at an overripe stage in 2010 to

examine the effects of extreme conditions on the phenolic composition of the grapes. After they had been picked, all the bunches were placed in crates and weighed to determine the yield per vine (kg). The number of bunches was also determined for each vine. For berry analyses, two bunches were collected randomly from each vine and placed in a separate crate. In 2010, berries were stripped from the bunches and mixed, and 3 x 200 berry samples were collected for berry mass/volume and ripeness parameter analysis and 3 x 200 berry samples for phenolic analysis. To improve the homogeneity of the different samples in 2011, the berries were sorted according to density (i.e., total soluble solids) and size classes. Density was estimated by flotation of berries in different sugar solutions and 6 x 200 berry samples with near-identical composition were compiled (Ojeda *et al.*, 2001).

3.2.6 Determination of grape chemical composition

Recently collected berry samples were lightly pressed by hand in a small plastic bag and the clarified juice was used to determine juice total soluble solids (TSS as °B) using an Atago PAL-1 pocket refractometer (Tokyo, Japan). Titratable acidity and pH were measured using an automatic titrator (Metrohm, 702 SM Titrino, Herisau, Switzerland) with 0.333 N NaOH. Fresh berry mass was determined by weighing 100 berries and the volume of 100 berries was determined by water displacement.

3.2.7 Determination of grape phenolic composition

3.2.7.1 Spectrophotometric analysis

Prior to phenolic analyses, fresh grape samples were weighed and then homogenised for four minutes using an Ultra-Turrax T 18b (IKA Labortechnik, Staufen, Germany) homogeniser. Fresh grape homogenate was used for the Glories method, while the rest was frozen immediately and stored at -20°C until the HPLC analysis was done.

The grape phenolic potential was determined according to the method described by Glories (Ribéreau-Gayon *et al.*, 2000), macerating fresh grape homogenate for four hours at pH 3.2 and 1.0. This method works on the assumption that, at pH 1.0, complete disruption of the vacuolar membrane takes place, facilitating the release of phenolic compounds. The pH of the macerating solution at 3.2 represents a similar cell degradation situation that occurs during maceration in winemaking (Glories & Saucier, 2000). In this study, the original pH 3.2 solution was exchanged for one of pH 3.6, which is better suited to the higher pH musts from the Stellenbosch region (WJ du Toit, personal communication, 2010). The absorbance of the pH 1.0 solutions at 520 nm on a Analytikjena Specord 40 UV/VIS spectrophotometer (Jena, Germany), using a method based on bleaching with sulphur dioxide (Ribéreau-Gayon & Stonestreet, 1965; Ribéreau-Gayon *et al.*, 1998), gives an indication of the maximum anthocyanin concentration of the grapes. This method measures mainly anthocyanins, but a small percentage of polymeric pigments may also be included. The total phenol content (TP%) was calculated by measuring the optical density of the

solution at pH 3.6 at 280 nm. The percentage of extractable anthocyanins was calculated as follows, where “Anth” represents the extractable anthocyanin concentration obtained in the specific buffer solution:

$$\text{Percentage extractable anthocyanins } \%EA = 1 - \frac{\text{Anth}_{pH1} - \text{Anth}_{pH 3.6}}{\text{Anth}_{pH 1.0}} \times 100$$

The ease of extractability is considered to increase when the difference between these two results is small and the percentage extractable anthocyanin therefore is high. The Adams-Harbertson Tannin Assay, a method that is based on the ability of tannin to complex and precipitate with protein (bovine serum albumin), was used to determine tannin concentration (Harbertson *et al.*, 2002). A homogenate sample of one gram was extracted in 10 mL of 50% ethanol:water for one hour, centrifuged for 5 min at 3500 rpm, and the supernatant was retained for analyses.

3.2.7.2 HPLC analysis

The grape homogenate was defrosted and one gram was extracted in 10 mL of 50% ethanol:water (adjusted to pH 2.0 with HCl) for one hour, according to the method of Iland *et al.* (2000). The samples were then centrifuged for 5 min at 3 500 rpm and the supernatant was retained for reverse-phase high-performance liquid chromatography (RP-HPLC) analysis performed on a Hewlett Packard Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data acquisition and processing were performed with ChemStation (Revision B.04.02 SP1) software (Hewlett Packard, Waldbronn, Germany). The analysis method was adapted from Peng *et al.* (2002). Separations were carried out on a polystyrene/divinylbenzene reverse-phase chromatographic column (PLRP-S, 100Å, 250 × 4.6 mm, 5 µm) protected with a guard cartridge (PLRP-S, 10 × 4.6 mm) with the same packing material (both Polymer Laboratories (Ltd), Shropshire, UK). The following mobile phases were used: mobile phase A: 1.5% v/v orthophosphoric acid (Reidel-de Haën) in de-ionised water, and mobile phase B: acetonitrile (Chromasolve, Reidel-de Haën). A linear gradient was used from 0 min, A 95%, B 5%; to 73 min, A 75.2%, B 24.8%; to 78 min, A 50%, B 50%, remaining constant for 8 min. Flow rate was 1 mL.min⁻¹ and the column temperature was kept constant at 35°C. The following standards were used: (+)-catechin hydrate (Fluka), (-)-epicatechin (Sigma), gallic acid (Fluka), caffeic acid (Sigma), *p*-coumaric acid (Sigma), malvidin-3-glucoside (Polyphenols Laboratories AS, Norway), quercetin-3-glucoside (Fluka) and quercetin (Extrasynthèse, France). The following wavelengths were used: monomeric and dimeric flavan-3-ols and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 1.5 mg/L, and epicatechin was quantified as epicatechin with a quantification limit of 1.5 mg/L. The quantification limit for gallic acid was 0.25 mg/L, also quantified at 280nm. Three hundred and twenty nm was used for the determination of cinnamic acids. Caftaric acid and caffeic acid were quantified as mg/L caffeic acid, while coumaric acid and *p*-coumaric acid were expressed as mg/L *p*-coumaric units.

Flavonol-glycosides and flavonol aglycones were quantified at 360 nm as mg/L quercetin-3-glucoside and mg/L quercetin respectively. Monomeric anthocyanins and polymeric pigments were quantified at 520 nm as mg/L malvidin-3-glucoside, with a quantification limit of 1.25 mg/L. The samples were defrosted and filtered through a 0.45 µm filter (Millipore) before injection. The limit of quantification was defined as a signal-to-noise ratio of 1/10, which represented the smallest area that could be integrated accurately (< 3% standard deviation).

3.2.8 Statistic and chemometric analysis

All analyses were done using Statistica 10. Mixed model repeated measures ANOVAs were used and Fisher's least significant difference (LSD) corrections were used for posthoc analyses. Significant differences were judged on a 5% significance level ($p < 0.05$).

3.3 RESULTS AND DISCUSSION

3.3.1 Climatic data

Marked differences in climatic data were found between the two growing seasons. The date of budburst for this vineyard differed between seasons, with the later budburst being recorded in the 2010/2011 growing season. A total of 91 mm of rainfall was recorded a month before budburst in the 2009/2010 growing season compared to only 23 mm in the 2010/2011 growing season. This most likely caused a lower soil temperature, which may have delayed budburst as well as initial shoot growth. Cool and rainy conditions prevailed until quite late during the 2009/2010 growing season, which is evident from the daily and accumulated thermal time results (data not shown). In general, temperatures were lower during most of the 2009/2010 growing season, whereas lower temperatures were recorded earlier (before 80 DAB) in the 2010/2011 growing season, with the latter part of the season showing the highest mean temperature in both seasons.

3.3.2 Leaf water potential (predawn)

Figures 4 and 5 (AE Strever, unpublished results) show results for the Ψ_{PD} measurements throughout the 2010 and 2011 seasons.

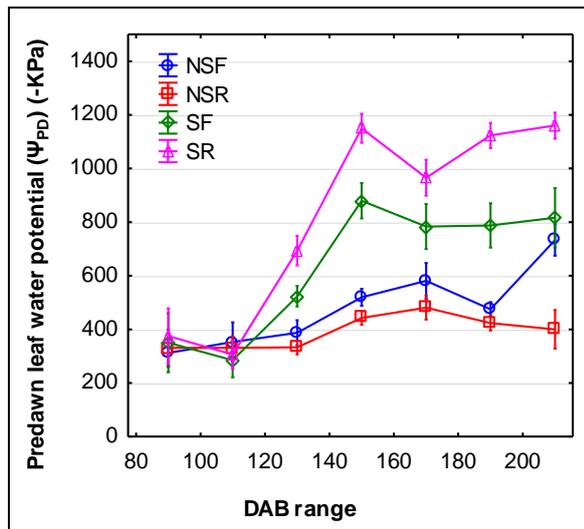


Figure 4 Predawn leaf water potentials (Ψ_{PD}) relative to date categories (20-day intervals) for the different treatments in the 2009/2010 growing season (means with +/- standard errors shown) (AE Strever, unpublished results).

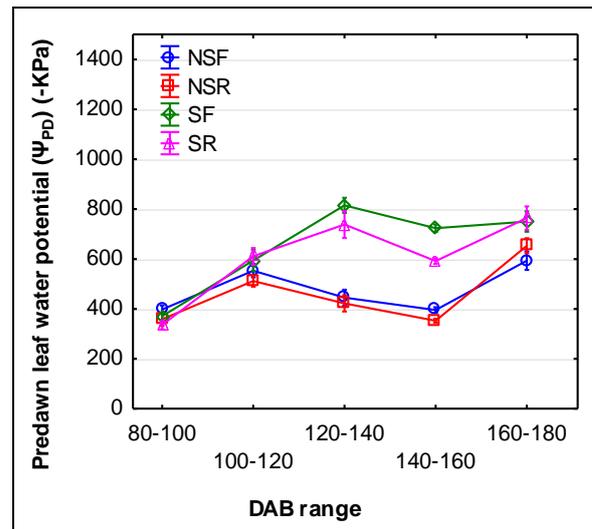


Figure 5 Predawn leaf water potentials (Ψ_{PD}) relative to date categories (20-day intervals) for the different treatments in the 2010/2011 growing season (means with +/- standard errors shown) (AE Strever, unpublished results).

The “stressed” treatments generally seemed to have more negative Ψ_{PD} values than the NS treatments in both seasons. The reduced canopies of the S treatments seemed to show more negative Ψ_{PD} values than the full canopies in the 2009/2010 but not in 2010/2011 growing season. The reduced canopies in the S treatment compensated via secondary shoot growth in the 2009/2010 season, leading to a potentially much larger canopy surface for transpiration and consequently higher water deficits in this season. In the 2010/2011 growing season, secondary shoots were removed, but also grew less due to the drier conditions, therefore not producing the same results as in the previous season. Although the 2009/2010 growing season was cooler and wetter than the 2010/2011 growing season, the highest Ψ_{PD} values in this season were registered for the S treatment. A higher crop load in terms of the yield:pruning mass ratio (data not shown) in the 2009/2010 growing season compared to the 2010/2011 growing season may have played a role here, as well as larger canopies in general (AE Strever, personal communication).

3.3.3 Grape chemical composition at harvest

Grape chemical and phenolic composition was assessed at three stages in the 2010 and 2011 seasons. Table 2 shows all the parameters at which a second-order interaction occurred between the harvest stages and treatments. Table 3 shows all the parameters at which a first-order interaction occurred between the harvest stages, and Table 4 shows all the parameters at which a first-order interaction occurred between the treatments.

The grapes from the full canopy treatment (SF and NSF) were harvested a few days later than those from the reduced canopy treatment (NSR and SR), as a delay in ripening was observed in the full canopy treatments (data not shown). This is in agreement with previous results (Spayd *et*

al., 2002; Chorti *et al.*, 2010) and could be the result of reduced photosynthesis in the shaded canopies slowing down ripening (Rojas-Lara & Morrison, 1989).

3.3.3.1 Accumulation of total soluble solids in berries

The TSS values for all treatments were significantly higher at the ripe stage compared to the unripe stage in 2010 and 2011 (Tables 2 and 3). As explained earlier, it turned out that the NSF and SF treatment grapes from the 2011 unripe stage had between 1 and 2 °B higher TSS concentrations than the NSR and SR treatment grapes respectively (Table 2). At the medium ripe stage, the grapes from the NSF treatment had higher concentrations than those from the other treatments, and at the ripe stage the NSR treatment had higher concentrations than the SF treatment (Table 2). The values of the treatments from the third harvest (ripe stage) of 2011, except for those of the NSR treatment, corresponded to the second harvest (ripe stage) of 2010 in terms of TSS, which must be taken into account when interpreting the results. In terms of ripeness, the full canopy grapes from the 2011 unripe harvest could also be compared to the reduced canopy grapes from the medium ripe stage of 2011 if their similar TSS concentrations are considered.

3.3.3.2 Berry growth

It seems as if the grapes from the NSF and NSR treatments had an overall higher volume than the stressed grapes in both seasons, although these differences were not significant in all cases (Table 2). In 2010 this difference was greater in the grapes from the full canopy treatment. Treatment differences occurred in grapes from the 2010 unripe and overripe stage, but not in the ripe grapes of 2010. Berry volume also decreased from the unripe to the ripe stage in all treatments from both seasons, although not significantly in the grapes from the SF and NSF treatments in 2010 and 2011 respectively (Table 2). Berry volume also decreased after the ripe stage in 2010, but not significantly in the NSF treatment. Treatment differences were observed for the medium ripe grapes in 2011, but not for the unripe and ripe grapes. The significantly higher TSS concentrations in the SF and NSF treatment grapes from the 2011 unripe stage, compared to the SR and NSR treatments respectively, could have masked the differences between the SR and SF as well as the SF and NSF treatments. When the full canopy grapes from the 2011 unripe harvest were compared to the reduced canopy grapes from the medium ripe harvest (due to similar TSS concentrations), the SR treatment grapes from the medium ripe stage had the lowest values. This could be attributed to a sharper decrease in values after the unripe stage in this treatment due to faster dehydration as a result of higher temperatures.

In 2010, berry fresh weight decreased significantly from the unripe to the ripe stage in all the treatments, and the overripe grapes had lower berry weight values than the ripe grapes (Table 3). In 2011, berry fresh weight also decreased significantly from the unripe to the ripe stage, except in the case of the NSF treatment (Table 2). Again, delayed ripening of the full canopy treatment at the unripe stage could have masked differences. When all three harvest stages in 2010 were taken

into consideration, the grapes from the NSF treatment had higher fresh berry weight values than the SF grapes, while there was no significant difference between the grapes from the NSR and SR treatments (Table 4). This seemingly reflects the same trend observed in the volume analysis. Significantly higher weights in the NSF treatment grapes compared to the SF treatment grapes in 2010 correspond to the results of a previous study, which found that berry weight was significantly lower in grapes from grapevines subjected to water stress (Chalmers *et al.*, 2010). The NSR treatment grapes from the 2011 unripe stage had significantly higher berry mass values than the SF treatment, which could have been due to the fact that the SF grapes had a higher TSS value than the grapes from the SR and NSR treatments, and therefore already had a lower mass. At the 2011 ripe stage it seemed as if the grapes from the NSF treatment had higher values than those from the SF treatment (as in 2010), but this difference was not significant. No significant treatment differences occurred between the full canopy grapes from the 2011 unripe stage and the reduced canopy grapes from the medium ripe stage.

3.3.3.3 Titratable acidity and pH

If all three harvest stages in 2010 are considered, the grapes from the SR and SF treatments had significantly higher TA values than those from the NSR and NSF treatments respectively (Table 4), and the NSR treatment had higher values than the NSF treatment, while there was no significant difference between the SR and SF treatments. The higher TA concentrations in the grapes from the NSR compared to those from the NSF treatment could have been due to the increased availability of potassium in the full canopy grapes and the possible decreased acidity due to tartaric acid salt formation (potassium bitartrate) (Smart & Coombe, 1983; Jackson & Lombard, 1993). The lower TA values in the non-stressed treatments compared to the stressed treatments are probably due to larger berries in the NS treatments, which could have given rise to a reduction in acid concentration due to dilution (Mullins *et al.*, 1992; Yuste *et al.*, 2004). The TA decreased significantly from the unripe to the ripe stage in all the treatments, and this was confirmed in 2011 (Tables 2 and 3). As in 2010, grapes from the SR treatment in 2011 had significantly higher TA concentrations than the grapes from the NSR treatment at all three stages of harvest. No significant difference was found between the NSF and SF treatments in 2011, and the grapes from the NSR treatment only had higher concentrations of TA than those from the NSF treatment at the unripe and medium ripe stages (Table 2). The SR treatment also had significantly higher values than the SF treatment at all three harvest stages. At the unripe stage, this could be attributed to the grapes from the SF treatment being riper at this stage and therefore already having lower values. According to the results obtained during ripening, the water-stressed treatments always had higher values than the non-stress treatments, with the SF treatment having the highest values in 2011 (data not shown). Delayed harvest of the full canopy grapes therefore seemed to have a big effect on the TA values of the grapes at harvest.

In 2010, grape pH increased significantly from the unripe to the ripe stage for all treatments, with the NSF treatment having significantly higher values than the NSR and SR treatment at the unripe stage (Table 2), which is consistent with a lower TA value in the grapes from the NSF treatment. Elevated pH is expected in luxuriously irrigated grapevines, due possibly to the increased availability of potassium and the decreased acidity as a result of tartaric acid salt formation (potassium bitartrate) in the must (Smart & Coombe, 1983; Jackson & Lombard, 1993). In 2010, the grapes from the ripe stage of the SF treatment had significantly higher values than those from the NSR treatment. Joscelyne *et al.* (2007) and Ristic *et al.* (2007) also reported that bunch exposure influenced pH values in grapes, suggesting higher concentrations of potassium ions in juice from the shaded berries (Ristic *et al.*, 2007). Higher pH values in the full canopy grapes could also be attributed to the fact that these grapes were harvested a few days later than those from the reduced canopy treatments, and therefore could have had higher values due to more advanced ripening. In 2010, pH values from the overripe stage in the reduced canopy grapes were significantly higher than those from the ripe stage, while the values in the full canopy grapes did not differ significantly between these two stages. No significant treatment effects were apparent from the pH values measured for the unripe and ripe stages in 2011 (Table 2). Again, a delay in the harvest of the full canopy grapes could have masked differences observed between the reduced and full canopy grapes during ripening (data not shown). In 2011, only grapes from the SR treatment showed a significant increase from the unripe to the ripe stage.

3.3.3.4 Ratio of total soluble solids to titratable acidity

No treatment differences in TSS/TA ratio occurred in the 2010 unripe grapes (Table 2), but full canopy grapes from the ripe stage had significantly higher values than the reduced canopy grapes. Values increased significantly from the unripe to the ripe stage and from the ripe to the overripe stage, except in the full canopy grapes. In 2011, values increased significantly from the unripe to the ripe stage when all treatments were taken into consideration (Table 3). In 2011, the grapes from the NSF treatment had the highest value, followed by those from the NSR and SF treatments, with SR having the lowest value (Table 4). This is not in accordance with results obtained during ripening, when the grapes from the NSR and SR treatments had higher TSS/TA ratio values than those from the NSF and SF treatments respectively (data not shown). Therefore, the delayed harvest of the full canopy grapes at each harvest stage greatly affected the treatment differences observed at each stage. Differences between the stressed and non-stressed treatments could have been due to seemingly lower TA values in the non-stressed grapes than in the stressed grapes, although these differences were not significant in all cases.

3.3.4 Determination of grape colour and phenolic composition

3.3.4.1 Monomeric anthocyanins (HPLC analysis)

The results of the HPLC monomeric anthocyanin analyses are shown in Tables 2-4. Generally the values seemed to be slightly lower in 2011 than in 2010, which could be attributed to the 2011 season being drier and warmer, indicating that temperatures could have exceeded the optimum value for anthocyanin accumulation in that season. There was a significant second-order interaction between the harvest stages and treatments in 2010 (Table 2). The values decreased significantly from the unripe to the ripe stage only in the reduced canopy treatments; after the ripe stage it decreased significantly in all except the NSR treatment. The NSR treatment significantly had higher monomeric anthocyanin concentrations than the NSF treatment at the unripe stage. No significant treatment differences were evident at the ripe stage in 2010, which could be attributed to values decreasing in the reduced canopy but not in the full canopy treatments after the unripe stage. This could be due to more extreme stress conditions being experienced by the reduced canopy treatment, possibly because of higher temperatures that had a negative effect on anthocyanin accumulation in these grapes (Bergqvist *et al.*, 2001).

In 2011, treatment differences were clearer (Table 4), which could have been due to the continual secondary shoot removal in the bunch zone, which was done in 2011 but not in 2010. This treatment intensified the light/temperature effect on anthocyanin accumulation in 2011. The SR treatment had significantly higher values than the SF, NSR and NSF treatments when all three harvest stages were taken into consideration. These results are consistent with the trends observed for malvidin-3-glucoside, the most abundant anthocyanin found in grapes. Values at the unripe and ripe stages, however, were not as significantly different (Table 3) as those observed for malvidin-3-glucoside, which could be due to the counteracting effect of the other anthocyanin derivatives in which concentrations were higher than or did not differ between these two stages (results not shown). Previous studies also reported a higher total anthocyanin concentration in grapes from reduced canopy treatments (Spayd *et al.*, 2002; Tarara *et al.*, 2008; Chorti *et al.*, 2010) and a decrease in total anthocyanin concentration just before harvest (Gonzalez-San Jose *et al.*, 1990; Ryan & Revilla, 2003) and/or during over-ripening (Roggero *et al.*, 1986; Fournand *et al.*, 2006). This could be due to the oxidative degradation of anthocyanins in the grapes (Ribéreau-Gayon *et al.*, 2000), indicating that the anthocyanin concentration could have reached its peak before the first harvest. Monomeric anthocyanin concentrations decreased significantly from the ripe stage (2010), except in the NSR treatment. Higher contents in the grapes from the SR treatment of the 2011 season correspond to previous studies reporting that anthocyanin content (mg/berry) was higher in grapes subjected to water deficit compared to a control treatment (Castellarin *et al.*, 2007a). In other studies in which anthocyanin concentration was analysed only in the berry skins, a higher concentration (mg per g fresh weight) was also reported in grapes subjected to water deficit (Ojeda *et al.*, 2002; Castellarin *et al.*, 2007a, 2007b). The activation of

anthocyanin pathway genes in grapes subjected to water deficit could be attributed primarily to increased solar radiation in the bunch zone due to reduced leaf turgor and leaf senescence, as well as increases in abscisic acid (ABA) (Castellarin *et al.*, 2007a, 2007b).

3.3.4.2 Anthocyanins and polymeric pigments (spectrophotometric analysis)

The results of the spectrophotometric anthocyanin analyses are shown in Tables 2 and 3.

The results from the Glories method show that there was a significant second-order interaction between the treatments and different harvest stages with respect to total anthocyanin concentration (Anth_{pH1}) in 2011 (when expressed as mg/L, mg/g fresh berry weight and mg/berry) and 2010 (only when expressed as mg/berry) (Table 2). Only a first-order interaction existed between harvest stages in 2010 when expressed as mg/L and mg/g fresh berry weight (Table 3).

No significant treatment differences in Anth_{pH1} values were observed at the ripe stage in 2010 when expressed as mg/L or mg/g fresh berry weight (Table 3), consistent with the monomeric anthocyanin results. Treatment differences in Anth_{pH1} values seemed to have been clearer in 2011, especially between the grapes from the reduced and full canopy treatments (Table 2). In 2011, the SR treatment had significantly higher concentrations than the other treatments at the unripe stage, which is consistent with the monomeric anthocyanin results. Concentrations decreased significantly from the ripe stage (2010) (Tables 2 and 3).

For the anthocyanin concentration retained in the pH3.6 buffer, which represents a similar cell degradation situation to that occurring during maceration in winemaking, there was a second-order interaction between treatment and harvest in both seasons (Table 2). In general, $\text{Anth}_{\text{pH3.6}}$ values seemed to have been slightly lower in 2011 than in 2010. As with the Anth_{pH1} results, it seems as if treatment differences in the $\text{Anth}_{\text{pH3.6}}$ values were clearer in 2011. Concentrations decreased significantly from the ripe stage (2010), except for the NSF treatment, in which the concentrations remained constant (when expressed in mg/L or mg/g fresh berry weight). Generally, this study confirms that increased fruit exposure to sunlight increases the concentrations of anthocyanins in the grapes (Smart *et al.*, 1985; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Joscelyne *et al.*, 2007). This could be due to the fact that reduced canopy grapes were probably more exposed during the green and lag stages of berry growth, which could have increased the initial concentration or activity of one or several of the anthocyanin biosynthetic enzymes (Takeda *et al.*, 1988). It is also possible that the maximum activity of these enzymes was maintained during ripening in 2011 (Dokoozlian & Kliewer, 1996) due to continual lateral shoot removal in the bunch zone.

No significant treatment differences in polymeric pigment concentrations occurred at the unripe and ripe stages in 2010 (Table 2). Concentrations increased significantly from the ripe stage for all treatments except the NSF treatment. In both seasons, polymeric pigment concentrations increased significantly from the unripe to the ripe harvest, and concentrations were higher in the grapes from the SF treatment than those from the SR treatment at all three harvests in 2011 (Table 2). The grapes from the unripe and medium ripe stage in the NSF treatment had significantly

higher concentrations than those from the NSR treatment (2011). The higher polymeric concentrations in the full canopy grapes in 2011 are not in accordance with previous studies, which reported higher concentrations in exposed fruit (Price *et al.*, 1995; Joscelyne *et al.*, 2007). Our findings could be attributed to the delayed ripening of the grapes from the full canopy treatment and polymerisation reactions that could have occurred to a larger extent in these treatments than in the reduced canopy treatments at the time of harvest. This delay was also longer in 2011 compared to 2010 because of the intensification of the treatments in 2011.

3.3.4.3 Percentage extractable anthocyanins (%EA)

The results of the Glories method are shown in Tables 3 and 4. In both seasons, the SF treatment had significantly higher %EA values than the NSF treatment, while there were no other consistent treatment differences (Table 4). These results are not in accordance with a previous study, which reported a lower extractability of phenolics, particularly anthocyanins and seed tannins, in a weak solvent (such as tartaric buffer, prepared with 5 g L⁻¹ of tartaric acid buffered at pH 3.2 with 1N NaOH) from grapes produced under conditions of water deficit (Sivilotti *et al.*, 2005). This study, however, reported increased anthocyanin concentration and extractability of these grapes in a 12% EtOH solution. The reason why there were no significant differences between the NSR and SR treatments could be due to greater areas for transpiration in the full canopy treatments, which intensifies the water deficit effect.

Although there was no interaction between harvest stages in 2011, the percentage extractable anthocyanins increased significantly from the unripe to ripe and overripe stage in 2010 (Table 3). An increase in the percentage of extractable anthocyanins over time could be due to the degradation of the cellular walls by pectolytic enzymes (Ribéreau-Gayon *et al.*, 2000), corresponding with the findings in the literature, which show that the extractability of anthocyanins increases as maturation progresses (Saint-Cricq *et al.*, 1998; Glories, 1999; Ribéreau-Gayon *et al.*, 2000, 2003). Differences in cell wall polysaccharide composition, together with the cellulose content and degree of methylation of the pectins, could also be responsible for the differences in extractability (Ortega-Regules *et al.*, 2006). The %EA did not seem to reflect trends in anthocyanin concentration (monomeric anthocyanins, A_{pH1} and $A_{pH3.6}$) observed between treatments. This could be due to the fact that the grapes were harvested at a %EA value of more than 60%, which is considered the minimum value necessary to obtain good extraction. This probably led to concentration being a more important factor than %EA in determining anthocyanin concentration. The increase in %EA between the unripe and overripe stages indicates an increase in the extractable fraction of anthocyanins. If $[Anth_{pH1}]$ decreased in the same proportion as $[Anth_{pH3.6}]$, the %EA would have decreased. Therefore, it can be concluded that the decrease in the $Anth_{pH1}$ value was greater than that of $Anth_{pH3.6}$, thus increasing the percentage of extractable anthocyanins.

3.3.4.4 Tannins

The results of the Adams-Harbertson Tannin Assay are shown in Table 2. Values for tannin concentration did not seem to differ much between the two seasons. Also, water deficit did not seem to have influenced the values, except at the overripe stage, when the SF treatment had significantly higher values than the NSF treatment. The SF treatment in 2010 experienced much more stress in comparison with the 2011 season, and a higher degree of leaf senescence could have resulted in more exposed conditions. In 2011, a significant increase in tannin concentration was observed from the unripe to the ripe stage only in the reduced canopy grapes, but in 2010 it was observed in all treatments, although not significantly in the case of NSF. This is in accordance with previous studies that found an increase in tannin concentration with maturation (Fournand *et al.*, 2006). Concentrations in the full canopy grapes, which did not increase significantly in all cases, are in accordance with studies indicating a stabilisation in tannin concentration at maturity (Kennedy *et al.*, 2000, 2001; Ojeda *et al.*, 2002). Ripe (2010 and 2011) and overripe (2010) NSR grapes had a significantly higher tannin concentration than NSF grapes, with no consistent significant difference between SR and SF. Higher tannin concentrations in NSR than NSF grapes could have been due to increased exposure in the reduced canopy treatment, as the berry mass and volume did not differ between the NSR and NSF treatments. Previous studies (Downey *et al.*, 2004; Cortell & Kennedy, 2006) also reported that tannin concentration was higher in exposed fruit compared to shaded fruit. This could be due to the fact that shading reduces the transcription of some structural genes in the biosynthetic pathways of several phenolics (Jeong *et al.*, 2004; Koyama & Goto-Yamamoto, 2008), thereby decreasing total phenols in the fruit (Morrison & Noble, 1990; Price *et al.*, 1995). The unripe grapes of the NSR treatment had significantly higher concentrations than the grapes of the SF treatment in 2010, but no treatment differences occurred in the unripe grapes in the 2011 season. This could be due to the higher concentration of TSS in the grapes from the SF treatment than those from the NSR treatment at the unripe stage in 2011, therefore masking the difference between treatments. After the ripe stage a significant increase in tannin concentration was only observed in the SF grapes.

3.3.4.5 Total monomeric flavan-3-ols and polymeric phenols

There was no interaction between treatments in 2010, but the grapes from the reduced canopy treatment had significantly higher monomeric flavan-3-ol concentrations than those from the full canopy treatment at the ripe stage of 2011, and significantly higher values compared to the unripe stage (Table 2). Lower total phenolics in fruit from the shaded treatment could be due to down-regulated flavonoid biosynthesis under decreased light conditions. In general, values were much lower in 2011 than in 2010 (Tables 2 and 3). Total monomeric flavan-3-ol concentrations decreased significantly from the unripe to the ripe and overripe stages in all treatments in 2010 (Table 3).

In 2010, the polymeric phenol concentrations increased significantly from the unripe to the ripe and overripe stages for all treatments (Table 2), whereas in 2011 the concentration of the reduced canopy grapes decreased after the unripe stage, but increased again after the medium ripe stage. The full canopy grapes from these two harvests in 2011 did not differ significantly in polymeric phenol concentration. Once again, this could be due to 2011 being drier and hotter than 2010. Temperatures could have exceeded the optimum value for polymerisation reactions in 2011. No treatment differences occurred at the ripe stages of both seasons and the unripe stage of 2010. For the unripe stage in 2011, however, the NSR treatments had higher concentrations than the NSF treatments, with no differences between SF and SR.

3.3.4.6 Total phenolic index (TP%)

The TP% values seemed to be slightly lower in 2011 compared to 2010 (Table 2). In 2011 the SR treatment had significantly higher values than the other treatments at the unripe stage, and in both seasons the NSR treatment significantly had higher concentrations than the NSF treatment at the ripe stage.

3.3.4.7 Flavonols

The total flavonol concentrations of the fruit harvested for winemaking, as measured by HPLC, are shown in Tables 3 and 4. No treatment interaction existed in 2010, but in 2011 the total flavonol concentrations were significantly higher in the reduced canopy treatment than in the full canopy treatment (Table 4), which could be due to increased expression of the gene encoding flavonol synthase in these fruit. This finding is in accordance with previous studies (Price *et al.*, 1995; Ristic *et al.*, 2007). In 2010, concentrations increased significantly from the unripe to the ripe stage, after which they remained relatively constant (Table 3). In 2011 there was no interaction between the various harvests and water deficit had no significant effect on total flavonol concentrations in both seasons.

3.4 CONCLUSIONS

This study has improved our understanding of how canopy management and irrigation could influence the phenolic composition of the grapes in very dense Shiraz canopies at different stages of ripening. Generally, canopy reduction by shoot removal led to an increase in the concentration of most flavonoids, with the effect being more pronounced than that of the water deficit treatment in most cases. The 2011 season was also drier and warmer than the 2010 season, which could have resulted in the generally lower values of certain phenolic parameters in 2011. Harvesting at different stages of maturation also affected the chemical and phenolic composition of the grapes. Future studies could investigate the effects of these treatments, applied at different stages during the growing season, on the phenolic composition of the grapes. These effects could also be

investigated in different wine regions and terroirs, and on different cultivars, row directions and trellising systems.

3.5 REFERENCES

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Table 1 Days after budburst (DAB) at which each harvest took place in the 2010 and 2011 seasons. For the 2010 and 2011 seasons, budburst occurred on 2009/09/04 and 2010/09/15 respectively. The total soluble solids and total soluble solids to titratable acidity ratios aimed for at each harvest stage, are also shown.

Harvest stage	Treatment	Season		TSS	TSS/TA
		2010	2011		
Unripe harvest	Reduced canopy	171	148	22	3.6-4.6
	Full canopy	176	166		
Medium ripe harvest	Reduced canopy		159	23-24	4.61-5.7
	Full canopy		168		
Ripe harvest	Reduced canopy	185	173	25-26	5.71-6.4
	Full canopy	193	189		
Overripe harvest	Reduced canopy	206		>27	>6.4
	Full canopy	208			

Abbreviations: TSS: total soluble solids; TA: titratable acidity

Table 2 Grape chemical composition and values of spectrophotometrically and HPLC-measured phenolic parameters of the Shiraz grapes from different treatments at harvest (2010 and 2011) (only parameters that show a significant second-order interaction, $p \leq 0.05$, are shown).

	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
TSS (°Balling)	2011	NSR	*22.0±0.38 ^{ab}	22.7±0.82 ^{abc}	27.0±0.46 ^d	
		SR	21.6±0.57 ^a	22.4±0.81 ^{abc}	26.0±0.10 ^{de}	
		SF	23.2±1.07 ^{bc}	23.3±0.50 ^{bc}	25.3±1.27 ^e	
		NSF	23.6±0.96 ^c	25.2±0.32 ^e	26.3±0.91 ^{de}	
Volume ^a	2010	NSR	142.00±2.00 ^a		120.00±4.00 ^b	96.00±14.42 ^c _d
		SR	137.33±4.62 ^a		109.33±8.33 ^{bc}	81.33±18.90 ^d
		SF	116.00±0.00 ^b		105.33±12.22 ^{bc}	84.00±10.58 ^d
		NSF	144.00±4.00 ^a		120.00±4.00 ^b	108.00±8.00 ^b _c
	2011	NSR	146.67±5.03 ^a	135.33±2.31 ^{ab}	120.67±4.16 ^{bc}	
		SR	150.67±25.48 ^a	106.00±9.17 ^c	116.67±8.08 ^{bc}	
		SF	129.33±4.62 ^{ab}	120.00±20.78 ^b _c	109.33±16.17 ^c	
		NSF	134.67±10.07 ^a _b	146.67±2.31 ^a	124.00±16.00 ^{bc}	
Fresh weight ^b	2011	NSR	162.68±6.00 ^a	150.22±1.09 ^{ab}	129.64±6.21 ^{cd}	
		SR	164.21±25.07 ^a	133.97±3.34 ^{bcd}	123.87±11.68 ^{cd}	
		SF	137.69±3.96 ^{bce}	128.19±20.75 ^b _{cd}	116.83±17.59 ^d	
		NSF	145.14±11.15 ^a _{bc}	159.57±0.61 ^{ae}	137.88±18.38 ^{bc} _d	
pH	2010	NSR	3.60±0.04 ^a		4.01±0.04 ^d	4.25±0.07 ^f
		SR	3.64±0.03 ^{ab}		4.09±0.06 ^{de}	4.22±0.13 ^f
		SF	3.77±0.06 ^{bc}		4.16±0.09 ^{ef}	4.17±0.03 ^{ef}
		NSF	3.83±0.10 ^c		4.07±0.12 ^{de}	4.17±0.08 ^{ef}
	2011	NSR	3.62±0.04 ^{ab}	3.74±0.02 ^{abc}	3.94±0.04 ^{ac}	
		SR	3.55±0.02 ^b	3.70±0.04 ^{abc}	3.89±0.05 ^{ac}	
		SF	3.84±0.11 ^{abc}	3.92±0.11 ^{bc}	4.00±0.14 ^c	
		NSF	3.70±0.04 ^{abc}	3.84±0.61 ^{bc}	3.98±0.12 ^c	
Titratable acidity ^c	2011	NSR	5.22±0.15 ^a	4.52±0.06 ^{bc}	4.10±0.11 ^{de}	
		SR	5.80±0.14 ^h	5.04±0.06 ^{af}	4.57±0.16 ^{bc}	
		SF	4.66±0.27 ^{bf}	4.28±0.4 ^{cdg}	4.01±0.49 ^{de}	
		NSF	4.32±0.25 ^{bcd}	3.91±0.49 ^{eg}	3.78±0.1 ^e	
TSS/TA	2010	NSR	3.62±0.27 ^a		5.34±0.53 ^b	6.77±0.39 ^c
		SR	3.69±0.17 ^a		5.18±0.43 ^b	6.21±0.38 ^{cd}
		SF	3.55±0.35 ^a		6.11±0.32 ^d	6.15±0.42 ^d
		NSF	4.00±0.46 ^a		6.22±0.09 ^{cd}	6.63±0.22 ^{cd}

	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
Monomeric anthocyanins ^d	2010	NSR	1.989±0.291 ^a		1.573±0.010 ^{bcd}	1.347±0.203 ^{de}
		SR	1.996±0.130 ^a		1.555±0.205 ^{bcd}	1.197±0.397 ^e
		SF	1.802±0.043 ^{ac}		1.627±0.086 ^{bc}	1.281±0.232 ^d
		NSF	1.518±0.059 ^{bcd}		1.604±0.161 ^{bcd}	1.281±0.072 ^d
Anth. _{pH1} (mg/L)	2011	NSR	1475.34±102.84 ^a	1419.82±126.94 ^a	1156.52±44.86 ^b	
		SR	1751.17±98.71 ^d	1522.52±83 ^a	1141.55±84.25 ^b	
		SF	1089.14±96.99 ^b	876.23±95.66 ^c	821.86±90.87 ^c	
		NSF	1082.90±198.87 ^b	1106.44±152.05 ^b	848.63±127.26 ^c	
Anth. _{pH1} (mg/g fresh berry weight)	2011	NSR	1.48±0.10 ^a	1.42±0.13 ^a	1.16±0.04 ^b	
		SR	1.75±0.10 ^d	1.52±0.08 ^a	1.14±0.08 ^b	
		SF	1.09±0.10 ^b	0.88±0.10 ^c	0.82±0.09 ^c	
		NSF	1.08±0.20 ^b	1.11±0.15 ^b	0.85±0.13 ^c	
Anth. _{pH1} (mg/berry)	2010	NSR	2.98±0.05 ^{ab}		2.07±0.08 ^{cd}	1.10±0.31 ^e
		SR	3.13±0.14 ^a		1.68±0.32 ^{cf}	0.72±0.42 ^e
		SF	2.28±0.15 ^{dg}		1.57±0.28 ^f	0.81±0.34 ^e
		NSF	2.66±0.18 ^{bg}		1.76±0.07 ^{cf}	1.10±0.24 ^e
	2011	NSR	2.40±0.03 ^f	2.10±0.21 ^a	1.49±0.11 ^{bc}	
		SR	2.86±0.30 ^g	2.00±0.04 ^a	1.40±0.17 ^{bc}	
		SF	1.50±0.08 ^{bd}	1.13±0.23 ^{ce}	0.96±0.06 ^e	
		NSF	1.52±0.36 ^b	1.77±0.25 ^{ab}	1.19±0.35 ^{cde}	
Anth. _{pH3.6} (mg/L)	2010	NSR	1169.39±76.72 ^{ab}		1302.00±204.17 ^{bc}	772.33±84.12 ^{fg}
		SR	1381.53±66.31 ^c		1023.17±80.20 ^{ad}	613.47±223.19 ^g
		SF	1096.67±31.25 ^{ad}		910.39±10.82 ^{de}	659.56±186.00 ^g
		NSF	980.58±70.44 ^a		808.11±30.04 ^{ef}	662.67±54.81 ^g
	2011	NSR	933.33±71.09 ^a	912.78±87.06 ^a	725.61±76.72 ^{cd}	
		SR	1067.79±135.25 ^b	938.68±80.94 ^a	698.72±81.54 ^{cd}	
		SF	804.27±129.43 ^{ac}	553.00±61.45 ^{ef}	581.26±53.10 ^{de}	
		NSF	694.37±111.90 ^{cde}	693.96±74.71 ^c	517.50±25.79 ^f	
Anth. _{pH3.6} (mg/g fresh berry weight)	2010	NSR	1.17±0.09 ^{ab}		1.29±0.22 ^a	0.76±0.08 ^{cd}
		SR	1.36±0.04 ^a		1.03±0.09 ^{be}	0.63±0.19 ^c
		SF	1.09±0.04 ^{bf}		0.91±0.01 ^{def}	0.68±0.17 ^{cg}
		NSF	0.99±0.07 ^{be}		0.86±0.06 ^{deg}	0.67±0.06 ^{cg}

	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
	2011	NSR	0.93±0.07 ^{ab}	0.91±0.09 ^a	0.73±0.08 ^{cd}	
		SR	1.07±0.14 ^b	0.94±0.08 ^a	0.70±0.08 ^{cde}	
		SF	0.80±0.13 ^{ac}	0.55±0.06 ^{ef}	0.58±0.05 ^{def}	
		NSF	0.69±0.11 ^{cde}	0.69±0.07 ^{cde}	0.52±0.03 ^f	
Anth. _{pH3.6} (mg/berry)	2010	NSR	1.81±0.05 ^{ab}		1.73±0.37 ^{ac}	0.82±0.17 ^{de}
		SR	2.07±0.03 ^b		1.24±0.19 ^{fg}	0.6±0.27 ^d
		SF	1.42±0.05 ^{cfh}		1.09±0.14 ^{eg}	0.66±0.22 ^d
		NSF	1.54±0.13 ^{acf}		1.14±0.08 ^{gh}	0.81±0.16 ^{de}
	2011	NSR	1.53±0.01 ^{ab}	1.35±0.14 ^{ac}	0.93±0.12 ^{de}	
		SR	1.73±0.27 ^b	1.27±0.08 ^{cf}	0.86±0.11 ^{deg}	
		SF	1.08±0.12 ^{df}	0.71±0.17 ^{eg}	0.65±0.08 ^g	
		NSF	1.00±0.23 ^d	1.10±0.13 ^{cd}	0.71±0.13 ^{eg}	
Polymeric pigments ^d	2010	NSR	0.094±0.017 ^a		0.175±0.020 ^b	0.268±0.068 ^c _d
		SR	0.103±0.006 ^a		0.199±0.033 ^{bc}	0.352±0.088 ^e
		SF	0.100±0.014 ^a		0.239±0.043 ^{bc}	0.305±0.054 ^d _e
		NSF	0.098±0.011 ^a		0.191±0.011 ^b	0.242±0.036 ^b _{cd}
	2011	NSR	0.073±0.002 ^a	0.087±0.004 ^{bc}	0.148±0.007 ^d	
		SR	0.071±0.005 ^{ab}	0.099±0.011 ^{ce}	0.148±0.010 ^d	
		SF	0.111±0.012 ^{ef}	0.118±0.009 ^f	0.169±0.003 ^g	
		NSF	0.108±0.014 ^{ef}	0.118±0.010 ^f	0.145±0.014 ^d	
Tannin (mg/L CE)	2010	NSR	126.11±15.98 ^a _b		162.94±4.61 ^{ef}	161.08±15.66 ^{ef}
		SR	117.18±2.59 ^{acd}		145.57±8.99 ^{be}	145.93±12.55 ^{be}
		SF	95.65±6.66 ^c		133.13±8.13 ^{bd}	172.80±39.56 ^f
		NSF	102.37±4.73 ^{ac}		126.10±6.86 ^{ab}	133.32±18.57 ^{bd}
	2011	NSR	118.37±7.15 ^a	143.12±5.58 ^{bc}	158.83±10.71 ^d	
		SR	137.54±20.28 ^a _b	142.46±18.54 ^b _{cd}	152.85±2.48 ^{cd}	
		SF	131.96±16.28 ^a _b	144.50±16.72 ^b _{cd}	137.55±9.18 ^{abc}	
		NSF	128.08±3.29 ^{ab}	131.34±3.00 ^{ab}	137.95±8.71 ^{abc}	
Monomeric flavan-3-ols ^d	2011	NSR	0.004±0.007 ^{ab}	0.016±0.003 ^c	0.017±0.001 ^c	
		SR	0.000±0.000 ^a	0.013±0.002 ^{cd}	0.015±0.001 ^c	
		SF	0.010±0.002 ^{de}	0.008±0.002 ^{be}	0.006±0.001 ^{be}	
		NSF	0.000±0.000 ^a	0.008±0.003 ^e	0.010±0.001 ^{de}	
Polymeric phenols ^d	2010	NSR	2.23±0.16 ^a		3.05±0.10 ^{bc}	3.61±0.40 ^{de}
		SR	2.37±0.14 ^a		2.98±0.13 ^b	4.02±0.30 ^d
		SF	2.14±0.18 ^a		3.34±0.31 ^{bce}	3.84±0.43 ^{df}

	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
	2011	NSF	2.05±0.07 ^a		2.97±0.08 ^b	3.46±0.25 ^{cef}
		NSR	4.86±1.17 ^g	1.63±0.02 ^a	2.39±0.10 ^{bcd}	
		SR	3.01±0.36 ^b	1.74±0.21 ^{ac}	2.33±0.14 ^{de}	
		SF	2.7±0.21 ^{bd}	1.95±0.18 ^{acef}	2.52±0.12 ^{bdf}	
		NSF	1.74±0.11 ^{ace}	1.84±0.07 ^{ace}	2.17±0.24 ^{acd}	
TP%	2010	NSR	51.20±3.95 ^{ab}		63.76±2.69 ^d	56.69±0.88 ^{bd}
		SR	49.78±3.40 ^{abc}		56.64±0.76 ^{bd}	62.47±19.01 ^d
		SF	47.44±1.89 ^{abc}		49.16±2.42 ^{abc}	51.98±3.31 ^{ab}
		NSF	42.51±1.65 ^{ac}		40.09±1.33 ^c	49.33±4.03 ^{abc}
	2011	NSR	39.11±1.51 ^a	46.82±4.01 ^b	38.38±5.64 ^a	
		SR	47.28±6.09 ^b	46.04±6.17 ^b	38.84±2.30 ^a	
		SF	37.39±2.63 ^a	24.80±0.80 ^c	35.05±0.67 ^a	
		NSF	33.26±3.61 ^a	38.28±2.72 ^a	26.50±0.98 ^c	

^aExpressed as the volume of 100 berries (ml)

^bExpressed as the weight of 100 berries (g)

^cExpressed as g/L of tartaric acid

^dExpressed as mg/g fresh berry weight

All values displayed in the table are the means of three repeats, with the standard deviation expressed after '±'.

*Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter and season

Abbreviations: TSS: total soluble solids; TA: titratable acidity; Anth._{pH1}: anthocyanins extracted at pH 1; Anth._{pH3.6}: anthocyanins extracted at pH 3.6; TP: total phenol content of the solution at pH 3.6; CE: catechin equivalents; nd: not detected

Table 3 Chemical characteristics and values of spectrophotometrically and HPLC-measured phenolic parameters of the Shiraz grapes at different harvest stages in the 2010 and 2011 seasons (only parameters that show a significant first-order interaction, $p \leq 0.05$, and for which no significant second-order interaction occurred, are shown).

	Season	Harvest stage			
		Unripe	Medium ripe	Ripe	Overripe
TSS ($^{\circ}$ Balling)	2010	*22.2±0.84 ^a		26.0±1.04 ^b	29.6±2.48 ^c
Fresh weight ^a	2010	154.16±5.80 ^a		126.89±10.41 ^b	105.34±17.15 ^c
Titrateable acidity ^b	2010	6.00±0.41 ^b		4.59±0.47 ^a	4.64±0.48 ^a
TSS/TA	2011	4.60±0.75 ^a	5.36±0.91 ^b	6.39±0.55 ^c	
Monomeric anthocyanins ^c	2011	1.440±0.189 ^{ab}	1.496±0.205 ^a	1.366±0.145 ^b	
Malvidin-3-glucoside ^c	2010	0.643±0.095 ^a		0.556±0.049 ^b	0.444±0.088 ^c
	2011	0.535±0.116 ^a	0.516±0.108 ^{ab}	0.479±0.082 ^b	
Anth. _{pH1} mg/L	2010	1871.63±181.68 ^a		1379.73±115.54 ^b	879.67±255.16 ^c
Anth. _{pH1} mg/g fresh berry weight	2010	1.87±0.18 ^a		1.38±0.12 ^b	0.88±0.26 ^c
%EA	2010	62.11±4.19 ^a		70.35±6.24 ^b	79.31±8.62 ^c
Monomeric flavan-3-ols ^c	2010	3.66±0.47 ^a		3.25±0.24 ^b	2.74±0.36 ^c
Total flavonols ^c	2010	0.08±0.02 ^a		0.09±0.01 ^b	0.08±0.01 ^{ab}

^aExpressed as the weight of 100 berries (g)

^bExpressed as g/L of tartaric acid

^cExpressed as mg/g fresh berry weight

All values displayed in the table are the means of four treatments, each of which has three field repeats, with the standard deviation expressed after '±'.

*Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter and season

Abbreviations: TSS: total soluble solids; TA: titrateable acidity; Anth._{pH1}: anthocyanins extracted at pH 1; Anth._{pH3.6}: anthocyanins extracted at pH 3.6; %EA: percentage extractable anthocyanins

Table 4 Chemical characteristics and values of spectrophotometrically and HPLC-measured phenolic parameters of the Shiraz grapes of the different treatments in the 2010 and 2011 seasons (only parameters that show a significant first-order interaction, $p \leq 0.05$, and for which no significant second-order interaction occurred, are shown).

	Season	Treatment			
		NSR	SR	SF	NSF
Fresh weight ^a	2010	*132.49±22.40 ^{ab}	122.28±26.95 ^a	121.31±24.47 ^a	139.11±17.97 ^b
Titratable acidity ^b	2010	5.11±0.81 ^a	5.46±0.56 ^b	5.13±0.88 ^{ab}	4.61±0.79 ^c
TSS/TA	2011	5.27±1.04 ^a	4.62±0.88 ^b	5.61±0.74 ^a	6.30±0.82 ^c
Monomeric anthocyanins ^c	2011	1.413±0.091 ^a	1.650±0.152 ^b	1.408±0.141 ^a	1.265±0.107 ^a
Malvidin-3-glucoside ^c	2011	0.531±0.044 ^a	0.637±0.066 ^c	0.463±0.073 ^{ab}	0.410±0.048 ^b
%EA	2010	70.54±8.43 ^a	75.41±7.52 ^a	71.95±12.45 ^a	64.46±6.97 ^b
	2011	63.04±3.08 ^a	60.59±2.93 ^a	68.435±5.595 ^b	63.31±2.26 ^a
Total flavonols ^c	2011	0.16±0.02 ^a	0.16±0.02 ^a	0.10±0.01 ^b	0.11±0.01 ^b

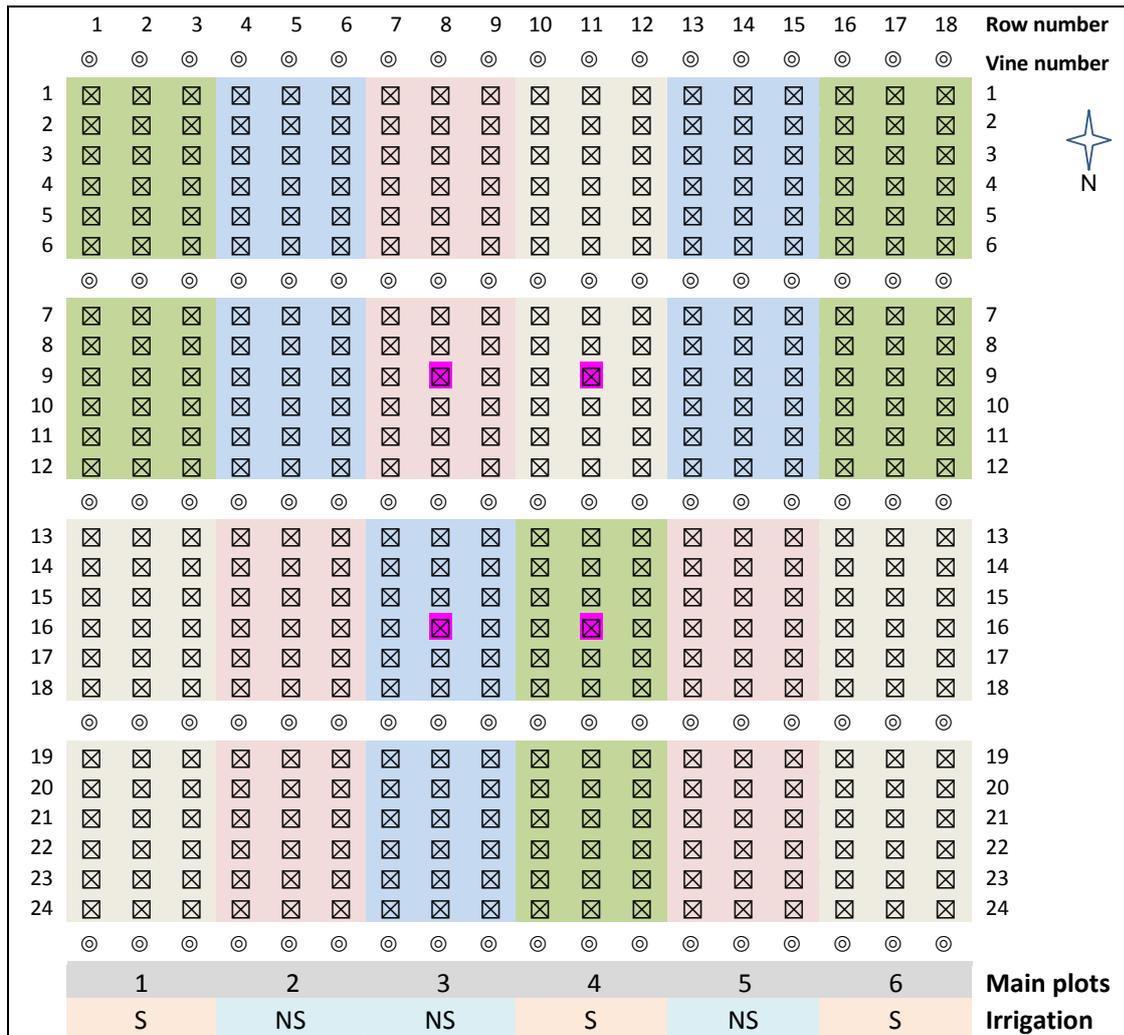
^aExpressed as the weight of 100 berries (g)

^bExpressed as g/L of tartaric acid

^cExpressed as mg/g fresh berry weight

All values displayed in the table are the means of three harvest stages, with the standard deviation expressed after '±'.

*Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter and season
Abbreviations: TSS: total soluble solids; TA: titratable acidity; %EA: percentage extractable anthocyanins



Irrigation	Canopy treatment
Non stressed	Full canopy
Non stressed	Reduced canopy
Stressed	Full canopy
Stressed	Reduced canopy
⊠ Neutron probe positions	

Figure 1 Experiment layout in the 2010 season according to a split-plot design. Main plots are shown and sub-plots are represented by the different canopy manipulation treatments shown within a main plot.

Chapter 4

Research results

Interactive effects of growth manipulations and water deficit in grapevine (*Vitis vinifera* L.) cv. Shiraz: Impact on wine phenolic and sensory characteristics

4. Research results

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The aim of this study was to determine how the interaction of bunch exposure and water deficit influences the flavonoid, colour and sensory characteristics of Shiraz wine made from grapes harvested at different stages of ripeness over two seasons. Generally, shoot removal increased the concentration of certain flavonoids, as well as colour intensity, astringency and body, in the resulting wines. In most cases, higher concentrations of flavonols, monomeric flavan-3-ols and monomeric anthocyanins in the grapes from the reduced canopy treatments (see Chapter 3) were reflected in the resulting wine. However, differences in the concentrations of certain other phenolic compounds were less clear in wines made from unripe grapes compared to wines from ripe grapes. Certain differences in phenolic and colour parameters in wines made from grapes from the reduced canopy compared to the full canopy treatments were also maintained during a period of wine ageing. Generally, ripening *per se* increased the concentrations of specific phenolic compounds, such as polymeric phenols and polymeric pigments, in the wines, and a higher concentration of polymeric pigments was also observed in riper grapes (see Chapter 3). This led to more astringent wines with higher colour density. However, overripe grapes resulted in wines with lower concentrations of important phenolic compounds, probably due to breakdown reactions in the former. The effects of water deficit on most of the colour and phenolic parameters of wines were not as prominent as those of the canopy manipulation treatment. Therefore, this study proved that some viticultural manipulations could be reflected in the resulting wines, which could support efforts to aim for a specific Shiraz wine style.

4.1 INTRODUCTION

Grape flavonoid compounds are important contributors to wine quality as they influence the colour, colour stability and sensory properties of a red wine (Glories, 1988). Anthocyanins, tannins, flavonols, flavan-3-ols and polymeric pigments are the main flavonoid compounds that are often investigated in red wines.

The colour of a young red wine is mainly due to monomeric anthocyanins or copigments, the latter accounting for 30 to 50% of the colour (Boulton, 2001). Anthocyanins can form interactions with themselves (self-association) or in complexes with other phenolic compounds (flavonol glycosides and cinnamic acids), known as copigmentation (Brouillard & Mazza, 1989; Boulton, 2001). Direct and indirect condensation of anthocyanins and flavanols also occur during the winemaking process, leading to more polymeric pigments in wine. As wine ages, a larger fraction of wine colour is thus due to stable polymeric pigments, the result of

polymerisation reactions and copigmentation associations that are more resistant to pH fluctuations, sulphur dioxide bleaching and increases in alcohol concentration (Boulton, 2001).

Fruit flavonoid concentration, the rate of extraction and fermentation conditions are important parameters that determine wine flavonoid concentration (Romero-Cascales *et al.*, 2005; Ristic *et al.*, 2007; Río Segade *et al.*, 2008), and fruit ripeness, ethanol content, berry size, water deficit (Sivilotti *et al.*, 2005) and sunlight (Cortell & Kennedy, 2006; Ristic *et al.*, 2007; Rustioni *et al.*, 2011) are known to influence the extraction of flavonoids (Canals *et al.*, 2005).

Recent investigations into the effects of light on flavonoid biosynthesis in red grape cultivars and the resulting wines have involved a range of approaches, particularly the application of physical shade treatments, including shade cloth (Joscelyne *et al.*, 2007), boxes made from white polypropylene sheeting and painted black on the inside (Ristic *et al.*, 2007), bird nets wrapped around the canopies (Smart *et al.*, 1985; Ristic *et al.*, 2010) and sampling different parts of the bunch where the light regime was perceived to be different (Price *et al.*, 1995). In addition, treatments have been applied at different developmental stages, from fruit set to véraison.

The literature suggests that extensive shading could result in wine with decreased concentrations of colour and phenolic compounds, which could be maintained during ageing (Smart *et al.*, 1985; Price *et al.*, 1995; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007, 2010). An informal tasting of Pinot noir wines made from shaded and exposed fruit indicated differences in the sensory characteristics of the wines (Price *et al.*, 1995). In another study, wine made from shaded fruit was rated lower for overall fruit flavour and fruit flavour persistence, as well as for a number of mouthfeel characteristics including overall astringency (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007). Excessive artificial shading was also found to produce wines with an intensified sensory detection of 'straw' and 'herbaceous' characters (Ristic *et al.*, 2010) and lower colour intensity, lighter body, shorter length and higher acid perception (Joscelyne *et al.*, 2007).

Increased phenolics in wines made from grapes subjected to deficit irrigation is believed to be caused by changes in anthocyanin and phenolic chemistry during winemaking, or by a concentration effect as a result of decreasing berry size with decreased water (Chalmers *et al.*, 2010). Recent studies report a positive effect of deficit irrigation on the phenolic composition of bottled and aged wine for up to 18 months (Matthews *et al.*, 1990; Kennedy *et al.*, 2002; Chapman *et al.*, 2005; Peterlunger *et al.*, 2005; Koundouras *et al.*, 2006; Bindon *et al.*, 2008, 2011; Chalmers *et al.*, 2010). Bindon *et al.* (2008) and Chalmers *et al.* (2010) have suggested that a higher anthocyanin concentration or percentage of polymeric or copigmented forms in these wines could cause a higher concentration of red pigments after six months of ageing, which is consistent with the findings of previous studies (Levengood, 1996; Levengood & Boulton, 2004). Recent research confirms that the application of water deficits can also

positively affect wine composition with regard to wine sensory properties (Matthews *et al.*, 1990; Escalona *et al.*, 1999; Koundouras *et al.*, 2006).

For a variety of reasons, the relationship between grape and wine anthocyanins is not always consistent. Some studies have reported little relationship (Price *et al.*, 1995; Ristic *et al.*, 2007; Bindon *et al.*, 2011), while others have found good correlations between grape and wine composition (Kennedy *et al.*, 2002; Cortell *et al.*, 2005; Peterlunger *et al.*, 2005; Koundouras *et al.*, 2006; Bindon *et al.*, 2008; Jensen *et al.*, 2008; Du Toit, 2011). This could mainly be ascribed to differing extraction conditions and media.

Currently there is not a clear understanding of the interactive effect of light and water deficit on grape composition, especially regarding phenolic and colour compounds, and how this is reflected in the corresponding wines. The effect of these changes on the sensory characteristics of wine, and how these phenolic and colour differences develop during wine ageing also need further attention. The aim of the current study was thus to track the changes in the fruit phenolic composition of *Vitis vinifera* cv. Shiraz in response to the interactive effect of irrigation and canopy modification treatments to wine phenolic composition and sensory properties for two consecutive seasons. The concentrations and composition of wine anthocyanins, flavan-3-ols, flavonols, polymeric pigments and tannin were thus monitored for two seasons after alcoholic fermentation, as well as after malolactic fermentation and six months' ageing for the first season. Chapter 3 provides detailed information on the viticultural treatments.

4.2 MATERIALS AND METHODS

Refer to Chapter 3 for more detailed information on the vineyard, experiment layout, climate measurements, predawn leaf water potential measurements, berry sampling and determination of grape chemical and phenolic composition.

4.2.1 Small-scale winemaking

Twenty kilogrammes of grapes from each treatment's field repeat (Table 1) were harvested at three stages (Table 2) and used for winemaking purposes. In 2010, the overripe stage yielded wines with very high alcohol levels that led to some stuck malolactic fermentations, and we thus decided to harvest the third stage in 2011 at lower soluble solid levels. Bunches were destemmed and crushed, and grape must samples were collected to determine pH and titratable acidity (TA), using an automatic titrator (Metrohm, 702 SM Titrano, Switzerland). Total soluble solids (TSS/°B) were determined with a PAL-1 pocket refractometer (Atago, Japan). A combination of an ammonium ion determination procedure and the NOPA procedure (which determines the free alpha amino acid content) was used by Vinlab Pty (Ltd), an accredited wine analysis laboratory in Stellenbosch, to determine the YAN (yeast assimilable nitrogen) levels. Sulphur dioxide (SO₂) was added (30 mg/L) to the must using a 2.5% potassium metabisulfite solution. Titratable acidity was corrected to 6 g/L, if required, using tartaric acid (Merck), and the

musts were then inoculated with active dry wine yeast (*Saccharomyces cerevisiae* strain NT 116) at 0.3 g/L according to the supplier's recommendations (Anchor Yeast, Biotechnologies, South Africa). Wines were fermented in a temperature-controlled room at approximately 25°C and the skins were mixed with the must during fermentation three times a day. After a decrease of 5 to 6 °B, Fermaid K and/or diammonium phosphate (DAP) were added according to the catalogue recommendations of Lallemand South Africa, which bases these additions on must YAN levels. Routine wine analyses after alcoholic fermentation (pH, TA, VA and sugar concentration) were done using a Grapescan™ FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004). The instrument utilises Fourier transform infrared spectroscopy (FT-IR). Samples were degassed and filtered before analysis, using the filtration unit (type 70-500, Foss Electric, Denmark) with filter paper circles graded at 20 to 25 µm and with a diameter of 185 mm. At the end of alcoholic fermentation (when the sugar concentration was less than 5 g/L), the wines were pressed to 0.5 bar in a small-scale basket press. Free-run and pressed wines were combined.

The wines were inoculated with CH16 (Christiaan Hansen) (*Oenococcus oeni*) at 1 g/hL, as well as with BACTIV-AID (0.2g/L). Malolactic fermentation was conducted at 20°C. Malic and lactic acid concentrations were monitored using a Grapescan™ FT 120 instrument (Foss Electric, Denmark) (Nieuwoudt *et al.*, 2004). After the completion of malolactic fermentation (malic acid concentration lower than 0.3 g/L), the wines were racked, 60 mg/L of SO₂ was added and the temperature was changed to 15°C. After one month the wines were again racked and the free sulphur content was determined by Vinlab using the aspiration method. The free sulphur and TA of the wines were adjusted to 40 mg/L and 6g/L respectively, and the wines were bottled in 750 mL dark green glass bottles, sealed with screw caps and stored at 15°C.

4.2.2 Wine sampling

One hundred mL wine samples for phenolic analysis were collected after alcoholic fermentation (after pressing, 2010 and 2011), after malolactic fermentation (before SO₂ addition, only 2010) and after six months' bottle ageing (2010). Samples were frozen immediately and stored at -20°C until time of analysis.

4.2.3 Spectrophotometric analyses

All spectrophotometric analyses were performed using an Analytikjena Specord 40 UV/VIS spectrophotometer (Jena, Germany). Depending on the composition of the wine or the required wavelength of the analysis, the following cuvettes were used: 1 mm and 10 mm quartz cuvettes, 1 mm glass cuvettes or 10 mm plastic cuvettes. All of the spectrophotometric analyses were done at the same time to quantify all samples under the same conditions, or with the same calibration curve, if necessary. All samples were centrifuged for 1 min at 2 500 rpm prior to analysis to remove any solid particles that could influence the measurement. All analyses of the treatment replicates were performed in duplicate. Different spectrophotometric analyses were

done according to Iland *et al.* (2000) (Table 3). These included wine colour density (CD), total red pigments, total phenolics and estimate of SO₂-resistant pigments. To compensate for any effects of pH and SO₂ concentration on colour in the different wines, modified wine colour density (MCD) was also measured after the adjustment of each wine to pH 3.60 and after the addition of acetaldehyde to remove any SO₂ bleaching effects (Somers & Evans, 1977). This approach allows for a more valid comparison of treatment effects on colour parameters, both between treatments and over time. Copigmentation complexes were also determined according to the method of Boulton (2001). Total anthocyanin concentration was analysed according to Ribéreau-Gayon *et al.* (2000), and the Adams-Harbertson Tannin Assay, a method that is based on the ability of tannin to complex and precipitate with protein (bovine serum albumin), was used to determine tannin concentration (Harbertson *et al.*, 2002).

4.2.4 HPLC analysis

Reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Hewlett Packard Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Hewlett Packard, Waldbronn, Germany).

The method for HPLC analysis was adapted from the method of Peng *et al.* (2002). Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100Å, 250 × 4.6 mm, 5 µm) protected by a guard cartridge (PLRP-S, 10 × 4.6 mm) with the same packing material (both Polymer Laboratories (Ltd), Shropshire, UK). The following mobile phases were used: mobile phase A: 1.5% v/v orthophosphoric acid (Reidel-de Haën) in de-ionised water, and mobile phase B: acetonitrile (Chromasolve, Reidel-de Haën). A linear gradient was used from 0 min, A 95%, B 5%; to 73 min, A 75.2%, B 24.8%; to 78 min, A 50%, B 50%, staying constant for 8 min. Flow rate was 1 ml/min and the column temperature was 35°C. The following standards were used: (+)-catechin hydrate (Fluka), (-)-epicatechin (Sigma), gallic acid (Fluka), caffeic acid (Sigma), *p*-coumaric acid (Sigma), malvidin-3-glucoside (Polyphenols Laboratories AS, Norway), quercetin-3-glucoside (Fluka) and quercetin (Extrasynthèse, France). The following wavelengths were used: monomeric and dimeric flavan-3-ols and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 1.5 mg/L, and epicatechin as epicatechin with a quantification limit of 1.5 mg/L. The quantification limit for gallic acid was 0.25 mg/L, also quantified at 280nm. A value of 320 nm was used for cinnamic acids. Caftaric acid and caffeic acid were quantified as mg/L caffeic acid, while coumaric acid and *p*-coumaric acid were expressed as mg/L *p*-coumaric units. Flavonol-glycosides and flavonol aglycones were quantified at 360 nm as mg/L quercetin-3-glucoside and mg/L quercetin respectively. Monomeric anthocyanins and polymeric pigments were quantified at 520 nm as mg/L malvidin-3-glucoside, with a quantification limit of 1.25 mg/L. The division of compounds or groups of compounds measured by HPLC is shown in Table 3. The samples were defrosted

and filtered through 0.45 µm filter (Millipore) before injection. Limit of quantification was defined as a signal-to-noise ratio of 1/10. This represents the smallest area that could be integrated accurately (< 3% standard deviation).

4.2.5 Sensory descriptive analysis

4.2.5.1 Experimental design

All four treatments and all three field replicates from the unripe (A) harvest stage (NSR 1-3A, SR 1-3A, SF1-3A and NSF1-3A) and two field replicates from each treatment from the ripe (B) harvest stage (NSR 2-3B, NSF 1-2B, SR 1,3B and SF 2-3B) were used for sensory analysis in 2010. The rest of the treatments from the ripe and overripe stage did not complete malolactic and alcoholic fermentation respectively, and were thus not used for sensory analyses. One bottle from each field repeat was used for the training sessions, and two bottles from each field repeat were used for the formal test sessions. In total, the number of bottles was $3 \times 20 = 60$. Each treatment field repeat was tasted three times during the formal test sessions and thus consisted of two different bottles.

4.2.5.2 Sensory evaluation procedure

All samples were evaluated with the use of discriminative and descriptive testing procedures. A preliminary discriminative test was used to establish whether differences could be perceived among the different treatments. Subsequently, the description of the wine sensory properties was achieved by using a trained panel. The sensory evaluation procedure that was followed is explained in terms of discriminative testing, sensory panel selection and training and, finally, the descriptive testing procedures used.

4.2.5.3 Preliminary discriminative testing

Initial sensory testing involved the use of a discriminative technique to establish if there were perceived differences between the treatments. A panel of seven individuals who regularly taste wines were asked if the differences observed were more prominent in terms of aroma (perceived smell), taste/mouthfeel (palate attributes) or both. The tasters were also prompted to provide a few descriptive terms that would capture the differences observed. These descriptive terms were used as an indication for possible reference standards to be prepared during the formal training of the panel.

4.2.5.4 Sensory panel selection and training

A panel consisting of 10 women (ages 24 to 50) were used for the wine evaluation. The panellists were selected on the basis of availability, having an interest in wine sensory evaluation and previous experience in wine evaluation. Panel members were trained according to the consensus method (Lawless & Heymann, 1998). Six one-hour training sessions took place over three days to train the panel. Two sessions took place per day with a 45 minute

break in between. During each session the panellists generated appropriate descriptive terms and gained familiarity with recognising and scoring the intensity of selected attributes. All 20 wines were tasted over two sessions, with 10/11 wines per session. Some aroma reference standards in a neutral wine (in covered wine-tasting glasses) were presented at subsequent sessions and modified in response to suggestions from the panellists (Table 4). This was used to encourage description of the specific wine and to determine whether the reference standards could be used for aroma recognition or confirmation purposes. No references were provided for the taste and mouthfeel attributes, as all the panellists had extensive training in that area of wine tasting. Although the focus of the sensory evaluation was on mouthfeel attributes, canopy manipulation and water deficit are often associated with a contribution to the aroma properties of wine (Matthews *et al.*, 1990; Chapman *et al.*, 2005; Koundouras *et al.*, 2006; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007, 2010). It therefore was decided to include aroma properties for sensory evaluation. For the purpose of this study, the mouthfeel descriptors (Gawel *et al.*, 2000) were kept very basic. The two mouthfeel descriptors included were body and fullness, bitterness and astringency. Definitions for this specific study are provided in Table 4.

Body or mouthfeel was described as the weight of the wine in the mouth and was anchored on the line scale by *thin* and *full* descriptors. Once the recognition and description of possible aroma nuances and mouthfeel properties had been finalised and the definitions had been confirmed (Table 4) with the panel, each group of samples was profiled on an unstructured line scale where 0 = *No intensity* and 100 = *Prominent intensity*. Intensity ranking was practised and discussed for six training sessions in order to calibrate the panel in the use of the line scale. Quantitative descriptive analysis training was regarded as complete when the panel members reached consensus on the range of sensory attributes, as well as on the actual intensity or scalar value of each attribute (Lawless & Heymann, 1998).

4.2.5.5 Descriptive testing

Quantitative descriptive analysis (QDA®) (Stone *et al.*, 1974) was used to describe the sensory attributes perceived in the wine samples as identified by the trained panel. Wine evaluation was performed in tasting booths equipped with the electronic data-capturing software, Compusense *five*® (Compusense Inc., Guelph, Canada). Three formal rating sessions were conducted in individual tasting booths under white light and in temperature-controlled conditions (21°C ± 2°C). Samples (30 mL) were presented in tulip-shaped standard clear ISO wine-tasting glasses covered with a Petri dish lid (Kimix, South Africa). At every rating session, the panellists received seven samples with three-digit codes in a completely randomised order according to a balanced complete block design. At the beginning of each session, the panellists familiarised themselves with the aroma standards and had access to these in their booths. The panel used a 100 mm unstructured line scale to analyse the five products for the 13 respective sensory attributes (Table 4). The panellists were asked to refresh their palate with distilled water and

unsalted crackers (Water Biscuits, Woolworths, Stellenbosch, South Africa) in between samples. The analysis was replicated during three identical, consecutive sessions for each assessor on the same day. A time delay of five minutes was included in the questionnaire to ensure a resting period between each of the three tasting sessions.

4.2.6 Statistical analysis of data

4.2.6.1 Statistical analysis of must chemical and wine phenolic characteristics

All analyses were done using Statistica 10. Mixed model repeated measures ANOVAs were used and Fisher's least significant difference (LSD) corrections were used for posthoc analyses. Significant differences were judged on a 5% significance level ($p < 0.05$).

4.2.6.2 Statistical analysis of sensory analysis data

The experimental design was a randomised complete block with four treatment combinations replicated in three blocks. The treatment design was a 2 x 2 factorial with two canopy management treatments (Full/Reduced) and two irrigation treatments (Stress/No stress). Observations were made on two harvest dates in each experimental unit.

Univariate analysis of variance was performed on all the variables accessed (sensory and instrumental) using the GLM (General Linear Models) Procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Sensory data was pre-processed by subjecting it to a test-retest analysis of variance (ANOVA), using SAS, to test for panel reliability. Judge*Replication and Judge*Sample interactions were used as measures of the temporal stability (precision) and internal consistency (homogeneity) of the panel respectively. Panel performance was also evaluated using *PanelCheck* Software (Version 1.3.2, Nofima Mat, Norway). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

In addition to the univariate ANOVAs, the data was also subjected to multivariate methods such as principal component analysis (PCA) (XLStat, Version 2011, Addinsoft, New York, USA) to visualise and elucidate the relationships between the samples and their attributes.

4.3 RESULTS AND DISCUSSION

Wine phenolic and colour composition were assessed at three stages: at the end of alcoholic fermentation (AF) (2010 and 2011), after malolactic fermentation (MLF) (2010) and after six months of bottle ageing (wines from the 2010 vintage). At each of these stages, wines made from shaded fruit exhibited a number of differences in phenolic and colour characteristics compared to wine made from the fruit exposed to sunlight.

Table 5 shows all the parameters in which a third-order interaction occurred (interaction between harvest stages, treatments and times of analysis during the winemaking process).

Table 6 shows all the parameters in which a second-order interaction occurred between the treatments and harvest stages. Values in Table 6 (‡) at only two harvest stages indicate the parameters measured in the wines from the unripe and ripe stages after AF, MLF and six months for which there were no third-order interaction. Values at three harvest stages in Table 6 indicate the parameters measured after AF only. These include the wines from the 2011 season, which were only measured after AF. They also include the wine from the overripe stage in 2010, which also was measured only after AF to examine the effect of overripe conditions. Table 7 shows all the parameters in which a second-order interaction occurred between harvest stages and times of analysis. Table 8 shows all the parameters in which a second-order interaction occurred between treatments and times of analysis. Table 9 shows all the parameters in which only a first-order interaction occurred between harvest stages, while Table 10 also shows the parameters in which only a first-order interaction occurred between treatments.

The results for each parameter are discussed according to the significance of the interactions that occurred, in order from higher-order interaction to lower-order interaction.

4.3.1 Phenolic composition of the grapes

Refer to Chapter 3 for more detail of the phenolic and colour composition of the grapes.

4.3.2 Must composition

Shiraz musts were analysed at the time of crushing to determine TSS, pH, YAN, TA and TSS/TA ratios (Tables 6, 9 and 10). Grapes from the full canopy treatments were harvested a few days later than grapes from the reduced canopy treatments because of delayed ripening in the full canopy treatments.

In 2010, the musts from NSR and NSF treatments had the lowest TSS concentrations at the unripe and overripe stage respectively (Table 6). These differences were not observed in the grape chemical composition data (see Chapter 3), which could be due to the different sampling and processing procedures of the grapes and must. However, certain chemical differences between the grapes and the must samples were not large (not more than 1°B for TSS, for instance; results not shown). Concentrations were significantly higher in all musts from the ripe stage than musts from the unripe stage in both seasons (Tables 6 and 9), and were significantly higher in all must treatments from the overripe stage compared to treatments from the ripe stage (except NSF) (Table 6).

The TA values are shown in Tables 6, 9 and 10. At the unripe and overripe stages in 2010, the SR and SF treatment musts had significantly higher TAs than the NSR and NSF treatment musts respectively (Table 6), while the SR treatment must from the 2010 ripe stage had significantly higher concentrations than the NSF and SF must, but not the NSR must. Although the NSR and NSF treatments had the lowest TSS concentration at the unripe and overripe stages in 2010 respectively, these treatments still had the lowest TA concentrations (Table 6).

The lower TA values in the non-stressed treatments compared to the stressed treatments are probably due to larger berries in the non-stressed treatments, where a reduction in acid concentration due to dilution could have been possible (Mullins *et al.*, 1992; Yuste *et al.*, 2004). In 2010 (Table 6) and 2011 (Table 9), the TA was significantly lower for all treatments in musts from the ripe stage compared to musts from the unripe stage, except for the NSR treatment from the 2010 vintage (Table 6). In 2011 there was no interaction between treatments and harvest stages and, when all three harvest stages were taken into consideration, the must from the SR and SF treatments had significantly higher TAs than those from the NSR and NSF treatments respectively (Table 10). Treatment differences in the must were negated before alcoholic fermentation through the addition of tartaric acid to the must.

For pH and YAN there was an interaction between harvest stage and treatment, and the values of the musts are shown in Table 6. The pH values increased significantly from the unripe to the ripe (2010 and 2011) and overripe stage (2010) in all treatments. The reduced canopy must from the unripe stage (2011) had a significantly lower pH than the full canopy must and, in both seasons, the SF treatment had a significantly higher pH than the NSR treatment at the ripe stage. The SF and both the full canopy musts from the ripe stage had a significantly higher pH than the non-stress and SR treatment musts in 2010 and 2011 respectively. These results are consistent with previous studies, which reported a lower pH in sunlight-exposed grapes (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007), suggesting higher concentrations of potassium ions in juice from shaded berries (Ristic *et al.*, 2007). Higher pH values in the grapes from the full canopy treatment could also be attributed to the fact that these grapes were harvested a few days later than those from the reduced canopy treatments and therefore could have had higher values due to more advanced ripening.

The SF treatment musts had the highest YAN concentration at the ripe and unripe stage in 2011. In addition in 2010, musts from both the water deficit treatments had significantly higher concentrations than the non-stress musts at the unripe stage. In general, YAN concentrations seem to have been higher in 2010 than in 2011, especially in the SF treatment.

4.3.3 Wine colour and phenolic composition

A PCA biplot of the wines from the 2010 season (Figure 1) showed differentiation amongst the different treatments, harvest stages and times of analysis in terms of phenolic attributes. The wines from the different harvest stages and canopy modification and water deficit treatments, analysed after alcoholic fermentation, grouped together along the bottom right part of the PCA and were associated with parameters such as CD, copigments and total flavonols (group A). According to the Pearson correlation matrix, these parameters showed a positive correlation with each other (data not shown). Wines from the unripe harvest (1) showed no clear differentiation between treatments, but wines from the ripe harvest (2) showed clear separation between the full and reduced canopy treatments, indicating that the wines from the latter

treatment had higher values of the attributes from Group A. According to PC1, the time of sampling during the winemaking process had a greater effect than the viticultural treatments on wines from the unripe stage.

Wines from the unripe harvest (1) showed no clear differentiation between treatments after malolactic fermentation (MLF). However, wines from the ripe harvest (2) showed a clearer separation after MLF between treatments, indicating that the wines from the reduced canopy treatments appeared to have higher values of SO₂-resistant pigments, polymeric pigments, hydroxycinnamic acids, polymeric phenols, tannins, total phenols and MCD (Group B) than those from the full canopy treatments. According to the Pearson correlation matrix, these parameters showed a positive correlation with each other (data not shown). Wines from the ripe stage in the full canopy treatment were also negatively associated with parameters from Group A after MLF. Wines from the unripe harvest were negatively associated with parameters from Group B after MLF and seemingly had lower values of parameters from Group A than after AF. The association of wines from the ripe stage of the reduced canopy treatment with phenolic and colour characteristics shifted from group A to group B from after AF to the completion of MLF.

Wines from the unripe harvest (1) showed no clear differentiation between treatments after six months' ageing, but wines from the ripe harvest (2) showed similar tendencies after six months' ageing as after MLF. According to PC1, wines from the unripe harvest seemingly had lower values of parameters from Group A after six months' ageing than after AF and MLF. The wines from the ripe stage of the reduced canopy treatment after six months' ageing seemingly had lower concentrations of parameters from Group A when compared with the analysis done after AF and MLF. This could have been due to polymerisation and precipitation reactions that occurred during ageing (Du Toit *et al.*, 2006, Fang *et. al*, 2008, García-Puente Rivas *et. al*, 2006, Moreno-Arribas *et. al*, 2008). It seems as if water deficit had little effect on the phenolic concentrations in wine after AF, MLF or six months.

The PCA biplot results for the wine from the 2011 season (Figure 2) showed differentiation amongst the different treatments in terms of phenolic attributes after AF and amongst wines from the different harvest stages. Wines from the first harvest grouped together along the left part of PC1 and were negatively associated with the phenolic parameters that were analysed. According to the Pearson correlation matrix, all of these parameters showed a positive correlation with each other (data not shown). According to PC2, reduced canopy wines from the unripe stage were positively associated with MCD, total flavonols, total monomeric anthocyanins, SO₂-resistant pigments, copigments, total anthocyanins, total flavan-3-ols, CD, total pigments and tannins (Group A), while wines from the full canopy treatments were negatively associated with these parameters. Total phenols, hydroxycinnamic acids, polymeric phenols, ethanol, pH and polymeric pigments grouped together along the bottom right part of the PCA (Group B).

Wines from the medium ripe harvest (2) showed the same differentiation between treatments, but it seems that the reduced and full canopy wines from this stage had higher values of the parameters from Group A and B respectively when compared to the wines from the unripe harvest.

According to PC1, reduced canopy wines from the ripe harvest stage (3) seemed to have higher values of the parameters from Group A and B than full canopy wines. According to PC2, wines from the ripe harvest could have had higher concentrations of parameters from group A due to the higher degree of polymerisation reactions than had taken place in the ripe grapes in comparison to the unripe grapes. Wines from the unripe stage (1) seem to have had the lowest values of the parameters from Group A and B when compared to wines from the medium ripe and ripe stages.

Some of the above observations are supported by the mixed model repeated measures ANOVAs, which are shown in Tables 5 to 10.

4.3.3.1 Total monomeric flavan-3-ols and total phenols

Total monomeric flavan-3-ol, total phenol and polymeric phenol concentrations are shown in Table 5, 6, 9 and 10.

After AF there were no clear treatment differences in monomeric flavan-3-ol concentrations in wines from the unripe stage of 2010 (Table 6), but the wines from the ripe stage of the SR treatment had significantly higher concentrations than those from the NSF treatment (Tables 5 and 6). These differences, however, diminished during ageing (Table 5). Concentrations of monomeric flavan-3-ols decreased significantly during ageing in all treatments, and this might be due to the polymerisation and precipitation reactions of phenolics that take place during ageing (Du Toit, 2011, Fang *et. al*, 2008, García-Puente Rivas *et. al*, 2006, Moreno-Arribas *et. al*, 2008, Perez-Magarino & Gonzalez-San Jose, 2006). In all the treatments in 2010, concentrations were significantly higher at the ripe stage than at the unripe stage after AF, except for the NSF treatment (Table 6). Concentrations were significantly higher in wines made from grapes from the overripe stage in the full canopy treatment when compared to grapes from the ripe stage, whereas concentrations did not differ significantly in their reduced canopy counterparts (Table 6). In 2011, concentrations were significantly higher in all treatments at the ripe stage than in the unripe stage (Table 9), and the wines from the reduced canopy treatment had significantly higher monomeric flavan-3-ol concentrations than those from the full canopy treatment after AF (Table 10). This was also observed in the grapes from the ripe stage of this season (see Chapter 3, Table 2). Water deficit had no clear effect on wine monomeric flavan-3-ol concentration.

Spectrophotometric analyses indicated that the SR treatment wines from the 2010 unripe stage had significantly higher total phenol concentrations than the NSF treatment wines, regardless of the time of analysis (Table 6). In both seasons, wines from the ripe stage had

significantly higher values than wines from the unripe stage (Table 6) and wines made from ripe grapes from the reduced canopy treatment had significantly higher total phenol concentrations than wines from the full canopy treatment in both seasons, regardless of the time of analysis (Table 6). Treatment differences at the unripe and ripe stage in 2010 were also observed in the grapes (total phenolic index) (see Chapter 3, Table 2). These results are consistent with previous studies, which observed higher values of total phenols in wines made from exposed grapes (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007). This could be due to the reduced transcription of some structural genes in the biosynthetic pathways of several phenolics in the grapes from the full canopy treatment (Jeong *et al.*, 2004; Koyama & Goto-Yamamoto, 2008), which decreased total phenol concentrations in the grapes (Morrison & Noble, 1990; Price *et al.*, 1995) and subsequent wine. No significant treatment differences were observed in wines from the 2011 unripe stage after AF (Table 6), and water deficit again had no clear effect on total phenol concentrations. Concentrations remained relatively constant between the ripe and overripe stage (2010) (Table 9).

4.3.3.2 Polymeric phenols and tannins

In 2011 there were no significant treatment differences in polymeric phenol concentrations between the unripe and medium ripe stage after AF, but NSR and SR treatment wines from the ripe stage had significantly higher concentrations than the NSF and SF treatment wines respectively, and the NSF wines had significantly higher concentrations than the SF wines (Table 6). Lower polymeric phenol concentrations have been found in wine made from shaded Pinot noir grapes after four months in a previous study (Price *et al.*, 1995). No significant interaction was observed between treatments or between treatments and harvest stages in 2010, which was also true for the unripe and ripe grapes from this season (see Chapter 3, Table 2). However, an interaction between the treatments and harvest stages was observed in 2011. This could be due to the intensified canopy modification treatment in 2011. Secondary shoots were continually removed from the bunch zone of the reduced canopy treatment in 2011, which counteracted the compensation effect of the reduced canopy vines. This led to increased light intensity being maintained in the reduced canopy treatment in 2011, causing a higher polymeric phenolic concentration in the wines made from the reduced canopy grapes than in the wines from the full canopy grapes of the 2011 ripe stage. However, no significant treatment differences were observed in the grapes from this stage. In all treatments from the 2010 vintage, polymeric phenol concentrations increased significantly during MLF, but decreased during ageing (Table 7). In 2011 (Table 6) and 2010 (Table 9), all treatments from the ripe stage had significantly higher concentrations than the treatments from the unripe stage, while it had lower concentrations than those treatments from the overripe stage. Water deficit does not seem to have influenced the polymeric phenol concentration in the wines, except that the NSF treatment from the 2011 ripe stage had significantly higher values than the SF treatment.

Tannin concentrations are shown in Tables 5 and 6. The NSR treatment wines from the 2010 ripe stage had the highest tannin concentrations compared to wines made from other treatments when all the stages of analysis during vinification are considered, while the SR treatment wines from the ripe stage had significantly higher concentrations than the SF treatment wines after AF and after six months' ageing (Table 5). This is consistent with previous studies, which found that tannin concentration was lower in wines made from shaded Shiraz grapes after AF (Ristic *et al.*, 2010) and after eight months' ageing (Ristic *et al.*, 2007). There were no significant tannin differences between treatments in wines from the unripe stage after AF (2010 and 2011) (Tables 5 and 6), which is consistent with no treatment differences in polymeric phenol concentrations at that stage. The wines from the SR treatment increased significantly in tannin concentration from the unripe to the ripe stage in 2010, but not in 2011 (Table 6). In both seasons, tannin concentration in the NSR wines was significantly higher in wines from the ripe stage compared to wines from the unripe stage after AF, while concentrations in the full canopy wines did not differ significantly between these stages (Table 6). Significantly higher tannin concentrations in the NSR treatment compared to the NSF treatment at the ripe stage in 2011 corresponds to the wine polymeric phenol and grape tannin results (see Chapter 3, Table 2). The condensed tannins in wine from the 2011 season were either more reactive with proteins or occurred at higher concentrations, therefore displaying higher values compared to the 2010 season. Differences in the conformation of tannin molecules can influence their reactivity towards BSA, leading to differences in precipitation and possibly in tannin concentration (Harbertson & Downey, 2009). The vintage effect can also play a role in the tannin concentration of wines, as was found by Van der Merwe *et al.* (2011).

4.3.3.3 Total flavonols and copigments

Total flavonol values are shown in Tables 6, 7 and 9. Wines from the reduced canopy treatment had significantly higher concentrations than those from the full canopy treatment after AF for all three harvest stages in 2011 (Table 6), and this was also observed in the grapes (see Chapter 3, Table 4). These treatments had the largest influence on quercetin-3-O-gluconoride in the wine, thereby influencing variances in total flavonol concentrations to a large extent (data not shown). According to the literature, flavonols are the group of flavonoids on which the positive effect of increased sunlight is the greatest (Price *et al.*, 1995). No treatment interaction was observed in wines from the 2010 vintage, consistent with the grape data (see Chapter 3). When all treatments are considered, concentrations of flavonols decreased significantly in the wines from both the unripe and ripe stage during MLF and after six months (Table 7). All wines from the ripe stage, except SF, had significantly higher concentrations than wines from the unripe stage after AF (2011) (Table 6), but all wines from the 2010 ripe stage had significantly higher total flavonol concentrations than wines from the unripe stage after AF (Table 9). The latter was also observed in the grapes from the 2010 season (see Chapter 3, Table 3). Concentrations were significantly lower in all wine treatments from the overripe stage compared

to wines from the ripe stage (2010) (Table 9). However, this significant difference was not observed in the grapes (see Chapter 3, Table 3).

Copigment concentration values are shown in Tables 5, 6, 9 and 10. Concentrations in all wine treatments from the 2010 vintage decreased significantly during MLF and then remained relatively constant during the ageing period, except for the wines from the ripe stage of the reduced canopy treatment, which showed a significant increase during this period (Table 5). No significant treatment differences were observed in wines from the unripe stage after AF, MLF and six months, but NSR and SR wines from the ripe stage had significantly higher copigment concentrations than the NSF and SF treatments respectively after AF and six months (Table 5), consistent with higher levels of total anthocyanins, phenols, pigments and monomeric anthocyanins in the wines from the reduced canopy treatment in this season. All wine treatments from the 2010 overripe stage, except for NSF, had significantly lower copigment values in comparison with wines from the ripe stage after AF, and water deficit had no clear effect on wines from the unripe and ripe stage of the 2010 season (Table 6). In 2010, increased values in wines from the ripe stage were observed in comparison with wines from the unripe stage, but only for the reduced canopy treatment (Table 6). When all treatments were considered in 2011, increased values were observed in wines from the ripe stage in comparison with wines from the unripe stage after AF (Table 9). When all three harvest stages were considered, the NSR and SR treatment wines had significantly higher copigment concentrations than the NSF and SF treatments respectively (Table 10). This was consistent with significantly higher levels of monomeric flavan-3-ols, total flavonols, total anthocyanins, monomeric anthocyanins and total pigments in the wines from the reduced canopy treatment in this season. These are compounds that take part in copigmentation reactions and contribute to the colour of a young red wine (Boulton, 2001). As in 2010, water deficit had no significant effect on wines from the unripe and ripe stages in this season (Table 10).

4.3.3.4 Anthocyanins, polymeric pigments, SO₂-resistant pigments and total red pigments

Anthocyanin concentrations measured spectrophotometrically (total anthocyanins) and with the HPLC (monomeric anthocyanins) are shown in Tables 6, 9 and 10.

When all the times of analysis during the vinification process are considered, it is clear that the NSR and SR treatment wines from the 2010 ripe stage had significantly higher total anthocyanin concentrations than the NSF and SF treatment wines respectively (Table 6), and the SR treatment wines from the unripe stage had significantly higher total anthocyanin concentrations than the SF, NSF and NSR treatment wines. This is consistent with previous studies, which observed higher values in wines made from exposed grapes after ageing (at bottling and for up to three years of ageing) (Price *et al.*, 1995; Joscelyne *et al.*, 2007; Ristic *et al.*, 2007). This could be due to the fact that early shoot removal in the reduced canopy treatment resulted in bunch exposure during the green and lag stages of berry growth, which

increased the initial concentration or activity of one or several anthocyanin biosynthetic enzymes (Takeda *et al.*, 1988). In 2011, the maximum activity of these enzymes was also maintained during ripening (Dokoozlian & Kliewer, 1996) due to continual secondary shoot removal in the bunch zone. No significant treatment differences in monomeric anthocyanins were found in wines from the 2010 unripe stage, but NSR and SR treatment wines from the 2010 ripe stage had significantly higher concentrations than the NSF and SF treatment wines respectively when all the times of analysis during the winemaking process are considered (Table 6). Concentrations of total and monomeric anthocyanins were significantly lower for all treatments in wines from the overripe stage (2010) than in wines from the ripe stage due to the breakdown reactions of anthocyanins in overripe grapes (Ribéreau-Gayon *et al.*, 2006) (Table 6). According to the second-order interaction between the treatments and all three harvest stages, total anthocyanin and monomeric anthocyanin concentrations in wines from the 2010 ripe stage did not differ significantly from wines from the unripe stage after AF (Table 6). In 2011, however, concentrations were significantly higher in all wines from the ripe stage (Table 9). However, this did not occur in the grapes (see Chapter 3, Table 3). After AF, the NSR and SR treatment wines from the 2011 vintage had significantly higher total anthocyanin concentrations than the NSF and SF wines respectively (Table 10), and the SR wines also had significantly higher concentrations than the NSR wines. There was no significant difference in concentration between the SF and NSF treatment wines. This was confirmed with the HPLC analysis of monomeric anthocyanins (Table 10). These differences were also observed in the grapes, except for the difference between NSR and NSF (see Chapter 3, Table 4). As observed in Chapter 3, values seem to have been slightly lower in 2011 than in 2010, which could have been attributed to the 2011 season being drier and warmer, with temperatures possibly exceeding the optimum value for anthocyanin accumulation in the grapes. The discrepancies between the wine and the grape data could be ascribed to the different extraction media used when extracting anthocyanins from grapes and wines.

Polymeric pigment values for wines from the 2010 and 2011 vintages are shown in Tables 6, 8 and 9. The SF treatment wines from the 2011 unripe stage had significantly higher concentrations than the SR and NSR treatment wines after AF, with no significant differences between the latter two wines and the NSF treatment (Table 6). Polymeric pigment concentrations increased significantly in all wine treatments from the unripe to the ripe stage in 2011 (Table 6), which is consistent with higher monomeric flavan-3-ols, total phenols, polymeric phenols, copigments, total anthocyanins, monomeric anthocyanins and total pigments in wines from the 2011 ripe stage. No significant treatment differences occurred in the wines from the 2010 season after six months (Table 8). As in 2011, polymeric pigment concentrations increased significantly in all wine treatments from the unripe to ripe and overripe stages of 2010 after AF (Table 9), consistent with higher SO₂-resistant pigment (Table 9) and polymeric phenol

concentrations in the wine from the overripe stage, and higher SO₂-resistant pigment (Table 9), flavonol, polymeric phenol and total phenol concentrations in wine from the ripe stage.

Total pigment concentrations are shown in Tables 6, 9 and 10. The same treatment differences observed for total anthocyanins measured spectrophotometrically after AF, MLF and six months (2010) were observed in terms of total pigments in the 2010 season (Table 6). Total pigment concentrations were significantly higher at the ripe stage than the unripe stage after AF only for the SR treatment wines from 2010 (Table 6). All treatments except NSF showed significant decreased concentrations at the overripe stage (2010) (Table 6), consistent with lower total anthocyanin, monomeric anthocyanin, flavonol and copigment concentrations in wines from this stage. After AF, all treatments from the 2011 ripe stage had significantly higher total pigment concentrations when compared to the unripe stage (Table 9). After AF in the 2011 season, the wines from the reduced canopy treatment had significantly higher total pigment concentrations than the full canopy treatments, which is consistent with the higher concentrations of total anthocyanins and monomeric anthocyanins in the reduced canopy treatment wines (Table 10).

4.3.3.5 Modified colour density and colour density

Modified colour density (MCD) and colour density (CD) values in the 2010 vintage wines are shown in Tables 5, 6 and 9. There were no significant treatment differences in CD in wines from the unripe stage (which was maintained during ageing) (Table 5), but the SR treatment wines from the ripe stage had a significantly higher CD than the SF treatment wines after AF, with no difference being observed between the NSF and NSR treatments (Table 5). After six months, the CD was significantly higher in both the reduced canopy wines made from ripe grapes than in their full canopy counterparts (Table 5). These results confirmed those of previous studies, which reported lower CD values (Smart *et al.*, 1985) for up to two years (Joscelyne *et al.*, 2007), and lower MCD values after AF (Ristic *et al.*, 2010) and up to three years (Ristic *et al.*, 2007) in wines made from shaded bunches. This is probably due to higher concentrations of anthocyanins being present in the wines from the reduced canopy treatment, with more of these anthocyanins becoming incorporated into more stable polymeric pigmented forms (Du Toit *et al.*, 2006, Fang *et al.*, 2008, García-Puente Rivas *et al.*, 2006, Moreno-Arribas *et al.*, 2008, Perez-Magarino & Gonzalez-San Jose, 2006). Another reason could be the higher flavonol content in the reduced canopy treatments, as flavonols are the copigments and will increase CD. The CD decreased significantly for all treatments during MLF but remained constant during ageing, except in the SF and NSR treatment wines from the unripe stage, in which values decreased significantly (Table 5). Previously, other workers have reported that MLF caused a decrease in colour (Rankine *et al.*, 1970; Husnik *et al.*, 2007). A sharp decrease in colour could be due to pH changes as a result of the conversion of malic acid to lactic acid and a subsequent shift in the equilibrium of the anthocyanins to the colourless form (Bousbouras & Kunkee, 1971;

Pilone & Kunkee, 1972). The specific *O. oeni* strain used in this study could also have had a more negative effect on the colour and phenolic composition than other strains. It is also known that MLF could prevent polymeric pigment formation by the degradation of pyruvic acid and acetaldehyde (Osborne *et al.*, 2006). Acetaldehyde plays a role in the formation of polymeric pigments by forming ethyl-linked bridges for flavanol and anthocyanin polymeric pigment reactions as well as links between anthocyanins (Cheynier *et al.*, 2006). In addition, acetaldehyde and pyruvic acid can also be incorporated into vitisins A and B, compounds with increased absorbance at 520 nm and resistance to bleaching by SO₂ (Schwarz *et al.*, 2003), contributing to stable red wine colour (Rentzsch *et al.*, 2010). The CD values were also significantly higher in wines made from the ripe stage compared to wines from the unripe stage, consistent with significantly higher polymeric pigment concentrations in the 2010 wines from the ripe stage (Table 9). Wines from the overripe stage had lower CD values when compared to wines from the ripe stage, although not significantly. (Table 9). This is consistent with lower total flavanol and anthocyanin concentrations in wines from the overripe stage.

After AF, wines made from ripe grapes from both the SR and NSR treatments in 2010 had significantly higher MCD than the SF and NSF treatment grapes respectively, (Table 6). This difference could probably be attributed to the higher pH values of the reduced canopy wines (data not shown); when reduced to 3.60, it could have had a larger impact on changing more anthocyanins to the red flavilium ion form in the reduced canopy than in the full canopy wines. The SR treatment wines from the 2010 unripe stage had significantly higher MCD values than the NSF wines after AF (Table 6), consistent with higher concentrations of total pigments, total anthocyanins and monomeric anthocyanins in the SR treatment wines. The MCD values were significantly higher in wines from the ripe stage compared to wines from the unripe stage, while the values were lower in wines made from the overripe stage, except for the SF treatment (Table 6).

The MCD and CD values of the wines from the 2011 vintage are shown in Tables 9 and 10. As in 2010, MCD and CD values after AF were significantly higher in wines from the ripe stage compared to wines from the unripe stage (Table 9), which is consistent with higher polymeric pigment, total pigment, total anthocyanin and monomeric anthocyanin concentrations in these wines. According to the first-order interaction between treatments, CD values in the reduced canopy wines were higher than that of their full canopy counterparts, although the difference between the NSR and NSF treatments was not significant (Table 10).

Generally, the water deficit treatment did not influence the phenolic composition of the wines to the same extent as the canopy modification effect in this study. The effect of extreme water deficit varied according to season, treatment and harvest stage, but had little or no effect in most cases, corresponding with previous studies (Sipiora & Gutiérrez Granda, 1998; Chalmers *et al.*, 2010).

4.3.4 Sensory analysis

Aroma and mouthfeel attributes (Table 3) were rated in certain 2010 wines after six months' ageing, as described in Materials and Methods.

4.3.4.1 Sensory differences imparted by the effect of different harvest stages on Shiraz wine from the 2010 season

Principle component analysis (PCA) showed differentiation between the different harvest stages (Figure 3) for the measured sensory attributes. Cumulatively, PC1 and PC2 explained 74.52% of the total variance for Shiraz of the 2010 vintage. Wine treatments from the unripe harvest (indicated with the letter A) grouped towards the left part of PC1 and were associated with fresh berry aroma attributes. Wines from the ripe harvest (indicated with the letter B) were positioned towards the right half of PC1 and seem to have been negatively associated with the fresh berry attribute and positively with the spicy, bitter, astringency, body and fullness and colour intensity attributes. Differentiation between wines from the two harvests along PC1 seems to have been driven by a negative association of wines from the unripe harvest with the body and mouthfeel/colour intensity/spicy attributes. There was no clear differentiation in terms of PC2.

Each sensory attribute was also tested for significant differences across the different wine treatments. In some cases the ANOVA results substantiate the PCA observations. These are summarised in Table 11 and discussed briefly. No significant differences were observed for stinky, berry jam, vegetative, raisin and smoky/savoury sensory attributes. The wine from the unripe harvest had significantly higher levels of perceivable fresh berry attributes than the wine from the ripe harvest. Wine from the ripe harvest exhibited significantly higher spicy, bitter, astringency, body and fullness and colour intensity attributes compared to wine from the unripe stage. The higher astringency of wines from the ripe stage corresponds to the higher tannin concentrations determined by the Adams-Harbertson Tannin Assay in these wines after six months (Table 5). Previous studies also found a significant increase in the extraction of skin and seed proanthocyanidins at higher ethanol concentrations, resulting in more tannic wines (González-Manzano *et al.*, 2004; Canals *et al.*, 2005). The Adams-Harbertson Tannin Assay has been reported to show a strong correlation with perceived wine astringency (Kennedy *et al.*, 2006; Mercurio & Smith, 2008). A higher colour intensity in wines from the ripe stage corresponds to the higher CD values measured in these wines (Table 5). Canals *et al.* (2005) observed that the colour intensity of wines increased throughout ripening due to a higher alcohol content in wines from riper grapes, which increased the extraction of anthocyanins.

4.3.4.2 Sensory impact of water deficit and canopy reduction on wine made from unripe grapes from the 2010 season

Differentiation in the sensory properties of different treatments from the unripe harvest in the 2010 Shiraz is evident from the PCA results shown in Figure 4. The SR treatments separated towards the right of PC1 and seemed to have strong association with the colour intensity,

astringency, body and fullness, vegetative, smoky/savoury and stinky attributes, while these attributes were negatively associated with wines from the non-stress (except NSR_A2) and SF treatments.

Some of these observations are supported by the ANOVA results, which are shown in Table 12. One-way ANOVA for the sensory attributes of the Shiraz wine from the first harvest in 2010 indicates that no significant differences were observed for the spicy, bitter and berry jam attributes. The SR treatment was perceived to be significantly more intense in stinky, vegetative and smoky/savoury attributes and the least intense in fresh berry aroma attributes than the other treatments. These results do not correspond to the study by Ristic *et al.* (2007), who reported an increase in reduced/sulphide aroma in wines made from artificially shaded fruit eight months after bottling. The SR treatment was perceived to be significantly more intense in the astringency, colour intensity, body and fullness mouthfeel attributes compared to the SF wines, with no significant differences observed between the NSR and NSF treatments. These results correspond to previous studies, which reported that wines made from artificially shaded fruit were significantly less astringent than wines made from exposed fruit (Joscelyne *et al.*, 2007; Ristic *et al.*, 2007).

4.3.4.3 Sensory impact of water deficit and canopy reduction on wine made from ripe grapes in the 2010 season

The PCA results for the wine from the ripe harvest stage of the 2010 season (Figure 5) showed differentiation between the different treatments in terms of sensory attributes. The wines from the reduced canopy treatment grouped together along the top part of PC2 and were positively associated with the mouthfeel attributes. The full canopy treatments separated towards the bottom part of PC2 and were positively associated with the stinky and vegetative aroma attributes. Most of the wines made from grapes subjected to water deficit were associated with the stinky attribute.

The ANOVA results for the 2010 Shiraz wines from the ripe stage are summarised in Table 13. Fresh berry, berry jam, vegetative and smoky/savoury aroma and flavour attributes showed no differences among the different treatments. Similarly, no differences were observed for bitter, colour intensity and body and fullness attributes. This was not confirmed by the spectrophotometric analysis, which showed differences between treatments (Table 5). Small, although significant, differences in the wine's colour density cannot always be observed visually (Du Toit, 2010). The wines from the SR treatment had the highest rating for the stinky attribute when compared to the other treatments, while the SF treatment wines had lower ratings for the astringency attribute when compared to the wines from the reduced canopy treatment. Tannin analysis confirmed this lower perceived astringency of the SF treatment wines (Table 5).

4.4 Conclusions

Canopy manipulation in the form of the removal of approximately half of the shoots and grapes at flowering in Shiraz generally led to increased concentrations of certain flavonoids. In addition, a reduction in canopy of the grapes subjected to water deficit, resulted in wines with increased colour intensity, astringency and body when produced from unripe grapes, in comparison with its full canopy treatment counterpart. For wines made from ripe grapes, this phenomena was only significant for the astringency parameter. In Chapter 3, grapes from the reduced canopy treatment had higher concentrations of certain phenolic compounds than grapes from the full canopy treatment, which carried through to the wines in most cases. Treatment differences in the concentrations of certain other phenolic compounds were seemingly less clear in wines made from unripe grapes than in wines from ripe grapes. Higher levels of some phenolic and colour parameters in Shiraz wines made from reduced canopy compared to full canopy treatments could also be maintained during an ageing period. Although wines from the reduced canopy treatment generally had higher colour intensity values, body and fullness than the wines from the full canopy treatment, the reduced canopy treatment grapes subjected to extreme water deficit, resulted in wines with generally higher values of negative sensory descriptives such as stinky, savoury/smokey, vegetative and bitter, compared to the other treatments.

Generally, the concentrations of specific phenolic compounds in the wines, such as polymeric phenols and polymeric pigments, increased with the harvesting of grapes at more advanced levels of ripeness, and this was also observed in the grapes (see Chapter 3). This could lead to more astringent and bitter Shiraz wines with higher CD values, body and fullness and colour intensity. Overripe grapes, however, may lead to breakdown reactions in the grapes, resulting in wines with lower concentrations of important phenolic compounds. The effects of water deficit on most of the colour and phenolic parameters in the wines were not as prominent as those of the canopy manipulation treatment. Therefore, this study proved that some viticultural manipulations could be reflected in the resulting wines, which could allow for the creation of a certain wine style. Future work should focus on the effect of canopy and water deficit treatments on other compounds in Shiraz wines, such as important volatile aroma compounds, as well as maturing these wines in oak barrels in order to resemble industrial winemaking conditions more closely.

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Table 1 Grape vineyard treatments of Shiraz grapes that were used for winemaking purposes.

Treatment ^a		Abbreviation	Field Repeat ^b
Reduced canopy	No water deficit	NSR	1
Reduced canopy	No water deficit	NSR	2
Reduced canopy	No water deficit	NSR	3
Reduced canopy	Water deficit	SR	1
Reduced canopy	Water deficit	SR	2
Reduced canopy	Water deficit	SR	3
Full canopy	Water deficit	SF	1
Full canopy	Water deficit	SF	2
Full canopy	Water deficit	SF	3
Full canopy	No water deficit	NSF	1
Full canopy	No water deficit	NSF	2
Full canopy	No water deficit	NSF	3

^a Indication of the specific canopy modification and water deficit treatment interaction. The predawn leaf water potential (Ψ_{PD}) target for the non-stressed grapevines (NS) was less negative than -400 KPa and for the stressed grapevines (S) less negative than -1700 KPa. For the canopy reduction treatment, shoot removal was performed at 55 to 60 days after budburst (DAB) in the 2010 and 2011 seasons by removing the apical shoot on a two-bud spur, followed by suckering to a single shoot per bearer. In the 2011 season, secondary shoots were removed continuously from the lower 25 to 30 cm of the reduced canopy treatment (bunch zone).

^b Treatment replicate in the vineyard

Table 2 Stages and average total soluble solid concentrations (TSS) at which Shiraz grapes were harvested for winemaking purposes.

Season	Harvest	Harvest stage description	Average TSS (°Balling)
2010	1	Unripe	21.8
	2	Ripe	26.3
	3	Overripe	30.0
2011	1	Unripe	22.4
	2	Medium ripe	23.3
	3	Ripe	26.6

Table 3 Divisions of the compounds or sensory attributes measured by Spectrophotometric, HPLC and Sensory analysis

Spectrophotometric analysis	HPLC analysis	Sensory analysis
Colour density (CD)	Monomeric anthocyanins	Bitter
Total red pigments	Polymeric pigments	Astringency
Total phenolics	Monomeric flavan-3-ols	Body and fullness
S ₀ ₂ -resistant pigments	Total flavonols	Colour intensity
Modified colour density (MCD)	Polymeric phenols	
Copigmentation complexes		
Total anthocyanins		
Condensed tannins		

Table 4 The aroma and by-mouth sensory attributes rated by the panel and the composition of the reference standards.

Attributes	Reference standard	Description
Aroma		
Berry jam	Berry jam	Aroma standard: 10 g mixed berry jam in 30 mL base wine*
Fresh berries	Blackcurrant	Aroma standard: Sensient blackcurrant essence
	Mixed berries	Aroma standard: 1 frozen blackberry and 1 frozen strawberry in 30 mL base wine*
	Blackberry	Aroma standard: 2 frozen blackberries in 30 mL base wine*
Raisins	Raisins	Aroma standard: 20 g raisins in 30 mL base wine*
Vegetative	Vegetative	Aroma standard: 1.5 g geranium leaves in 30 mL base wine*
Spicy	Spicy	Aroma standard: ground pimento allspice in 30 mL base wine*
Smoky	Smoke essence	Aroma standard: 1 drop 4-ethylguaiacol in 30 mL base wine*
By mouth		
Astringency		Overall level of all astringent sensation; feelings of lack of lubrication in the mouth, a sensation of dryness.
Bitterness		No standard
Body and fullness		Roundness and fullness of wine in mouth, from empty and thin to full and robust
Colour intensity		No standard

* Base wine was a commercial 2 L cask of "Tassenberg" dry red wine blend with an alcohol content of 12.5%.

Table 5 Phenolic and colour parameter values for each treatment of the unripe and ripe harvest stages of only the 2010 vintage after alcoholic fermentation (AF), malolactic fermentation (MLF) and six months ageing (only parameters that show a significant third-order interaction, $p \leq 0.05$, between the harvest stages, treatments and times of analysis are shown). Wines from the overripe stage were not included, as they did not complete MLF.

Parameter	Season	Treatment	Time of analysis					
			AF		MLF		6 months	
			Harvest		Harvest		Harvest	
			Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
Monomeric flavan-3-ols (mg/L)	2010	NSR	*35.68±27.73 ^{abc}	89.38±4.10 ^{df}	63.74±4.97 ^{de}	99.28±7.13 ^{fg}	16.65±1.31 ^{ac}	23.21±0.07 ^{ach}
		SR	43.33±12.44 ^{abe}	103.22±21.43 ^f	76.33±4.83 ^{dfi}	76.16±23.34 ^{dfi}	18.71±1.64 ^{ac}	25.20±8.27 ^{ach}
		SF	23.47±5.99 ^{ach}	84.98±49.74 ^{df}	69.26±10.09 ^{de}	72.02±28.90 ^{deg}	13.93±1.81 ^{ac}	16.59±2.17 ^{ach}
		NSF	60.21±12.78 ^{bd}	53.21±9.22 ^{behi}	66.20±5.44 ^{de}	72.73±19.09 ^{deg}	10.53±1.39 ^c	16.43±2.70 ^{ac}
Tannin (mg/ L CE)	2010	NSR	138.91±83.3 ^{abc}	362.24±20.98 ^{gh}	68.79±40.65 ^{def}	332.68±55.46 ^{gi}	73.60±46.13 ^{acde}	401.43±80.59 ^h
		SR	102.09±38.03 ^{acdik}	253.16±47.21 ^{il}	43.92±23.28 ^{de}	48.01±21.14 ^{de}	68.85±40.93 ^{acde}	210.46±69.85 ^{bl}
		SF	70.83±43.79 ^{acde}	137.39±96.28 ^{abf}	7.73±8.35 ^{em}	61.24±51.71 ^{cde}	5.58±2.36 ^{em}	100.14±32.91 ^{acdjk}
		NSF	90.36±25.37 ^{acdm}	171.22±109.93 ^{bkl}	43.65±42.73 ^{de}	49.71±2.75 ^{def}	20.17±12.71 ^{ej}	182.3±23.83 ^{bkl}
SO ₂ - resistant pigments (AU)	2010	NSR	1.4±0 ^{ab}	2.4±0.04 ^{cd}	1.4±0 ^{ab}	3.4±0.08 ^c	1.2±0.01 ^{ab}	2.7±0.02 ^e
		SR	1.4±0.01 ^{ab}	2.7±0.07 ^{de}	1.5±0.01 ^{ab}	2.8±0.01 ^{cd}	1.2±0.01 ^{ab}	3.1±0.1 ^{cde}
		SF	1.1±0.01 ^{ab}	2.6±0.06 ^{cde}	1.1±0 ^{ab}	2.4±0.02 ^{cd}	1.5±0.04 ^{ab}	2.8±0.06 ^{cd}
		NSF	1.1±0.01 ^{ab}	2.4±0.03 ^b	1.3±0.01 ^a	2.5±0.03 ^{cd}	1.4±0.05 ^{ab}	1.7±0.01 ^{cd}
Copigments (AU)	2010	NSR	0.86±0.05 ^{ab}	1.18±0.06 ^f	*0.62±0.01 ^{cd}	0.67±0.01 ^{cd}	0.69±0 ^{cde}	0.85±0.06 ^{ab}
		SR	0.88±0.03 ^{ab}	1.19±0.18 ^f	0.65±0.07 ^{cd}	0.71±0.02 ^{cde}	0.72±0.04 ^{ce}	0.82±0.08 ^{ab}
		SF	0.82±0.05 ^{ab}	0.9±0.04 ^a	0.61±0.01 ^d	0.63±0.03 ^{cd}	0.65±0.02 ^{cd}	0.71±0.05 ^{cde}
		NSF	0.79±0.08 ^{be}	0.85±0.09 ^{ab}	0.61±0.04 ^d	0.66±0.03 ^{cd}	0.67±0.03 ^{cd}	0.67±0.03 ^{cd}
CD (AU)	2010	NSR	*16.7±0.14 ^{abc}	17.2±0.27 ^{aci}	9.5±0.01 ^{def}	14.2±0 ^{bj}	7.2±0.03 ^{gh}	14.8±0.18 ^{bkl}
		SR	16.4±0.1 ^{abck}	19.6±0.21 ⁱ	9.8±0.02 ^{defg}	14.3±0.29 ^{bjl}	8.4±0.07 ^{degh}	14.0±0.12 ^{jk}
		SF	15.2±0.19 ^{abk}	16.9±0.29 ^{aci}	9.2±0.08 ^{degm}	10.6±0.11 ^{df}	6.7±0.06 ^h	10.6±0.08 ^{df}
		NSF	14.3±0.18 ^{bjl}	18.8±0.2 ^{ci}	9.2±0.08 ^{deghm}	12.0±0.15 ^{fj}	7.3±0.08 ^{egh}	11.2±0.05 ^{fm}

All values displayed in the table are the means of three repeats, with the standard deviation expressed after '±'

* Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter

Abbreviations: AU: absorbance units; CE: catechin equivalents; CD: colour density

Table 6 Values of physiological and phenolic parameters in wines and musts of each treatment from the 2010 and 2011 vintages (only parameters that show a significant second-order interaction between the harvest stages and treatments, $p \leq 0.05$, but no significant third-order interaction, are shown). Values at only two stages (‡) indicate mean values for each treatment after AF, MLF and six months' ageing for the 2010 season wines (wines from the overripe stage were excluded from these analyses). Values for a specific parameter at three harvest stages indicate means for each treatment measured only after alcoholic fermentation (AF) to determine the effect of overripe conditions.

Parameter	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
TSS (°Balling)	2010	NSR	*18.4±1.94 ^e		26.6±0.9 ^a	29.4±2.45 ^{bc}
		SR	22.1±0.96 ^d		27.1±1.1 ^{ab}	30.5±2.03 ^c
		SF	21.0±0.99 ^d		26.4±1.46 ^a	30.1±1.56 ^c
		NSF	22.2±0.15 ^d		25.3±0.15 ^a	26.6±1.90 ^a
Titratable acidity**	2010	NSR	3.98±0.08 ^{abc}		4.21±0.06 ^{acde}	3.73±0.21 ^{bfg}
		SR	5.27±0.39 ^h		4.55±0.11 ^{di}	4.53±0.30 ^{di}
		SF	4.94±0.28 ^{hi}		3.80±0.65 ^{abg}	4.33±0.41 ^{cd}
		NSF	4.24±0.20 ^{acdf}		3.76±0.26 ^{be}	3.33±0.29 ^g
pH	2010	NSR	3.65±0.01 ^a		3.95±0.04 ^b	4.22±0.06 ^{cd}
		SR	3.60±0.05 ^a		3.96±0.10 ^{be}	4.18±0.06 ^c
		SF	3.61±0.04 ^a		4.06±0.06 ^e	4.29±0.09 ^d
		NSF	3.70±0.06 ^a		3.94±0.10 ^b	4.24±0.03 ^{cd}
	2011	NSR	3.48±0.03 ^{ab}	3.62±0.02 ^{ac}	3.95±0.05 ^{de}	
		SR	3.42±0.03 ^b	3.58±0.03 ^{ac}	3.81±0.11 ^{df}	
		SF	3.67±0.06 ^{cf}	3.56±0.22 ^{abc}	4.15±0.03 ^g	
		NSF	3.7±0.05 ^{cf}	3.8±0.05 ^f	4.04±0.11 ^{eg}	
YAN (g/L)	2010	NSR	213.33±15.28 ^a		266.67±25.17 ^{bc}	
		SR	353.33±37.86 ^d		370.00±26.46 ^{ab}	
		SF	333.33±40.41 ^d		370.00±81.85 ^d	420.00±62.45 ^d
		NSF	306.67±5.77 ^b		276.67±15.28 ^{ac}	270.00±26.46 ^{ab}
YAN (g/L)	2011	NSR	200±0 ^{ab}	213.33±11.55 ^a	180±20 ^b	
		SR	226.67±23.09 ^{ac}	260±10 ^{de}	220±0 ^a	

Parameter	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
		SF	266.67±28.87 ^{df}	340±20 ^g	350±17.32 ^g	
		NSF	226.67±28.87 ^{ae}	260±10 ^{cd}	233.33±32.15 ^{ae}	
Monomeric flavan-3-ols (mg/L)	2010	NSR	35.68±27.73 ^a		89.38±4.10 ^{bc}	101.03±50.26 ^{bde}
		SR	43.34±12.44 ^{af}		103.22±21.43 ^{bde}	114.89±26.13 ^{bd}
		SF	23.47±5.99 ^a		84.98±49.74 ^b	139.01±27.67 ^d
		NSF	60.21±12.78 ^{ace}		53.21±9.22 ^{ac}	100.96±20.05 ^{bd}
Total phenols (AU) (‡)	2010	NSR	40.4±0.04 ^{abc}		57.57±0.04 ^d	
		SR	43.43±0.04 ^{ace}		59.59±0.07 ^d	
		SF	38.38±0.03 ^{ab}		47.47±0.07 ^e	
		NSF	37.37±0.02 ^b		45.45±0.04 ^{ce}	
Total phenols (AU)	2011	NSR	37.28±0.03 ^{ab}	41.64±0.01 ^{abc}	61.02±0.07 ^d	
		SR	39.39±0.08 ^{ab}	45.81±0.04 ^{ce}	62.07±0.01 ^d	
		SF	35.01±0.06 ^{af}	42.04±0.02 ^{bc}	47.11±0.04 ^{ce}	
		NSF	34.69±0.02 ^a	41.69±0.02 ^b	49.15±0.02 ^e	
Polymeric phenols (mg/L)	2011	NSR	235.79±13.05 ^{ab}	315.92±20.63 ^{ac}	740.45±65.38 ^d	
		SR	199.13±39.83 ^b	347.48±42.22 ^{ce}	687.9±46.79 ^{df}	
		SF	285.94±52.13 ^{abc}	320.03±33.77 ^{ac}	467.55±52.35 ^g	
		NSF	274.55±35.49 ^{abe}	372.21±36.55 ^c	637.68±111.37 ^f	
Tannin (mg/L CE)	2010	NSR	138.92±83.30 ^{ab}		362.25±20.98 ^c	144.08±103.27 ^{ab}
		SR	102.09±38.03 ^a		253.16±47.21 ^{bc}	314.85±46.73 ^{cd}
		SF	70.83±43.80 ^a		137.40±96.28 ^{ab}	224.21±11.90 ^{bde}
		NSF	90.36±25.37 ^a		171.22±109.93 ^{ab}	123.02±94.75 ^{ae}
	2011	NSR	475.91±12.75 ^{ab}	485.38±26.73 ^{ab}	673.94±68.04 ^d	
		SR	475.87±32.47 ^{ab}	517.19±50.59 ^a	503.30±20.85 ^{ac}	
		SF	424.43±48.02 ^b	449.76±32.43 ^{ab}	467.57±22.59 ^{ab}	
		NSF	433.29±13.11 ^{bc}	493.23±45.45 ^{ab}	426.76±64.41 ^b	
Total Flavonols (mg/L)	2011	NSR	92.5±9.75 ^a	87.68±18.86 ^{ab}	114.02±9.13 ^{cd}	
		SR	84.78±28.96 ^{ab}	94.49±21.17 ^{ac}	126.95±16.25 ^d	

Parameter	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
		SF	40.33±8.92 ^{ef}	46.32±7.09 ^{efg}	43.44±7.9 ^{efg}	
		NSF	39.8±3.69 ^e	54.97±4.05 ^{fg}	65.29±8.91 ^{bg}	
Copigments (AU)	2010	NSR	0.86±0.05 ^{ab}		1.18±0.06 ^c	0.70±0.09 ^{de}
		SR	0.88±0.03 ^a		1.19±0.18 ^c	0.54±0.11 ^f
		SF	0.82±0.05 ^{abd}		0.90±0.04 ^a	0.59±0.15 ^{ef}
		NSF	0.79±0.08 ^{abd}		0.85±0.09 ^{abd}	0.71±0.01 ^{bde}
Total Anthocyanins (mg/L) (‡)	2010	NSR	764.87±131.48 ^a		842.48±157.12 ^b	
		SR	846.83±150.19 ^b		899.85±167.67 ^b	
		SF	744.16±128.70 ^a		718.00±146.51 ^a	
		NSF	734.44±86.91 ^a		699.92±181.58 ^a	
Total Anthocyanins (mg/L)	2010	NSR	934.35±59.32 ^{ab}		1022.58±93.10 ^{ac}	566.49±109.74 ^{de}
		SR	1026.96±31.70 ^{ac}		1103.72±54.27 ^c	455.29±149.10 ^d
		SF	886.81±65.78 ^{ab}		893.38±114.49 ^{ab}	448.34±113.94 ^d
		NSF	828.04±49.53 ^b		933.92±22.32 ^{ab}	668.31±107.48 ^e
Monomeric anthocyanins (mg/L) (‡)	2010	NSR	594.31±144.73 ^a		613.16±166.94 ^a	
		SR	641.41±162.58 ^a		640.19±192.03 ^a	
		SF	587.36±125.42 ^a		484.59±114.71 ^b	
		NSF	596.77±143.63 ^a		479.14±130.18 ^b	
Monomeric anthocyanins (mg/L)	2010	NSR	777.69±72.84 ^{ab}		796.04±123.43 ^{ab}	360.44±137.73 ^{cd}
		SR	839.83±88.96 ^a		879.67±81.59 ^a	208.86±139.10 ^c
		SF	728.88±67.70 ^{ab}		623.62±30.34 ^{be}	229.66±127.15 ^c
		NSF	769.19±104.40 ^{ab}		646.64±53.12 ^b	469.31±125.90 ^{de}
Polymeric pigments (mg/L)	2011	NSR	27.36±2.10 ^a	35.33±0.57 ^{ab}	80.14±13.03 ^c	
		SR	26.62±5.68 ^a	39.96±6.32 ^b	75.22±1.13 ^c	
		SF	38.41±8.45 ^b	42.72±1.71 ^b	59.06±5.13 ^d	

Parameter	Season	Treatment	Harvest			
			Unripe	Medium ripe	Ripe	Overripe
Total pigments (AU)	2010	NSF	35.44±3.29 ^{ab}	45.33±3.56 ^b	70.44±10.06 ^c	
		NSR	38.38±0.02 ^{ab}		44.44±0.03 ^{ac}	28.28±0.04 ^{de}
		SR	40.40±0.01 ^{af}		48.48±0.03 ^c	26.26±0.07 ^d
		SF	35.35±0.02 ^{bfg}		38.38±0.06 ^{abg}	26.25±0.06 ^d
		NSF	32.32±0.02 ^{be}		37.37±0.02 ^{bfg}	31.31±0.03 ^{deg}
MCD (AU)	2010	NSR	15.61±0.12 ^{abc}		24.25±0.17 ^d	16.60±0.08 ^{abc}
		SR	17.45±0.11 ^{abe}		23.65±0.21 ^d	15.79±0.01 ^{abc}
		SF	14.74±0.20 ^{ac}		18.57±0.29 ^b	16.87±0.37 ^{abe}
		NSF	13.62±0.11 ^c		17.77±0.12 ^{ab}	14.28±0.17 ^{ce}

** : Expressed as g/L of tartaric acid

All values displayed in the table, except at certain parameters (‡), are the means of three repeats after alcoholic fermentation, with the standard deviation expressed after '±'. Values at ‡ indicate means for three repeats over three stages of analysis during the winemaking process

* Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter.

Abbreviations: TSS: total soluble solids; YAN: yeast assimilable nitrogen; AU: absorbance units; CE: catechin equivalents; MCD: modified colour density

Table 7 Total flavonol and polymeric phenol values of wines from the unripe and ripe harvest in 2010 after alcoholic fermentation (AF), malolactic fermentation (MLF) and six months' ageing (only parameters that show a significant second-order interaction, $p \leq 0.05$, between the harvest stages and times of analysis are shown. There were no significant third-order interaction between the harvest stages, treatments and times of analysis for these parameters).

Parameter	Season	Harvest	Time of analysis		
			AF	MLF	6 months
Polymeric phenols (mg/L)	2010	Unripe	*235.25±80.01 ^a	283.00±27.87 ^b	169.48±21.23 ^c
		Ripe	593.83±119.33 ^d	685.90±120.93 ^e	421.90±65.30 ^f
Total flavonols (mg/L)	2010	Unripe	41.67±9.67 ^a	36.39±6.46 ^b	18.04±4.37 ^c
		Ripe	54.83±17.13 ^d	44.31±13.79 ^{ab}	17.99±6.06 ^c

All values displayed in the table are the means of four treatments (NSR, NSF, SF, SR), each of which has three field repeats, with the standard deviation expressed after '±'

* Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter

Table 8 Polymeric pigment values (mg/L) for the unripe and ripe harvest stages of each treatment of the 2010 wines after alcoholic fermentation (AF), malolactic fermentation (MLF) and six months' ageing, showing a significant second-order interaction, $p \leq 0.05$, between treatments and times of analysis. There was no significant third-order interaction between treatments, harvest stages and times of analysis for this parameter.

Parameter	Season	Treatment	Time of analysis		
			AF	MLF	6 months
Polymeric pigments (mg/L)	2010	NSR	*45.77±29.07 ^{ab}	62.42±36.17 ^{cd}	56.75±32.87 ^{ef}
		SR	65.81±37.73 ^{cddeg}	73.75±42.77 ^{ce}	62.37±40.24 ^{abdf}
		SF	51.47±24.94 ^{abfg}	59.23±32.65 ^{ace}	46.44±28.59 ^{bf}
		NSF	49.98±17.94 ^{afg}	58.83±28.50 ^{bce}	47.76±22.32 ^{af}

All values displayed in the table are the means of three repeats over two harvest stages, with the standard deviation expressed after '±'

* Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter

Table 9 Phenolic and colour parameter values of wines from the unripe, medium ripe, ripe and overripe stages only after alcoholic fermentation (in the case of wine parameters) (only parameters that show a significant first-order interaction, $p \leq 0.05$, between harvest stages and for which there was no significant second-order interaction are shown).

Parameter	Season	Harvest			
		Unripe	Medium ripe	Ripe	Overripe
TSS (°B)	2011	*22.4±0.92 ^a	23.3±0.85 ^b	25.6±1.20 ^c	
Titrateable acidity**	2011	5.19±0.61 ^a	4.86±0.60 ^b	4.23±0.57 ^c	
Monomeric flavan-3-ols (mg/L)	2011	10.25±1.99 ^a	10.00±2.45 ^a	12.86±3.33 ^b	
Total Phenols (AU)	2010	43.43±0.04 ^b		57.57±0.08 ^a	55.55±0.04 ^a
Polymeric phenols (mg/L)	2010	235.25±80.01 ^a		593.83±119.33 ^b	1011.84±276.56 ^c
SO ₂ -resistant pigments (AU)	2010	1.3±0.03 ^a		2.6±0.08 ^b	3.3±0.10 ^c
SO ₂ -resistant pigments (AU)	2011	1.3±0.03 ^b	1.5±0.03 ^a	1.6±0.03 ^a	
Total flavonols (mg/L)	2010	41.67±9.67 ^a		54.83±17.13 ^b	27.10±6.60 ^c
Copigments (AU)	2011	0.77±0.15 ^a	0.87±0.13 ^b	1.04±0.14 ^c	
Total anthocyanins (mg/L)	2011	710.28±123.83 ^a	802.40±82.24 ^b	905.42±116.56 ^c	
Monomeric anthocyanins (mg/L)	2011	547.63±101.87 ^a	613.66.67 ^b	667.61±87.68 ^c	
Total pigments (mg/L)	2011	27.27±0.05 ^a	31.31±0.03 ^b	37.37±0.05 ^c	
Polymeric pigments (mg/L)	2010	30.67±10.22 ^a		75.85±19.14 ^b	141.93±46.52 ^c
CD (AU)	2010	15.64±0.17 ^a		18.12±0.24 ^b	16.56±0.26 ^{ab}
CD (AU)	2011	14.60±0.28 ^a	15.83±0.22 ^a	20.10±0.31 ^b	
MCD	2011	11.06±0.29 ^a	12.29±0.23 ^{ab}	12.69±0.28 ^b	

** : Expressed as g/L of tartaric acid

All values displayed in the table are the means of four treatments, each of which has three field repeats, with the standard deviation expressed after '±'

* Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter.

Abbreviations: TSS: total soluble solids; AU: absorbance units; CD: colour density; MCD: modified colour density

Table 10 Treatment differences in phenolic and colour parameters in wines from the 2011 season after alcoholic fermentation (only parameters that show a significant first order interaction, $p \leq 0.05$, between treatments and for which no significant second-order interaction existed, are shown).

Parameter	Season	Treatment			
		NSR	SR	SF	NSF
Titrateable acidity**	2011	*4.65±0.52 ^{ab}	5.34±0.62 ^c	4.90±0.57 ^{ac}	4.15±0.60 ^b
Monomeric flavan-3-ols (mg/L)	2011	13.22±2.85 ^a	12.99±2.12 ^a	8.84±1.38 ^b	9.10±1.64 ^b
Copigments (AU)	2011	0.96±0.12 ^{ab}	1.02±0.17 ^a	0.83±0.16 ^{bc}	0.76±0.13 ^c
Total anthocyanins (mg/L)	2011	829.02±99.54 ^a	926.74±143.53 ^c	738.50±90.35 ^{ab}	729.89±101.68 ^b
Monomeric anthocyanins (mg/L)	2011	628.88±57.06 ^b	708.96±99.66 ^c	555.88±65.50 ^a	545.18±69.82 ^a
Total pigments (AU)	2011	32.32±0.060 ^a	36.36±0.07 ^a	29.29±0.04 ^b	29.29±0.04 ^b
CD (AU)	2011	17.73±0.31 ^{ab}	19.32±0.40 ^a	15.60±0.35 ^b	14.72±0.25 ^b

** : Expressed as g/L of tartaric acid

All values displayed in the table are the means of three harvest stages and three field repeats, with the standard deviation expressed after '±'. * Different letters indicate significant differences according to LSD ($p < 0.05$) for the specific parameter

Abbreviations: AU: absorbance Units; CD: colour density

Table 11 Sensory attribute intensities perceived by the trained panel and ANOVA results for the unripe and ripe harvest stages evaluated in the Shiraz 2010 wine after six months' ageing.

	Shiraz 2010	
	Harvest stage	
	Unripe (A)	Ripe (B)
AROMA		
Stinky	17.39a*	25.53a
Fresh berries	28.60a	18.10b
Berry jam	16.81a	24.11a
Vegetative	12.92a	19.90a
Raisin	6.68b	9.25b
Spicy	7.70c	9.85b
Smoky/savoury	12.75a	17.18a
TASTE AND MOUTHFEEL		
Bitter	3.67b	8.40a
Astringency	23.21b	32.22a
Body and fullness	25.61b	39.32a
Colour Intensity	38.79b	64.85a

*Means with different letters (a, b) in a row are significantly different ($p < 0.05$)

Table 12 Sensory attribute intensities as perceived by the trained panel and ANOVA results for the different wine treatments from the unripe harvest (A) evaluated in the Shiraz wine from the 2010 vintage.

	Shiraz 2010			
	Unripe harvest (A)			
	Treatments			
	NSR	SR	SF	NSF
AROMA				
Stinky	15.02 ^{b*}	31.83 ^a	13.23 ^b	9.27 ^b
Fresh berries	33.55 ^a	20.16 ^b	29.88 ^a	30.89 ^a
Berry jam	18.70 ^a	13.62 ^a	17.54 ^a	17.38 ^a
Vegetative	8.87 ^b	19.94 ^a	12.08 ^b	10.79 ^b
Raisin	6.52 ^b	8.25 ^a	7.19 ^{ba}	4.86 ^c
Spicy	9.08 ^a	6.88 ^a	8.15 ^a	6.72 ^a
Smoky/savoury	10.58 ^b	21.16 ^a	10.83 ^b	8.42 ^b
TASTE AND MOUTHFEEL				
Bitter	3.87 ^a	4.18 ^a	3.23 ^a	3.37 ^a
Astringency	24.04 ^{ba}	25.46 ^a	19.94 ^b	23.39 ^{ba}
Body and fullness	25.32 ^{ba}	27.59 ^a	24.22 ^b	25.27 ^{ba}
Colour Intensity	40.71 ^{ba}	44.14 ^a	33.37 ^c	36.93 ^{bc}

*Means with different letters (a, b) in a row are significantly different ($p < 0.05$)

Table 13 Sensory attribute intensities as perceived by the trained panel and ANOVA results for the different wine treatments from the ripe harvest evaluated in the Shiraz wine from the 2010 season.

	Shiraz 2010			
	Ripe harvest (B)			
	Treatments			
	NSR	SR	SF	NSF
AROMA				
Stinky	13.48 ^{b*}	40.14 ^a	25.35 ^{ba}	23.14 ^{ba}
Fresh berries	19.17 ^a	17.63 ^a	15.81 ^a	19.80 ^a
Berry jam	27.73 ^a	19.02 ^a	24.08 ^a	25.60 ^a
Vegetative	10.79 ^a	25.25 ^a	20.15 ^a	23.41 ^a
Raisin	13.29 ^a	5.89 ^b	9.53 ^{ba}	8.20 ^{ba}
Spicy	10.42 ^a	8.04 ^b	10.01 ^a	10.92 ^a
Smoky/savoury	17.73 ^a	21.35 ^a	14.23 ^a	15.33 ^a
TASTE AND MOUTHFEEL				
Bitter	7.99 ^a	11.89 ^a	7.77 ^a	5.97 ^a
Astringency	33.53 ^a	34.09 ^a	28.58 ^b	32.69 ^a
Body and fullness	41.41 ^a	42.37 ^a	36.33 ^a	37.16 ^a
Colour intensity	72.20 ^a	69.90 ^a	54.48 ^a	62.68 ^a

*Means with different letters (a, b) in a row are significantly different ($p < 0.05$).

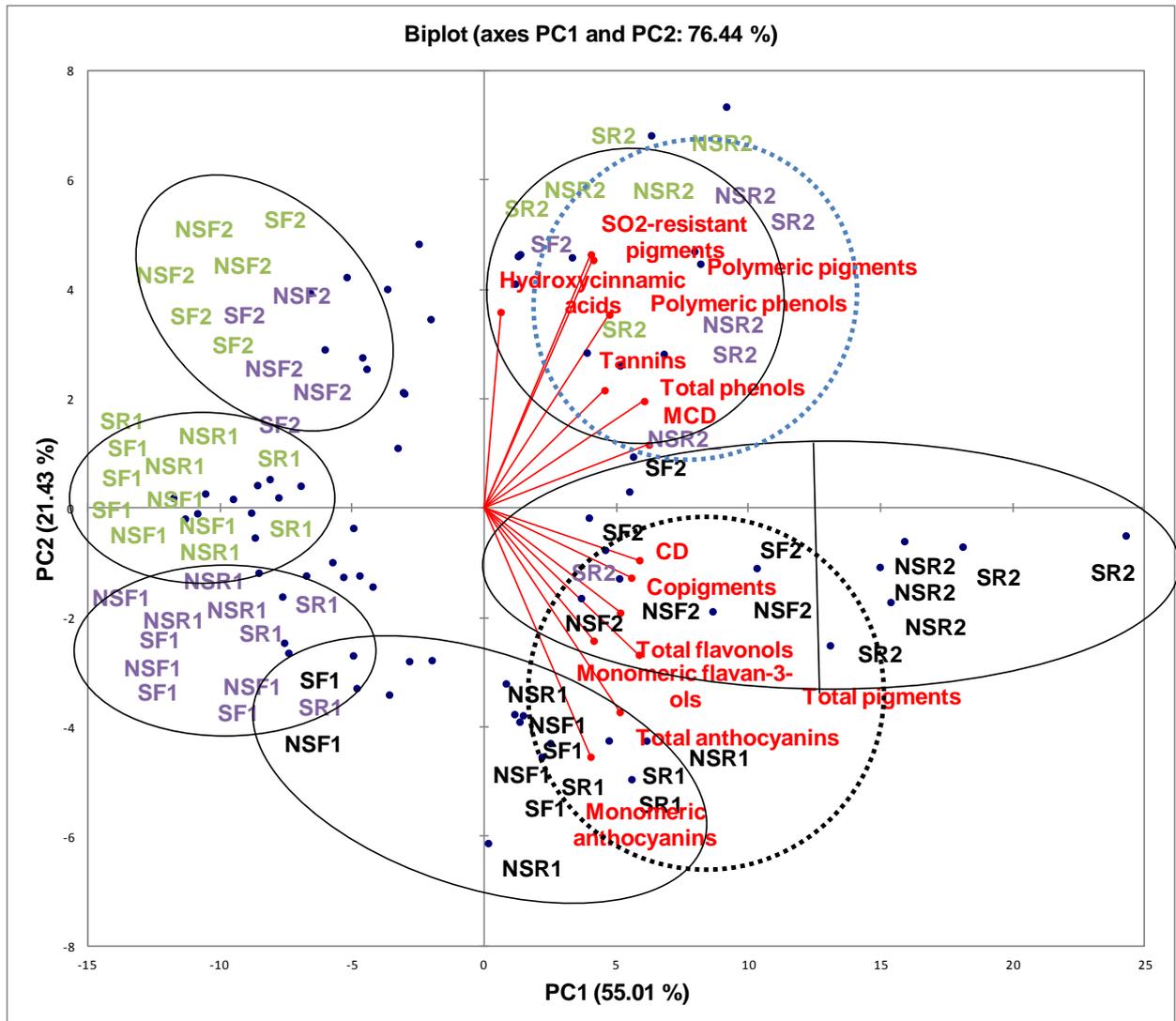


Figure 1 The PCA biplot for Shiraz wines from the unripe (1) and ripe (2) harvest stages of the 2010 vintage, based on the combination of phenolic parameters measured after alcoholic fermentation (AF) (samples indicated in black), malolactic fermentation (MLF) (samples indicated in purple) and six months' ageing (samples indicated in green). Samples include all three replicates of each treatment (NSR, NSF, SF and SR). Parameters grouping together in the bottom right part of the PCA are circled with a black dashed line (Group A), and parameters grouping together in the top right part of the PCA are circled with a blue dashed line (Group B). Abbreviations: MCD: modified colour density; CD: colour density

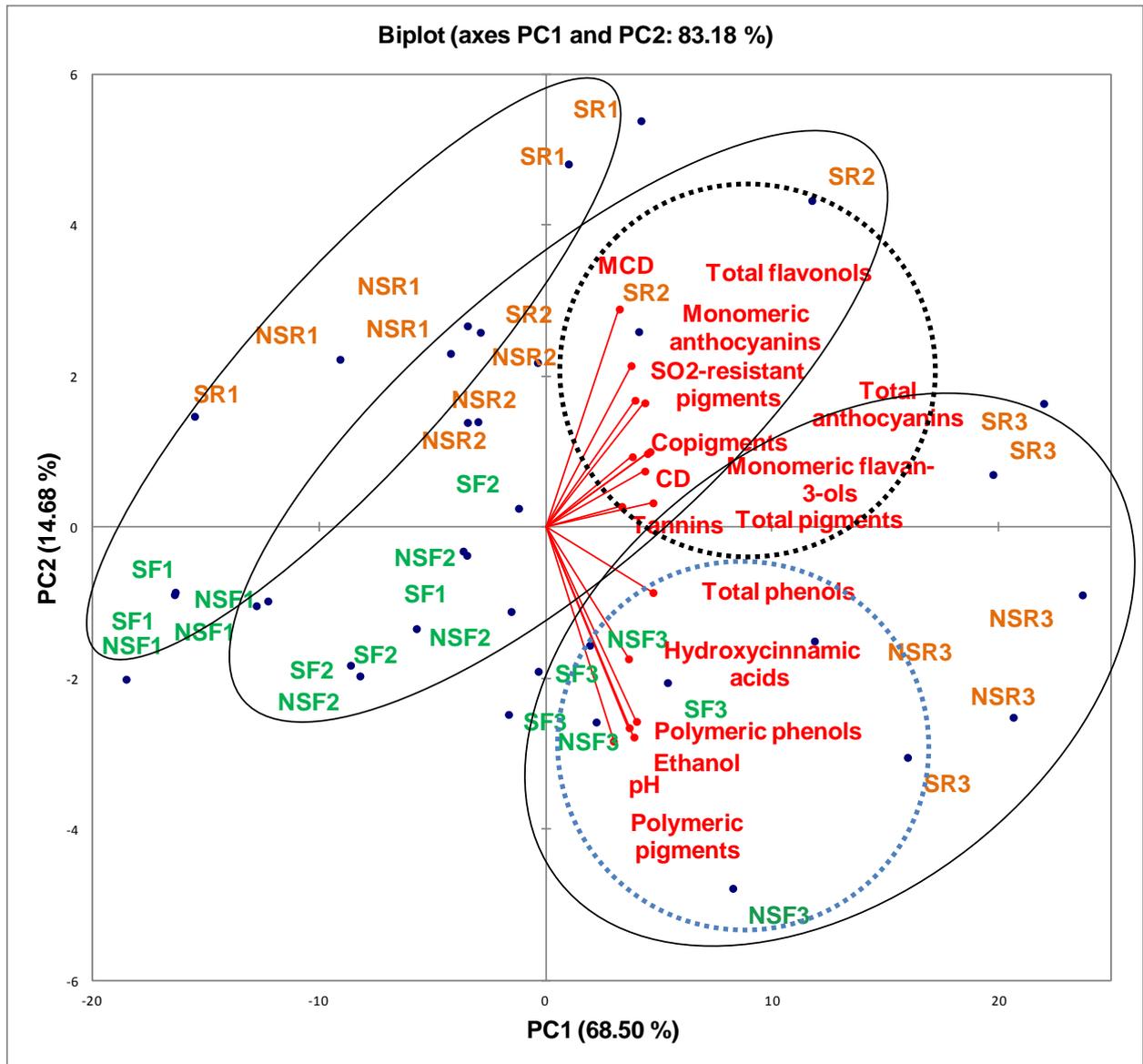


Figure 2 The PCA biplot for Shiraz wines from the unripe (1), medium ripe (2) and ripe harvest (3) stages of the 2011 vintage based on the combination of phenolic parameters measured after alcoholic fermentation (AF). Samples include all three replicates of each treatment (NSR, NSF, SF and SR). Parameters grouping together in the top right part of the PCA are circled with a black dashed line (Group A) and parameters grouping together in the bottom right part of the PCA are circled with a blue dashed line (Group B). Abbreviations: MCD: modified colour density; CD: colour density.

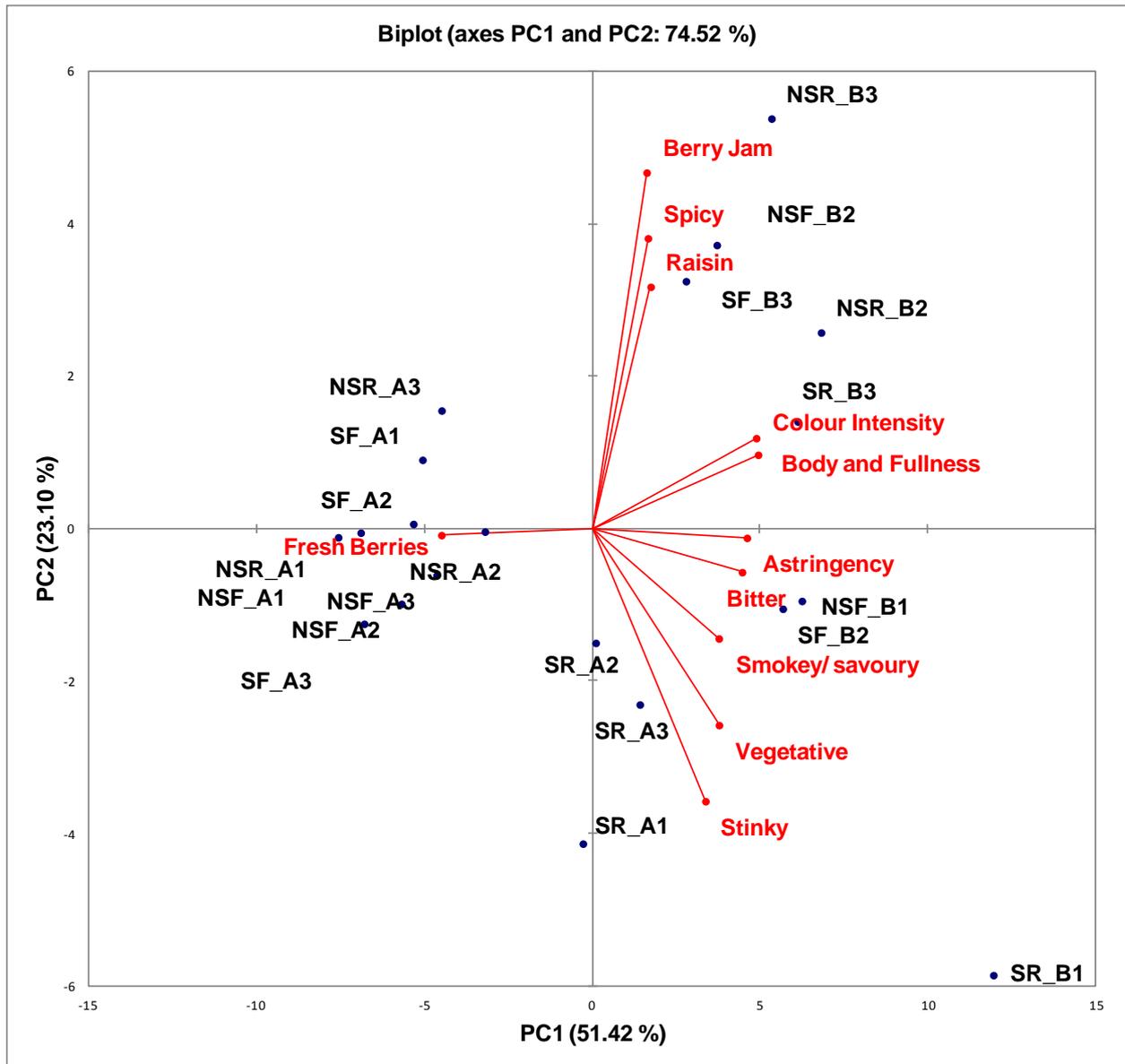


Figure 3 The PCA biplot for the 2010 Shiraz wine samples of the unripe (A) and ripe (B) harvest stages based on the perceived sensory attributes.

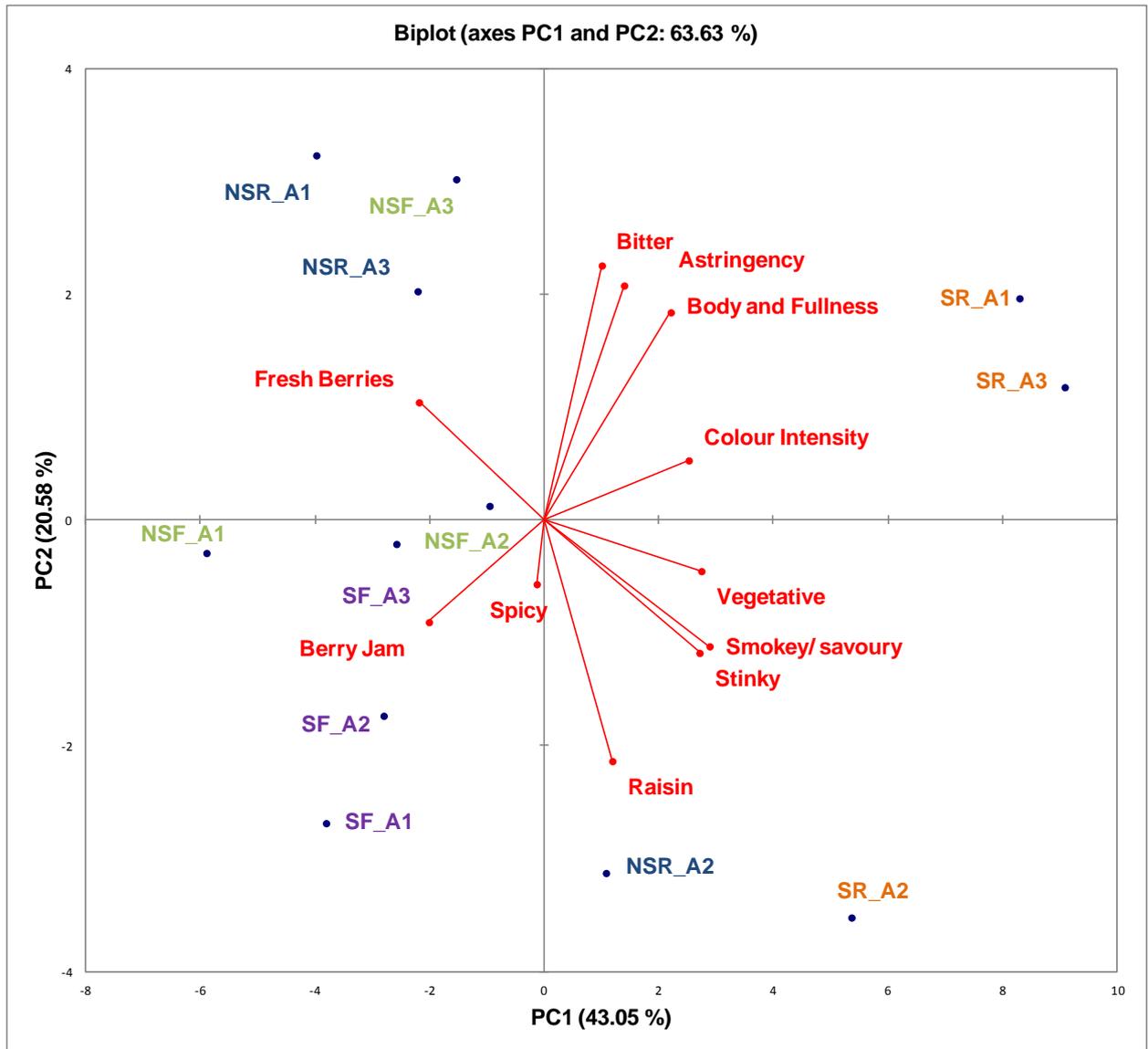


Figure 4 The PCA biplot for Shiraz samples from the 2010 unripe harvest (A) based on the perceived sensory attributes.

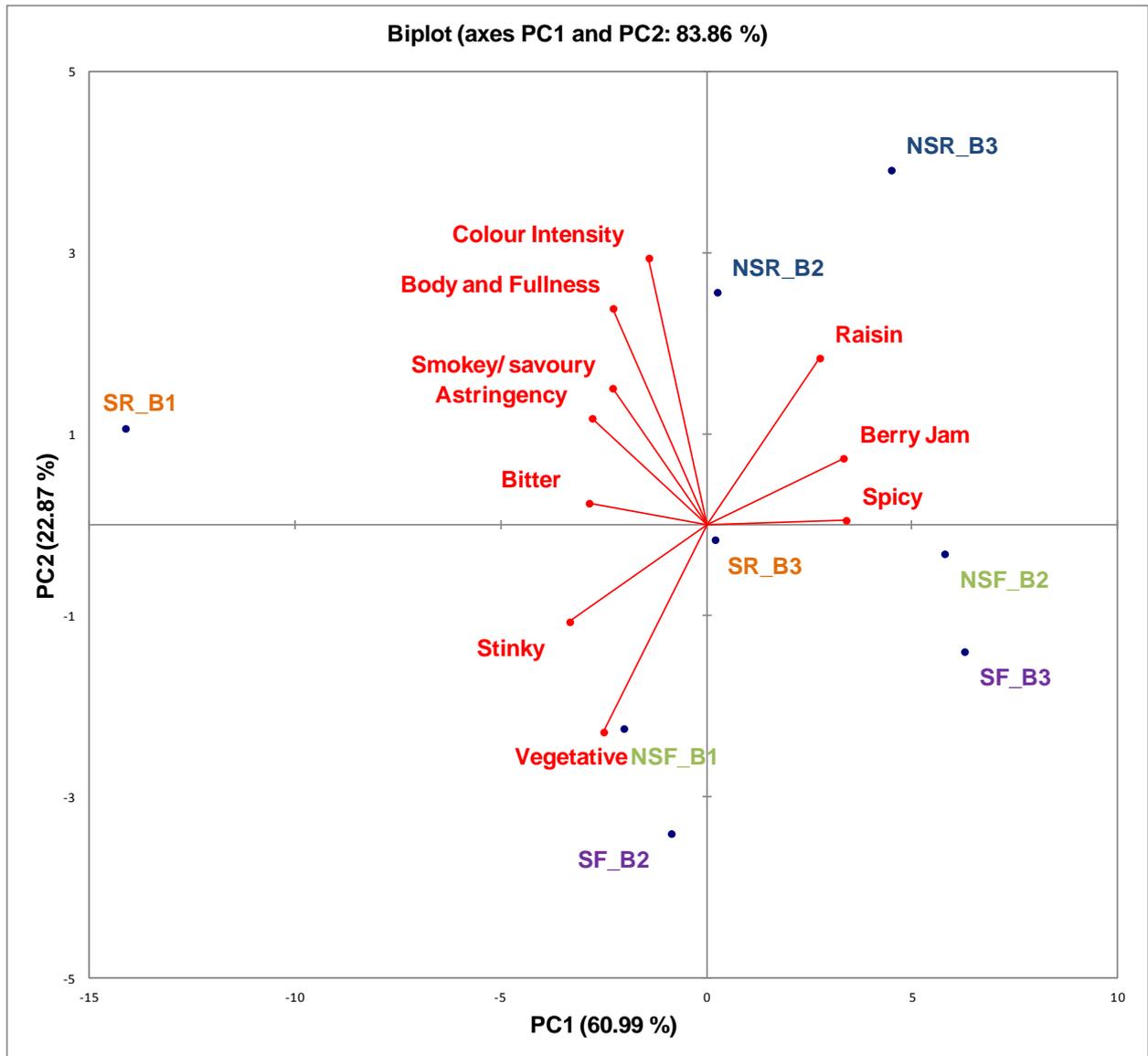


Figure 5 The PCA biplot for Shiraz samples from the 2010 ripe harvest (B) based on the perceived sensory attributes.

Chapter 5

General discussion and conclusions

5. General discussion and conclusions

5.1 CONCLUSIONS AND FUTURE PROSPECTS

In South Africa, grapevines that are too vigorous could result in excessively dense canopies, which may have a negative effect on the quantity and quality of the grapes produced. Viticultural practices, such as judicious canopy management and irrigation regimes, are employed to control vine vigour and yield and to improve fruit ripening and colour development (Hunter *et al.*, 2004).

The overall aim of this study was to investigate the interactive effect of canopy modification and water deficit on grape and wine flavonoid composition and wine sensory properties. There are a large number of studies on the effects of sunlight exposure and water deficit on grape flavonoid composition, but the reflection of these effects in the resulting wine after alcoholic fermentation, malolactic fermentation and ageing needs further attention.

This study investigated the interactive effect of shoot removal (performed during flowering) and water deficit on grape flavonoid composition and extractability in a vigorous Shiraz vineyard in the Stellenbosch area. The effects of these viticultural treatments were also investigated in the resulting wine. Generally, a reduction in canopy density increased the concentration of certain flavonoids in the grapes (tannins, total phenol index values and total flavonols), and increased the concentrations of certain flavonoids (total monomeric flavan-3-ols, total phenols, polymeric phenols, tannins, total flavonols, copigments, total anthocyanins and total pigments) as well as colour intensity, astringency and body in the resulting wines. These observations, however, were not significant at all harvest stages, seasons and stages during the winemaking process. Increased concentrations of certain flavonoids in the wines from the reduced canopy treatment were also maintained for an ageing period of six months. In some cases, higher concentrations of flavonols, monomeric flavan-3-ols and monomeric anthocyanins in the grapes from the reduced canopy treatment were also reflected in the resulting wines. Continuous secondary shoot removal in the bunch zone intensified the light/temperature effect on flavonoid accumulation, while the water deficit effect on most phenolic parameters measured in the grapes and wine was not as prominent as that of the canopy manipulation treatment. However, greater areas for transpiration in the full canopy treatment intensified the effect of water deficit on some parameters and at some harvest stages, which intensified the treatment differences more than in the case of canopies of which the size was reduced. Although wines from a reduced canopy treatment could generally have better colour intensity values and mouth feel properties than the wines from the full canopy treatment, the reduced canopy treatment grapes, when subjected to extreme water deficit, could result in wines with increased negative sensory descriptors such as stinky, savoury/smokey, vegetative and bitter. The importance of the correct vine water status in especially vineyards in which vine canopies is reduced, is therefore

emphasized in order to provide a wine with increased mouth feel but without negative flavour attributes.

Harvesting at different ripeness levels also affected the chemical and phenolic composition of the grapes and resulting wines. A general decrease in berry volume, fresh mass and titratable acidity was observed at a riper stage, while total soluble solids and certain polymeric fractions of phenolics increased in the grapes with ripening. An increase in these polymeric fractions seemingly also was observed in the resulting wines, with anthocyanin levels also varying in the wines made from different ripeness levels. These changes resulted in more astringent wines with higher colour density values and improved mouthfeel properties. Although harvesting at an overripe stage seemed to have led to very high percentages of extractable anthocyanins, it was also shown to result in grapes and wine with lower monomeric anthocyanin levels, possibly due to breakdown reactions occurring in these grapes.

It is a common fact that red wine quality and style are among the key drivers of consumer choice. This study improved our understanding of how an improvement in the microclimate in very dense Shiraz canopies could be reflected in the phenolic composition of wines, and how this could help to aim for a certain wine style. This could possibly be used to fulfil consumer and market demands in the future.

This was one of the first studies conducted under South African conditions to investigate the interactive effect of canopy manipulation and water deficit on the colour and phenolic composition of Shiraz wine. Similar studies only investigated the phenolic and colour composition after alcoholic fermentation, but our study included analyses after malolactic fermentation and six months' ageing, which are winemaking steps often employed by the industry when producing red wine. This study could therefore supply South African wine producers with valuable information regarding the cultivation and production of Shiraz grapes and wine in the Stellenbosch region.

Due to different microclimate conditions obtained by different canopy manipulations, future studies could investigate the effects of shoot or leaf removal, applied at different stages during the growing season, on the phenolic and sensory properties of the resulting wines. These effects could also be investigated in different wine regions and terroirs, and on different cultivars, row directions and trellising systems, to provide wine producers with guidelines on how to improve the grape microclimate under specific conditions. Future work could also include ageing of the wines in barrels in order for the results to be more representative of industrial winemaking conditions.

5.2 REFERENCES

- Hunter, J.J., Volschenk, C.G., Marais, J. & Fouché, G.W., 2004. Composition of Sauvignon blanc grapes as affected by pre-véraison canopy manipulation and ripeness level. *South African Journal of Enology and Viticulture* 25, 13-18.