INFLUENCE OF SUSTAINED DEFICIT IRRIGATION ON PHYSIOLOGY AND PHENOLIC COMPOUNDS IN WINEGRAPES AND WINE

Yasmin Michelle Chalmers

School of Agriculture and Wine
Discipline of Wine and Horticulture
The University of Adelaide

A thesis submitted to The University of Adelaide in fulfilment of the requirement for the degree of Doctor of Philosophy

February 2007

TABLE OF CONTENTS

ABSTRACT **iv**
DECLARATION **vii**
ACKNOWLEDGEMENTS **viii**
LIST OF ABBREVIATIONS **ix**
LIST OF FIGURES **xi**
LIST OF TABLES **xvi**

CHAPTER 1: General Introduction **1**
1.1 Background 1
1.2 Deficit Irrigation Strategies for Winegrapes 3
1.3 Water Relations in Grapevines 6
1.3.1 Water deficit effects on grapevine physiology 7
1.4 Grape and Wine Quality 9
1.4.1 Water deficit effects on yield components and berry size 12
1.4.2 Water deficit effects on grape and wine composition 14
1.5 General Conclusions and Aims of the Study 16

CHAPTER 2: General Materials and Methods **17**
2.1 Field Sites 17
2.1.1 Trial design 19
2.2 Glasshouse Trials 22
2.3 Definitions 24
2.4 Statistical Analyses 24

CHAPTER 3: Leaf Water Potential, Stomatal Conductance and ABA Responses of Red Winegrapes to Water Deficit **26**
3.1 Introduction 26
3.2 Materials and Methods 28
3.2.1 Physiology and hormone measurements 28
3.2.2 Grapevine growth and canopy development 30
3.3 Results 32
3.3.1 Effect of SDI on grapevine physiology and hormonal responses 32
3.3.2 Leaf area index and pruning weights 46
3.4 Discussion 47
3.4.1 Physiological response of grapevine varieties to water deficit 48
3.4.2 Hormonal response of grapevine varieties to water deficit 52

3.4.3 LAI and pruning weights of SDI-treated vines 56
3.5 Conclusions 58

CHAPTER 4: Changes in Yield Components and Berry Composition of Red Wine Grapes Exposed to SDI 59
4.1 Introduction 59
4.2 Materials and Methods 61
4.2.1 Determination of yield components and berry composition parameters. . 61
4.3 Results 62
4.3.1 Effect of SDI on Cabernet Sauvignon 62
4.3.2 Effect of SDI on Shiraz 66
4.3.3 Interaction between SDI, yield components and berry composition parameters 71
4.4 Discussion 76
4.4.1 Effect of SDI on yield and its components 76
4.4.2 Effect of SDI on berry composition 79
4.4.3 Management of SDI in the vineyard 80
4.5 Conclusions 82

CHAPTER 5: Composition of Phenolic Compounds in Berries from SDI-Treated Vines 83
5.1 Introduction 83
5.2 Materials and Methods 85
5.2.1 Determination of total anthocyanins and total phenolics 85
5.2.2 Extraction of anthocyanins and flavonols from berry skins for HPLC analysis 87
5.2.3 HPLC analysis of anthocyanin and flavonol profiles 87
5.2.4 Total tannin analysis methods for berry skins 90
5.3 Results 91
5.3.1 Changes in phenolic concentration during berry ripening 91
5.3.2 Changes in tannin concentration during berry ripening 94
5.3.3 Differences in anthocyanin and phenolic composition at harvest 94
5.3.4 Differences in total tannin at harvest 100
5.3.5 Differences in flavonol content at harvest 100
5.3.6 Interaction between SDI, berry weight and phenolic composition 102
5.4 Discussion 104
5.4.1 Effect of SDI on anthocyanins and total phenolics 104
5.4.2 Effect of SDI on tannin concentrations 108
5.4.3 Effect of SDI on flavonol levels at harvest 109

5.4.4 Is it berry weight or SDI influencing phenolic composition of berries? 110
5.5 Conclusions 111

CHAPTER 6: Influence of SDI on Red Wine Composition 113
6.1 Introduction 113
6.2 Materials and Methods 115
6.2.1 Micro-wine ferments 115
6.2.2 Wine analyses 118
6.3 Results 123
6.3.1 Changes in red wine colour composition of SDI-treated wines 123
6.3.2 HPLC anthocyanidin profiles of SDI-treated wines 127
6.3.3 Changes in tannin concentration of SDI-treated wines 130
6.3.4 Influence of SDI on wine copigmentation 130
6.4 Discussion 133
6.4.1 Wine colour 134
6.4.2 Wine phenolic composition 135
6.4.3 Wine copigmentation 136
6.4.4 Influence of berry size on wine composition 138
6.5 Conclusions 139

CHAPTER 7: General Discussion and Conclusions 141
7.1 Physiology and Growth 141
7.2 Yield and Yield Components 144
7.2 Berry Composition 146
7.3 Wine Composition 147
7.5 Management and Economic Implications of SDI 150
7.6 Future Recommendations 154

REFERENCES 156

APPENDICES 172

ABSTRACT

Wine grape production in the semi-arid regions of Australia is successful due to the availability of irrigation water. Whilst water is a natural resource it is also becoming extremely valuable. In the hot and semi-arid regions of Australia, the prospect of water restrictions from drought and intensifying horticultural and domestic competition for water has prompted the grape and wine industry to implement strategic deficit irrigation practices to try and maintain sustainable wine grape production. Sustained deficit irrigation (SDI) differs significantly in its management to partial rootzone drying and regulated deficit irrigation and is a technique that could potentially be easily adopted across the winegrape industry if water allocations were reduced. With SDI, the water deficit is not created by withholding water, but rather, by applying a lesser volume of water at each irrigation event for the entire irrigation season.

This study aimed to understand the physiological behaviour of wine grape cultivars to SDI and how this deficit irrigation strategy would influence yield and composition of the grapes and wine. The trials were conducted during 2003-2006 on the cultivars Cabernet Sauvignon and Shiraz grafted to 140 Ruggeri (*V. berlandieri* x *V. rupestris*) rootstock and grown in the Murray-Darling region of Australia. Furthermore, while Cabernet Sauvignon and Shiraz are the main red winegrape varieties grown in the Murray-Darling region, anectodally they are observed to respond differently to hot, dry conditions when managed under similar irrigation regimes. The vines were drip irrigated providing 100% of estimated ET_c (control) and three graded sustained water deficits (Cabernet Sauvignon 70%, 52% and 43% of the control; Shiraz 65%, 45% and 34% of the control). For each season, the volume of actual water applied (ML/ha) was calculated for each irrigation treatment and varied depending on seasonal and vineyard conditions. To further explore vine responses to water deficit, glasshouse studies on four own-rooted *Vitis vinifera* L. cultivars, including Cabernet Sauvignon, Shiraz, Grenache and Tempranillo were also conducted.

Deficit irrigation management, whilst controlling vegetative growth and manipulating berry composition, may not always produce consistent outcomes among grapevine varieties. This has lead to the observation that deficit irrigation management strategies may need to be tailored to individual grape cultivars. Consequently, an understanding as to how certain

grapevine varieties respond to water deficit, particularly in relation to physiological responses, could assist with linking any impacts that water deficits may have on grape and wine composition. Field-grown Cabernet Sauvignon and Shiraz exposed to approximately 50% SDI experienced significant reductions in leaf water potential and stomatal conductance compared to the control. By contrast, xylem sap abscisic acid (ABA) levels increased significantly for the SDI-treated vines compared to the control that is probably related to root to shoot signals and canopy-derived ABA. Under field situations, Cabernet Sauvignon displayed physiological responses more typical of an isohydric-like (drought avoiding) vine, compared to the anisohydric-like (drought tolerant) responses of Shiraz. These responses may also be supported by the pattern of xylem sap [ABA] production. The differences in canopy development (leaf area index and pruning weights) for Cabernet Sauvignon and Shiraz may be a reflection of the isohydric-like and anisohydric-like responses of these grape varieties to water deficit, thereby influencing carbohydrate dynamics and long-term viability of vine health under SDI.

After three seasons, the SDI treatments significantly reduced yield of the field-grown vines, primarily due to a reduction in berry weight that tended to occur from the beginning of veraison through to harvest. SDI reduced yield (t/ha) by up to 30% in Cabernet Sauvignon and Shiraz, when applied at approximately 50% of the control irrigation (ML/ha). Irrespective of the yield reductions, water use efficiency was improved between 40-50% for the SDI-treated Cabernet Sauvignon and Shiraz, compared to the control. The lighter berries from SDI-treated vines tended to have increased pH and decreased titratable acid levels than the control. The SDI treatments applied at approximately 50% of the control increased the concentration of total anthocyanins in Cabernet Sauvignon and Shiraz berries by 22% and 15% respectively. As less water was applied there was an increase in total malvidin concentration for both varieties, with less effect on delphinidin, peonidin, petunidin and cyanidin for Cabernet Sauvignon and peonidin, petunidin, delphinidin and cyanidin for Shiraz. The increase in total anthocyanin and total phenolic concentrations for the SDI treatments than the control is attributed more to factors such as water deficit, canopy light penetration and/or changes in phenolic synthesis, than to differences in berry size (skin surface area to pulp volume ratio).

Differences in grape anthocyanins and phenolics between the irrigation treatments were not the same as those measured in the wine. A decrease in berry weight did not alter the skin

weight to berry weight ratios, and were therefore unlikely to be the cause of the altered composition of SDI wines. The increases in wine colour with SDI treatment may be the result of biochemical changes in the flavonoid pathway as a result of altered grapevine physiology responses to the SDI. Alternatively, the increases in red wine colour could possibly be due to a change in chemical properties of the anthocyanins to copigmented forms that may have influenced extractability efficiency during the winemaking or ageing process.

This research showed that an SDI of approximately 50% less water could be applied over one or two seasons with improvements in water use efficiency (t/ML) and berry composition compared to fully irrigated vines. Furthermore, for Cabernet Sauvignon exposed to 70% and 52% SDI there tended to be improvements in the overall wine composition and sensory ranking than the control. However from an economic perspective, net returns were not largely affected by using SDI based on the current grape prices. If water becomes a more highly valued resource and priced accordingly, then a larger increase in net return will result from SDI. Additionally, wineries would need to offer price incentives to produce lower yields that may result from adopting SDI. Overall, if the wine industry was faced with reductions in water allocations of 50% or more in a particular season, then the adoption of SDI may be a feasible solution to maintaining winegrape production for the short-term. Through understanding the translation of grape composition into wine, these findings should be able to provide additional knowledge to the Australian grape and wine industry as to how SDI can be used to manipulate grape composition for the production of sustainable wine styles.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed: ……. Date:……

Yasmin Chalmers

ACKNOWLEDGEMENTS

- My supervisors Assoc. Prof. Peter Dry, Dr. Brian Loveys, Dr. Mark Krstic and Dr. Mark Downey for their supervision and constructive comments.
- The Victorian State Government (Our Rural Landscapes) and the Grape and Wine Research and Development Corporation (GWRDC) for financial support.
- The Department of Primary Industries (DPI-Mildura) for use of the Shiraz trial site and laboratory facilities.
- Mr. John Webley for collaborative support and management of the Cabernet Sauvignon site, Dareton, NSW.
- The Victorian and Murray Valley Vine Improvement Association (VAMVVIA) and Mr. Julian Connellean for use of hot-water treatment and glasshouse facilities, and advice on glasshouse grown vines.
- Ms. Nardia Baker, Ms. Marica Mazza, Ms. Glenda Kelly for their help and enthusiasm with the field, glasshouse and laboratory trials.
- Dr. Masoud Edraki for management and irrigation technical advice of the DPI Shiraz site.
- Ms. Debra Partington for assistance with experimental design and biometrics.
- Ms. Sue Maffei and Dr. Chris Soar for their assistance with ABA analyses and advice on field and glasshouse physiology measures.
- Dr. Patrick Iland and Dr. Helen Holt for assistance and advice with performing micro-wine ferments.
- Ms. Lyn McMahon for assistance with proof reading.
- Ms. Florence Juillet, Ms. Sara Melillo and Mr. Fabien Collerais for their technical assistance with processing grape material.
- Dr. Fiona Constable for her invaluable technical advice, support and friendship.
- Thanks to my family and friends for help and encouragement.
- Finally, I thank my husband and children - Paul, Liam, Niah and Eva for their support, patience and love.

LIST OF ABBREVIATIONS

ABA	abscisic acid
au	absorbance units
^{o}C	degrees Celsius
CE	catechin equivalents
cm	centimetres
CS	Cabernet Sauvignon
df	degrees of freedom
Epan	pan evaporation
ET_c	crop evapotranspiration under
ET_o	reference crop evapotranspiration (grass reference crop)
g	grams
g_s	stomatal conductance
GC-MS	gas chromatography/mass spectroscopy
GDD	growing degree days
GR	Grenache
h	hour
ha	hectare
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
kg	kilograms
kPa	kilopascals
L	litre
LAI	leaf area index
LSD	least significant difference
LWP	leaf water potential
m	metre
mg	milligram
mm	millimetres
min	minute
mL	millilitre
ML	megalitre

MPa	megapascals
n	number of samples
ng	nanogram
nm	nanometre
ns	not significant
NSW	New South Wales
P	probability for data
pH	$-\log[H^+]$
ppm	parts per million
PRD	partial rootzone drying
r	correlation coefficient
r^2	coefficient of determination
RDI	regulated deficit irrigation
rpm	revolutions per minute
s	second
SC	stomatal conductance
SDI	sustained deficit irrigation
s.e.	standard error of the mean
SHZ	Shiraz
t	tonnes
TA	titratable acidity (g/L tartaric acid)
TE	Tempranillo
TSS	total soluble solids (^{o}Brix)
µL	microlitre
UV	ultra-violet
Vic	Victoria
VPD	vapour pressure deficit
WUE	water use efficiency
Ψ_l	leaf water potential

LIST OF FIGURES

Figure 1.1 Geographic location of the Murray-Darling region in Australia. Map provided by the Murray Valley Winegrape Growers Corporation (2005). **2**

Figure 1.2 Relationship of soil water tension (kPa) to a grapevine's response towards the available soil moisture (adapted from Goodwin, 1995). **4**

Figure 1.3 Schematic representation of the flavonoid pathway showing the production of anthocyanins, tannins and flavonols, including the general structure for each phenolic compound (adapted from Robinson & Walker, 2006). **10**

Figure 1.4 Sigmoidal pattern of canopy growth and grape berry development and ripening. The stages of water application are also depicted for the deficit irrigation techniques of regulated deficit irrigation (RDI), partial rootzone drying (PRD) and sustained deficit irrigation (SDI) (adapted from Coombe, 1992; Coombe & McCarthy, 2000). **13**

Figure 2.1 Satellite image showing location of Shiraz (Irymple, Vic) and Cabernet Sauvignon (Dareton, NSW) vineyards used for the field trials in the Murray-Darling region, Australia. Image provided by ©Google Earth, NASA, 2006. **17**

Figure 2.2 Cabernet Sauvignon (left) and Shiraz (right) are the premium, red wine grape varieties grown in the Murray-Darling region. Cultivars were grafted to 140 Ruggeri rootstock and trained on a two-wire vertical trellis. **18**

Figure 2.3 Irrigation design for the Cabernet Sauvignon and Shiraz SDI trial sites during 2003-2006. The insert shows the number of vines per treatment, with the test vines (shaded) in the middle row surrounded by the buffer vines. **20**

Figure 2.4 Cumulative Enviroscan data showing changes in soil water content (mm) for each irrigation treatment at the Shiraz trial site during 2004-2005. **21**

Figure 2.5 Cumulative Gbug data showing changes in soil matric potential (kPa) for Cabernet Sauvignon (100%, 70%, 52% and 43% irrigation treatments) and Shiraz (100%, 65% and 34% irrigation treatments) during 2004-2005. **22**

Figure 2.6 Glasshouse trials established in August 2004 (left) and August 2005 (right) on own-rooted Shiraz, Cabernet Sauvignon, Grenache and Tempranillo at the Department of Primary Industries. **23**

Figure 3.1 Diurnal changes in stomatal conductance (g_s) from Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse control (full irrigation) vines measured on 12 November 2005. LSD bars indicate significance at P=0.05. (n=5). **32**

Figure 3.2 Leaf water potential (Ψ_l) of leaves of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005 at A) predawn (0500 h) and B) 1400 h. Means for the control or deficit vines

followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10). **33**

Figure 3.3 Stomatal conductance (g_s) at 1400 h from leaves of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005. Means for the control or deficit vines followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10). **34**

Figure 3.4 Petiole xylem sap abscisic acid [ABA] collected from Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005 at A) predawn (0500 h) and B) 1400 h. Means for the control or deficit vines followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10). **35**

Figure 3.5 Linear regression of leaf water potential (Ψ_l) and stomatal conductance (g_s) of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured at 1400 h on the 22 and 28 November 2005. Regression lines represent both control and deficit treatments, (n=10). **36**

Figure 3.6 Linear regression of leaf water potential (Ψ_l) and xylem sap abscisic acid [ABA] of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured on the 22 and 28 November 2005 at A) 0500 h and B) 1400 h. Regression lines represent both control and deficit treatments, (n=10). **37**

Figure 3.7 Exponential regression of stomatal conductance (g_s) and abscisic acid [ABA] of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured on the 22 and 28 November 2005 at 1400 h. Regression lines represent both control and deficit treatments, (n=10). **38**

Figure 3.8 Diurnal leaf water potential (Ψ_l) for field-grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) irrigated at a full irrigation (100% control) and a deficit irrigation (43% or 45%). Measurements for Cabernet Sauvignon and Shiraz were taken on the 13 and 18 January 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference (P=0.05) at that time of day, (n=8). **39**

Figure 3.9 Diurnal stomatal conductance (g_s) for field-grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) irrigated at a full irrigation (100% control) and a deficit irrigation (43% or 45%). Measurements for Cabernet Sauvignon and Shiraz were taken on the 13 and 18 January 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference (P=0.05) at that time of day, (n=8). **40**

Figure 3.10 Diurnal leaf water potential (Ψ_l) for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference at that time of day (P=0.05), (n=8). **41**

Figure 3.11 Diurnal stomatal conductance (g_s) for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference at that time of day (P=0.05), (n=8). **41**

Figure 3.12 Diurnal xylem sap abscisic acid [ABA] levels for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Data points represent mean values ± s.e. (n=8). **42**

Figure 3.13 Linear regression of leaf water potential (Ψ_l) and xylem sap abscisic acid [ABA] of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control (100%) irrigation or SDI (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8). **43**

Figure 3.14 Linear regression of stomatal conductance (g_s) and abscisic acid (ABA) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control (100%) irrigation or SDI (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8). **43**

Figure 3.15 Relationship between adjusted leaf water potential (LWP - Ψ_l) and abscisic acid (ABA) measured at different times of the day (0900, 1130, 1400 and 1630 h) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8). **44**

Figure 3.16 Relationship between stomatal conductance (g_s) and abscisic acid [ABA] measured at different times of the day (0900, 1130, 1400 and 1630 h) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8). **45**

Figure 3.17 Leaf area index (LAI) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control and SDI treatments during 2005/2006 (Year 3). Significance indicated by LSD bar for irrigation treatment at P=0.05, (n=4). **46**

Figure 4.1 Effect of SDI on 2005 and 2006 mean berry ripening parameters for Cabernet Sauvignon exposed to a control and SDI irrigations 70%, 52% and 43%. A + B) total soluble solids, C + D) berry weight, and D + E) titratable acidity (dotted lines) and juice pH. LSD bars indicate treatment significance at P=0.05, (n=4). **65**

Figure 4.2 Effect of SDI on 2005 and 2006 mean berry ripening parameters for Shiraz exposed to control and SDI irrigations (65%, 45% and 34%). A + B) total soluble solids, C + D) berry weight, and D + E) titratable acidity (dotted lines) and juice pH. LSD bars indicate treatment significance at P=0.05, (n=4). **69**

Figure 4.3 Regression analyses of the quantity of irrigation water applied (ML/ha) and A) yield, B) berry weight, C) average bunch weight, D) bunch number, E) juice pH and F) titratable acidity for Cabernet Sauvignon (CS) and Shiraz (SHZ). Data points represent the treatment means for 2005 and 2006 seasons, (n=8). **74**

Figure 5.1 HPLC chromatogram of a Cabernet Sauvignon grape skin extract, recorded at 520nm and illustrating the separation and identification of the A) anthocyanins and B) flavonols measured during the analyses. **88**

Figure 5.2 Standard curve for calculating concentration of mg of malvidin-3-*O*-glucoside per mL of grape skin extract. **89**

Figure 5.3 Standard curve for calculating concentration of mg of quercitin-3-*O*-glucoside per mL of grape skin extract. **89**

Figure 5.4 Effect of SDI during berry ripening on berry total anthocyanin concentration (mg/g) and berry total phenolic concentration (au/g) for Cabernet Sauvignon exposed to a control and SDI irrigations 70%, 52% and 43%. A) 2005 berry total anthocyanin concentration (mg/g), B) 2006 berry total anthocyanin concentration (mg/g), C) 2005 berry total phenolic concentration (au/g) and D) 2006 berry total phenolic concentration (au/g). LSD bar indicates treatment significance at P=0.05, (n=4). **91**

Figure 5.5 Effect of SDI during berry ripening on berry total anthocyanin concentration (mg/g) and berry total phenolic concentration (au/g) for Shiraz exposed to a control and SDI irrigations 65%, 45% and 34%. A) 2005 berry total anthocyanin concentration (mg/g), B) 2006 berry total anthocyanin concentration (mg/g), C) 2005 berry total phenolic concentration (au/g) and D) 2006 berry total phenolic concentration (au/g). LSD bar indicates treatment significance at P=0.05, (n=4). **93**

Figure 5.6 Effect of SDI during berry ripening on the development of total skin tannin concentration (mg catechin equivalent/g skin) from A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) grape skins exposed to a control and SDI irrigations. Berries were collected between veraison and harvest in 2006. LSD bar indicates treatment significance at P=0.05, (n=4). **94**

Figure 5.7 Linear regression of total flavonols (mg/g of skin) vs leaf area index (leaf area:soil area) for Cabernet Sauvignon berries exposed to a control and SDI irrigations 70%, 52% and 43% during 2004-2006, (n=8). **101**

Figure 5.8 Regression analyses of A), C) and E) total anthocyanins (mg/g) and B), D) and F) total phenolics (au/g) against berry weight, amount of irrigation water applied (ML/ha) and yield per vine (kg) for Cabernet Sauvignon (CS) and Shiraz (SHZ). Data was pooled over 2005 and 2006 seasons, (n=8). **103**

Figure 6.1 Grapes were crushed and fermented in plastic containers with gas traps (above left). Ferments were plunged daily to ensure even skin contact during the maceration process (above right). Wine was pressed off into glass Schott bottles and capped with gas traps to allow the gross lees to settle before racking and cool storage (below left). After 2 weeks cool storage the wine was bottled (below right). **117**

Figure 6.2 Standard curve for calculating concentration of mg of malvidin-3-*O*-glucoside equivalents per litre of wine extract. **119**

Figure 6.3 Development of wine colour from Cabernet Sauvignon micro-ferments at bottling and 6 month age for A) wine colour (au), B) wine hue (A_{420}/A_{520}), C) total anthocyanins (mg/L) and D) total phenolics (au). Grapes were collected at the 2006 harvest from Control (100%), 70%, 52% and 43% SDI treatments. Means at bottling or 6 months followed by the same letter are not significantly different at P=0.05. Data are represented as mean values ± s.e. (n=4). **124**

Figure 6.4 Development of wine colour from Shiraz micro-ferments at bottling and 6 month age for A) wine colour (au), B) wine hue (A_{420}/A_{520}), C) total anthocyanins (mg/L) and D) total phenolics (au). Grapes were collected at the 2006 harvest from Control (100%), 65%, 45% and 34% SDI treatments. Means at bottling or 6 months followed by the same letter are not significantly different at P=0.05. Data are represented as mean values ± s.e. (n=4). **126**

Figure 6.5 Regression analyses of monomeric anthocyanins, anthocyanins in the copigment complex, polymeric pigments and total colour against the amount of irrigation water applied for Cabernet Sauvignon (CS) and Shiraz (SHZ) wines after 6 months. A) monomeric anthocyanins, B) copigment anthocyanins, C) polymeric pigment anthocyanins and D) total colour. (n=4) **131**

Figure 6.6 Percent colour due to monomeric, copigment complex and polymeric pigment anthocyanins of Cabernet Sauvignon wines at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 70%, 52% and 43% SDI treatments. **132**

Figure 6.7 Percent colour due to monomeric, copigment complex and polymeric pigment anthocyanins of Shiraz wines at bottling and 6 month age**.** Grapes were collected at the 2006 harvest from control (100%), 65%, 45% and 34% SDI treatments. **133**

Figure 7.1 Net return per hectare for the control and SDI treatments for A) Cabernet Sauvignon and B) Shiraz during 2003-2006. **152**

LIST OF TABLES

Table 1.1 Percentage of total wine, drying and table grape production for each state of Australia in 2005. Data were compiled from the Australian Bureau of Statistics vineyard survey (Australian Bureau of Statistics (ABS), 2006). **1**

Table 2.1 Irrigation regimes established for the Cabernet Sauvignon and Shiraz trial sites from November 2003 until December 2006. **19**

Table 3.1 Mean pruning weights for Cabernet Sauvignon and Shiraz taken during 2004-2006. Means followed by the same letter are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row. **47**

Table 4.1 Yield components and berry composition parameters from Cabernet Sauvignon bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (70%, 52% and 43% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row. **63**

Table 4.2 Water applied and WUE for the different irrigation treatments for Cabernet Sauvignon between 2004-2006. Figures followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row. **66**

Table 4.3 Yield component and berry composition parameters from Shiraz bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (65%, 45% and 34% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row. **67**

Table 4.4 Water applied and WUE for the different irrigation treatments for Shiraz between 2004-2006. Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row. **70**

Table 4.5 Correlation matrices of berry composition for Cabernet Sauvignon treated with A) 100% (control) irrigation, B) 70%, C) 52% and D) 43% SDI irrigation. Data were pooled over three seasons from 2004 to 2006. Since the df = 10, then $P<0.05$ when $r>0.576$ (no shade); $P<0.01$ when $r>0.708$ (grey shade). **72**

Table 4.6 Correlation matrices of berry composition for Shiraz treated with A) 100% (control) irrigation, B) 65%, C) 45% and D) 34% SDI irrigation. Data were pooled over three seasons from 2004 to 2006. Since the df = 10, then $P<0.05$ when $r>0.576$ (no shade); $P<0.01$ when $r>0.708$ (grey shade). **73**

Table 5.1 Solvent gradient for HPLC to separate anthocyanin profiles in grapes using 10% formic acid (solvent A) and 10% formic acid:methanol (solvent B). **89**

Table 5.2 Berry total anthocyanin and berry total phenolic content and concentration for Cabernet Sauvignon berries harvested in 2004-2006 and exposed to a full irrigation

(100% control) and SDI irrigations (70%, 52% and 43% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row. **95**

Table 5.3 Berry total anthocyanin and berry total phenolic content and concentration from Shiraz bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (65%, 45% and 34% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row. **96**

Table 5.4 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Cabernet Sauvignon berry skins collected at the 2005 harvest from Control (100%) and SDI treatments 70%, 52% and 43%. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8). **97**

Table 5.5 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Cabernet Sauvignon berry skins collected at the 2006 harvest from Control (100%) and SDI treatments 70%, 52% and 43%. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8). **98**

Table 5.6 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Shiraz berry skins collected at 2005 harvest from Control (100%) and SDI treatments 65%, 45% and 34% expressed as mg/g of skin. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8). **99**

Table 5.7 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Shiraz berry skins collected at 2006 harvest from Control (100%) and SDI treatments 65%, 45% and 34% expressed as mg/g of skin. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8). **99**

Table 5.8 Concentration of total tannins from Cabernet Sauvignon and Shiraz berry skins collected at the 2005 and 2006 harvest from Control (100%) and SDI treatments expressed as mg of catechin equivalent/g of skin. Means ± s.e. (n=8). **100**

Table 5.9 Concentration of skin total flavonols from Cabernet Sauvignon and Shiraz berry skins collected at the 2005 and 2006 harvest from Control (100%) and SDI treatments expressed as mg/g of skin. Significance indicated by different letters. Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row. **101**

Table 6.1 Mean concentration of total red pigments (au), degree of red pigment colouration (%) and ionised pigments (mg/L) from Cabernet Sauvignon micro-ferments at bottling and 6

month age. Grapes were collected at the 2006 harvest from Control (100%), 70%, 52% and 43% SDI treatments. Means followed by the same letter in a row are not significantly different at P=0.05. (n=4). **125**

Table 6.2 Mean concentration of total red pigments (au), degree of red pigment colouration (%) and ionised pigments (mg/L) from Shiraz micro-wines at bottling and 6 month age. Grapes were collected at the 2006 harvest from Control (100%), 65%, 45% and 34% SDI treatments. Means followed by the same letter in a row are not significantly different at P=0.05, (n=4). No letters signify ns between irrigation treatments within a row. (n=4). **127**

Table 6.3 Mean concentration of total wine anthocyanins and parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidins from Cabernet Sauvignon wines expressed as mg/L of wine at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 70%, 52% and 43% SDI treatments. Means followed by the same letter in a row are not significantly different at P=0.05. (n=4). **128**

Table 6.4 Mean concentration of total wine anthocyanins and parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidins from Shiraz wines expressed as mg/L of wine at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 65%, 45% and 34% SDI treatments. Means followed by the same letter in a row are not significantly different at P=0.05. (n=4). **129**

Table 6.5 Mean concentration of total wine tannins from Cabernet Sauvignon and Shiraz grapes harvested in 2006 from Control (100%) and SDI treatments expressed as mg of catechin equivalent/L of wine. Means followed by the same letter in a row are not significantly different at P=0.05. (n=4). **130**

Table 6.6 Means of berry weight, total anthocyanins and total phenolics from grapes collected at the 2006 harvest. Total wine anthocyanin and phenolic concentrations are from micro-ferment wines after 6 months ageing. Grapes were collected from Cabernet Sauvignon and Shiraz exposed to a control (100%) and SDI treatments. Means followed by the same letter in a row are not significantly different at P=0.05 (n=8*, n=4). **139**

Table 7.1 Wine ranking scores for small-scale wines produced from a control (100%) and SDI treated Cabernet Sauvignon (70%, 52%, 43%) and Shiraz (65%, 45%, 34%) grapes harvested in 2006. The lower the score represents the more preferred wine. (n=4). **149**

CHAPTER 1: General Introduction

1.1 Background

Australian viticulture dates back to the late 1800s and since then has predominantly evolved in the southeastern parts of the continent. Currently winegrapes are grown in every state, with drying grapes and tablegrape production mainly occurring in Victoria and New South Wales (Table 1.1). In 2005 the proportions of total grape production (t) across all of Australia were 90% for winegrapes, 6% for drying grapes and 4% for tablegrapes (Australian Bureau of Statistics (ABS), 2006). Since the establishment of pumped, channel irrigation systems by the Chaffey Brothers in the late 1880s, much of Australia's current viticultural industries have had the opportunity to thrive in arid or semi-arid regions (Iland, 2004). One area in particular is the Murray-Darling region that extends along the Murray River and includes vineyards in Victoria and NSW (Figure 1.1). In 2006 the Murray-Darling region crushed approximately 180, 000 t of red wine grapes and 236, 000 t of white wine grapes (comp. Crothers, 2006). Currently the major red winegrape varieties grown in the Murray-Darling region include Shiraz, Cabernet Sauvignon and Merlot, whereas the white varieties are Chardonnay, Colombard, Semillon, Sultana and Muscat Gordo Blanco (comp. Crothers, 2006).

Table 1.1 Percentage of total wine, drying and table grape production for each state of Australia in 2005. Data were compiled from the Australian Bureau of Statistics vineyard survey (Australian Bureau of Statistics (ABS), 2006).

	% of total grape production (,000 t) in 2005		
Australian State	**Wine**	**Drying**	**Tablegrape**
Victoria (Vic.)	22	81	67
New South Wales (NSW)	26	16	15
South Australia (SA)	47	2	3
Western Australia (WA)	4	0.8	6
Queensland (Qld)	0.4	0.2	7
Tasmania (Tas)	0.3	-	-

Figure 1.1 Geographic location of the Murray-Darling region in Australia. Map provided by the Murray Valley Winegrape Growers Corporation (2005).

The environment in the Murray-Darling region is considered hot and semi-arid with low humidity and low rainfall associated with high temperatures and solar radiation (long-term annual rainfall 285 mm; growing season rainfall, 133 mm; mean January temperature 23.6^{o}C; annual evaporation 1542mm), (Gladstones, 1999; Iland, 2004). Successful winegrape production from this region relies on irrigation water from the Murray, Darling and Murrumbidgee River systems. Prior to pressurised irrigation systems the majority of the vineyards were furrow or flood irrigated that tended to result in over-irrigation. Vineyards that are exposed to over-irrigation can experience problems with unbalanced canopy micro-climates (pest and disease problems), rising water tables, soil salinisation, water logging and soil sodicity problems (Jackson & Lombard, 1993; Maschmedt, 2004). Furthermore, the excess water can have repercussions on the environment by carrying extra nutrients and chemicals that eventually drain back into water-courses (rivers, creeks, wetlands) leading to contamination issues (Walker et al., 2005b). To overcome this, the rapid conversion of

vineyards from traditional furrow irrigation to pressurised irrigation systems, especially drip irrigation and micro sprinklers has assisted in more efficient irrigation management of winegrapes. The changes in irrigation infrastructure have resulted in better vineyard water use and in conjunction with advancements in the understanding of strategic irrigation practices using soil moisture monitoring equipment, there have been improvements in canopy management and increases in winegrape composition and wine quality. Despite the benefits from decreased vineyard water use, the Australian wine and grape industry is currently responding to increasing water-related pressures from urban, industrial and environmental sectors that are driving the need for the continued development of more efficient irrigation management practices (Meyer, 1999; Davies et al., 2002).

The prospect of water restrictions caused by prevailing drought and intensifying competition for water from other horticultural industries has prompted the Australian wine and grape industry to develop strategic irrigation practices that will improve water use efficiency (WUE; tonnes of fruit per megalitre of applied irrigation, t/ML) and preserve vineyard sustainability. Fortunately the advancement in irrigation systems (infrastructure and monitoring) has benefited the development of strategic deficit irrigation practices.

1.2 Deficit Irrigation Strategies for Winegrapes

With the realisation that some form of deficit irrigation management will be necessary to produce sustainable wine grape production into the future, there needs to be greater understanding as to how a managed soil water deficit will affect yield, quality and long-term vineyard sustainability. A soil water deficit can refer to any combination of restricted water supply to the soil, either as a result of crop water use (evapotranspiration), low rainfall, reduced irrigation or poor soil structure (Goodwin, 1995; Atwell et al., 1999). Understanding how much soil moisture is available to a grapevine's root system is critical to successfully applying a deficit irrigation strategy. The relationship between available soil moisture and the ability of a grapevine to extract water from the soil is schematically represented in Figure 1.2. As soil water tension (kPa) increases, the force exerted by a vine to extract water from the soil profile must also increase. The soil water tension is not related to soil depth or the pattern of root growth as may be interpreted from the diagram in Figure 1.2, but is associated with soil

properties (water infiltration, storage, texture) in conjunction with environmental conditions (Smart & Coombe, 1983). Field capacity is the water available for vine use after drainage of gravitational water from a saturated soil. Permanent wilting point is when the soil moisture content is too low for a vine to remove water from the soil pores. Most crops are permanently damaged if the soil moisture content is allowed to reach wilting point. Deficit available water (DAW) is the amount of water available in the soil between a minimal or no stress situation and a desirable level of water stress (Goodwin, 1995). This is the water tension range that current deficit irrigation strategies work within.

Figure 1.2 Relationship of soil water tension (kPa) to a grapevine's response towards the available soil moisture (adapted from Goodwin, 1995).

When applying a water deficit there is potential to initiate so called "hydraulic lift" (Burgess et al., 1998; Caldwell et al., 1998; Burgess & Bleby, 2006). This phenomenon has been observed in *Eucalyptus* sp. and *Vitis* sp. whereby water is absorbed by the deep roots that have access to moist soil and then redistributed to the finer roots in the dry, shallow surface soil layers

(Burgess et al., 1998; Smart et al., 2005). While water has been found to passively move between soil profiles, there is evidence of horizontal transfers of water from irrigated to unirrigated roots when there are soil water disconuities created by differential extraction by roots or point-source irrigation (Stoll et al., 2000a; Smart et al., 2005; Burgess & Bleby, 2006). The maintenance of an active root system may be an important mechanism for drought avoidance, particularly by plants grown in semi-arid regions or exposed to water deficit, thereby allowing the root system to take advantage of infrequent rainfall events.

Currently the Australian wine industry has widely adopted regulated deficit irrigation (RDI) and partial rootzone drying (PRD) to strategically apply soil water deficits in anticipation of manipulating grapevine growth and fruit development and improving WUE (Davies et al., 2002; Dry et al., 2002; Kreidemann & Goodwin, 2003). A common feature of these irrigation techniques is the reduction in available soil water, but how the water is applied is fundamentally different. In the case of RDI, a controlled application of irrigation water at less than the crop water use is applied at a specific vine growth stage (temporal) (Kriedemann & Goodwin, 2003). By contrast with PRD, the irrigation water is manipulated over the soil area (spatial) by applying alternate irrigations to specific sides of the grapevine, thus creating discrete wet and dry zones around the root system (Dry et al., 1996; McCarthy, 1998). Whilst both deficit irrigation techniques reduce vineyard water application, there are differences in the physiological responses of the grapevines to these strategically applied water deficits that produce specific canopy and grape development responses (Dry et al., 1999).

Across the Murray-Darling region, RDI and PRD have been applied commercially and at times appear to produce variable responses with respect to measurable grape yield and compositional parameters, even when applied to winegrapes under similar management and environmental conditions. More recently, there has been an increasing trend towards applying deficit irrigation all season in anticipation of conserving water. The idea of a sustained deficit irrigation (SDI) that is applied throughout the irrigation season is the focus of this project. With SDI the water deficit is not created by withholding water, but rather, by applying a lesser volume of water at each irrigation event for the entire irrigation season. This means SDI irrigation could potentially create a higher soil water tension by not re-filling the entire rootzone during an irrigation event, thereby providing an insufficient amount of water to meet transpirational demands of the grapevine. However, when using SDI the water may be

restricted to the upper soil layers that potentially could lead to a build-up of salts or other compounds around the rootzone due to a lack of deep drainage (leaching) to flush them away.

Irrespective of how RDI, PRD or SDI create a soil water deficit there is potential for a grapevine to produce a water stress response. Whilst these novel irrigation methods are capable of improving WUE and potentially the value per tonne of grapes (due to decreased water costs or improved berry composition), there is scope to further explore the physiological responses of grapevine varieties to deficit irrigation in anticipation of optimising future deficit irrigation strategies.

1.3 Water Relations in Grapevines

Grapevine physiology is an important factor in helping to understand how deficit irrigation may influence grape and wine composition. Overall, grapevine water relations are complex and dynamic systems that have evolved a high degree of adaptability to different environmental conditions. Grapevines control water loss by varying the aperture of the leaf stomata (measured as stomatal conductance), which in turn regulate transpiration and prevents leaf water potentials decreasing too low to harm the hydraulic system (Tyree & Sperry, 1989; Jones & Sutherland, 1991). In grapevines the stomata are located on the under-surface of the leaves (hypostomatous) and vary in frequency and distribution depending on variety, environmental conditions and stage of growth (Mullins et al., 1992). Stomata also have to remain sufficiently open to diffuse carbon dioxide from the atmosphere for photosynthesis (Smart, 1974; Jones, 1998; Escalona et al., 1999; Medrano et al., 2002). Consequently stomata have a dual role of balancing transpiration and CO_2 exchange to prevent excessive water loss, whilst also maintaining adequate photosynthesis for healthy vine function and reproduction.

Water moves through the soil:plant:air continuum along a gradient from high to low water potential. It is at the leaf surface that the steepest gradient occurs indicating the importance of stomata in regulating water relations of the plant. Under field conditions there can be large diurnal changes in leaf water potential (Williams & Matthews, 1990). Diurnal changes in leaf water potential can be influenced by temperature, ambient radiation and vapour pressure deficit (Smart & Coombe, 1983). Measurements of leaf water potential across a grapevine

canopy can provide information on the vine water demand, soil water availability, stomatal regulation and plant hydraulic conductivity. Williams and Araujo (2002) concluded that measures of midday leaf water potential were equally similar to predawn leaf water potential measures as they similarily accounted for the amount of soil moisture and vine physiology stress responses due to water stress. Conversely others have found that measures of pre-dawn (before sunrise) leaf water potential are better indicators of vine water status than midday leaf water potential as it is postulated the vine has equilibrated with the available soil water during the dark period (Winkel & Rambel, 1993; Schultz, 1996; Loveys et al., 2005).

1.3.1 Water deficit effects on grapevine physiology

Most adaptations to drought or water stress favour plant survival so that when water becomes limited the productivity or yield decreases (Jones, 1980). Soil water deficits can reduce photosynthesis (Kriedemann & Smart, 1971; Liu et al., 1978) thereby affecting vegetative growth (van Zyl, 1984; Matthews et al., 1987a; Schultz & Matthews, 1988) and berry development and composition (Kliewer et al., 1983; Bravdo et al., 1985; Hepner et al., 1985; Matthews & Anderson, 1989). Overall, grapevines tend to tightly regulate stomatal closure when grown in soils of low water availability, which leads to a rapid reduction in stomatal conductance as pre-dawn leaf water potential decreases (Schultz, 2003; Cifre et al., 2005). Various studies have reported that differences in midday leaf water potential as a result of water deficit treatments can affect the rate of shoot growth (van Zyl, 1984; Matthews et al., 1987a). Furthermore, differences in stomatal conductance of grapevines in response to soil drying have been linked to variation in hydraulic (changes in leaf turgor) (Davies et al., 2002; Schultz, 2003) and non-hydraulic responses (hormonal responses) (Loveys & Düring, 1984; Correia et al., 1995; Davies et al., 2002; Soar et al., 2006b).

Under field situations the physiological response of a grapevine to soil water deficit, particularly after midday, can lead to lower leaf water potential due to greater evaporative demand (driven by radiation and atmospheric conditions) exceeding the ability of the root system to extract sufficient water from the soil to meet the evaporative demand. This lowering of the leaf water potential can sometimes be offset by stomatal closure (hydraulic response) (Liu et al., 1978; Schultz, 1996; Schultz & Matthews 1988; Comstock, 2002). However other deficit irrigation research, particularly PRD studies, utilise the production of non-hydraulic

chemical signals to control stomatal aperture (Loveys & Düring, 1984; Dry & Loveys, 1999; Stoll et al., 2000b). Plant roots that come into contact with drying soil are known to respond by synthesising abscisic acid (ABA) that is in turn transported via the xylem to the leaves (Loveys & Düring, 1984; Zhang et al., 1987). Overall, much is known about the production of ABA and its role in stomatal closure to minimise water loss (Loveys & Kriedemann, 1973; Loveys & Düring, 1984; Correia et al., 1995; Peterlunger et al., 2000; Loveys et al., 2005). The amount of xylem ABA could be used as an indicator of soil drying, however the concentration of ABA measured from xylem sap can not solely explain differences in leaf physiology as a result of soil drying (Munns & King, 1988; Wilkinson & Davies, 2002). This is because the amount of ABA detected in the leaf, as opposed to the amount released by the roots, can be affected by xylem pH (Tardieu & Davies, 1993), enzymatic breakdown (Hartung et al., 2001), cellular acidification (Hartung & Radin, 1989) or changes in atmospheric VPD (Tardieu et al., 1993).

Since grapevine varieties have been grown in diverse environments, vine cultivars have evolved a high degree of adaptability to different soils, climates and water availability. These variations in grapevine origin and genotype have ultimately influenced their physiological responses to water stress to the point of potentially categorising grape cultivars based on their stomatal response to soil water deficits (Schultz & Matthews, 1993; Schultz, 1996; Schultz, 2003; Soar et al., 2006b). Research by Schultz (2003) and Soar et al. (2006b) on Grenache and Shiraz have shown that these varieties respond differently to potentially stressful environmental conditions (water stress and changes in atmospheric conditions) thereby grouping these varieties based on their isohydric or anisohydric responses to water stress. In brief, isohydric plants tend to maintain a more constant water status by controlling stomatal conductance from an interaction between hydraulic and chemical signals, whereas anisohydric species tend to have less rigid stomatal control which allows a greater fluctuation in leaf water potential with decreasing soil water potential (Lambers et al., 1998; Tardieu & Simonneau, 1998) or increased evaporative demand (Soar et al., 2006). Therefore, the Grenache responded to the stressful conditions by tightly regulating the stomata (isohydric/pessimist) during the day as opposed to Shiraz that maintained a higher rate of gas exchange (anisohydric/optimist) (Loveys et al., 2005, Soar et al., 2006b). Additionally, the differences in stomatal control between winegrape varieties may be linked to the presence or absence of an interaction between hydraulic and non-hydraulic signals (Davies & Zhang, 1991; Tardieu & Simmoneau,

1998). Further work by Soar et al., (2006b) noted that irrespective of whether these winegrape varieties were field or glasshouse grown, the concentration of xylem sap ABA was a key factor in determining the isohydric or anisohydric behaviour.

Studies by Soar et al., (2006b) have noted that despite differences in the concentration of xylem sap ABA levels between Shiraz and Grenache when experiencing conditions of high evaporative demand, it is still unclear whether those differences are due to differences in root responses or differences in ABA synthesis within the canopy itself. Gene expression results from pot experiments indicated that changes in xylem sap ABA levels between Shiraz and Grenache were driven by the regulation of genes in the leaf tissue, but not in root tissue (Soar et al., 2006b). Nevertheless, an understanding of ABA synthesis as a result of a hormonal response to soil water deficits has been particularly important to the Australian wine industry in developing irrigation techniques such as PRD (Dry et al., 2000; Stoll et al., 2000a, 2000b; Loveys et al., 2005).

1.4 Grape and Wine Quality

Grape and wine quality is complex and includes a combination of the berry composition (sugar, acid and juice pH) and secondary metabolite compounds that ultimately have a role in influencing the appearance and sensory characteristics of a wine. These secondary metabolites are of great interest to winemaking, especially as it is becoming more evident that viticultural practices, such as irrigation management, can manipulate the concentration of these compounds in different parts of the grape berry during berry development and ripening .

Across the Australian wine industry, the common parameters for assessing grape quality include total soluble solids (^{o}Brix or Baumé), titratable acidity (TA) and juice pH (Iland et al., 2004). Total soluble solids (TSS), which is a measurement of the concentration of sugars (glucose, sucrose and fructose) per unit of water in the grape berry is used as a common guide for the Australian wine industry to determine fruit ripeness and harvest date (Krstic et al., 2003). TSS are expressed as ^{o}Brix (degrees Brix) or ^{o}Baumé (degrees Baumé) which is an estimation of the likely alcohol content when the grapes are fermented to completion (Jackson & Lombard, 1993). In the Murray-Darling region, premium red winegrapes such as Shiraz and Cabernet Sauvignon are generally harvested between 13.5 to14.5 ^{o}Baumé.

Secondary metabolites in wine grapes comprise a range of compounds that are of particular interest to winemaking as they contribute to the colour, mouth-feel, astringency and aroma of a wine. Many of these belong to a class of compounds broadly known as phenolics. Phenolic compounds can be divided into non-flavonoid and flavonoid compounds (Mullins et al., 1992; Ojeda et al., 2002). Non-flavonoid compounds are mainly comprised of phenolic acids and stilbenes (Ojeda et al., 2002), whereas the flavonoids include anthocyanins, flavonols and tannins (Figure 1.3) (Stafford, 1991; Shirley, 1996).

Figure 1.3 Schematic representation of the flavonoid pathway showing the production of anthocyanins, tannins and flavonols, including the general structure for each phenolic compound (adapted from Robinson & Walker, 2006).

Anthocyanins are the main pigments responsible for grape and wine colour in black and red wine grape varieties. (Ribereau-Gayon, 1982; Robinson & Davies, 2000). They are a group of

compounds comprised of the anthocyanidins (cyanidin, peonidin, delphinidin, petunidin and malvidin) in three glycosidic forms: the glucoside, acetylglucoside and coumaroylglucosides (Mazza, 1995; Brossaud et al., 1999; Ojeda et al., 2002). Malvidin 3-glucoside is the main anthocyanin detected in *Vitis vinifera* red wine grapes (Mullins et al., 1992; Iland, 1998). Anthocyanins begin to accumulate in the skin of a grape berry at veraison and accumulation tends to continue until harvest (Figure 1.4) (Somers, 1976). Anthocyanin accumulation has been closely associated with sugar levels from grape berries, but may not necessarily be associated with sugars from the grape berry as a whole (Pirie & Mullins, 1976; Pirie & Mullins, 1977).

Flavonols are another group of secondary metabolites that are non-coloured organic compounds belonging to the polyphenols. The most common flavonol found in grapes is quercetin comprising mainly of quercitin-3-O-glucoside and -3-O-glucuronide (Figure 1.3) (Cheynier & Rigaud, 1986; Price et al., 1995, Downey et al., 2003b). Flavonols are thought to act as UV protectants by accumulating in the epidermal layers of the berry skin in response to sun exposure (Flint et al., 1985; Price et al., 1995; Price et al., 1996; Haselgrove et al., 2000; Downey et al., 2004). Flavonols are also particularly effective as cofactors in copigmentation of anthocyanins in flowers and fruit (Asen et al., 1972; Boulton, 2001). Copigmentation is a phenomenon that can occur between flavonols and anthocyanins whereby they bind to form a new molecular complex that enhances the anthocyanin pigment, thus making it more stable in the wine (Boulton, 2001). In young wines (soon after fermentation) copigmentation accounts for up to 50% of the observed colour, however this colour complex depends on the concentration of the pigment, the ratio of the cofactor to the pigment and wine pH (Boulton, 2001).

Tannins can be divided into two classes known as hydrolysable tannins and condensed tannins (Figure 1.3). Grape tannin can be isolated from skins and seeds (Amrani-Joutei et al., 1994; Downey et al., 2003a), with total tannin being higher in the seed than skins (Prieur et al., 1994; Thorngate & Singleton, 1994; Souquet et al., 1996). Factors that can affect seed tannin levels include the number of seeds set per berry and the berry size (Harbertson et al., 2002). In wine, tannins influence the texture and palate feel of a wine whilst also contributing to bitter and astringent taste sensations (Thorngate, 1997; Gawel et al., 2000; Iland & Gago, 2002).

In Australia, the majority of grapevines used for commercial wine grape production are varieties of *Vitis vinifera* L. that have either originated from Europe or Western Asia (Dry, 2004). These cultivated grapevines have been selected based on their growth and fruiting capacity for winemaking and consequently have evolved differences based on exposure to environmental conditions and selection processes. Overall, grape and wine quality can be influenced by many different factors including sunlight exposure (Price et al., 1995; Dokoozlian, 1996; Bergqvist et al., 2001; Spayd et al., 2002; Downey et al., 2004), diurnal temperatures (Buttrose et al., 1971; Kliewer & Torres, 1972; Hale & Buttrose, 1974; Kliewer, 1977; Spayd et al., 2002), canopy management (Archer & Strauss, 1989; Haselgrove et al., 2000), soil type (Jackson & Lombard, 1993), water status (plant and soil) (Hepner et al,. 1985; Ginestar et al., 1998; Esteban et al., 2001; Ojeda et al., 2002) and irrigation management practices (Sipiora & Gutiérrez Granda, 1998; Esteban et al., 2001; Roby et al., 2004; Roby & Matthews, 2004).

1.4.1 Water deficit effects on yield components and berry size

It is widely known that water deficit can influence grapevine canopy structure, berry size and composition. The strategic irrigation management techniques of RDI and PRD are currently adopted commercially to manipulate grape composition and wine quality. In a semi-arid region, such as the Murray-Darling, irrigated grapevines generally have higher yields, larger berries and increased canopy vigour than grapevines exposed to a soil water deficit (Kliewer et al., 1983). By contrast, grapevines exposed to PRD tend to maintain yield levels by retaining berry size even though the irrigation volume may have been reduced (Dry et al., 2000).

Various studies have used different irrigation regimes to manipulate canopy vigour and have noted that yield and quality at harvest are dependent on when the irrigation is applied in relation to the stage of berry growth (Hardie & Considine, 1976; Goodwin & Jerie, 1992; McCarthy, 1997b; McCarthy, 2000; Kennedy et al., 2002). Figure 1.4 illustrates the different growth stages that occur in a grapevine and where some of the current deficit irrigation strategies can be applied in relation to these stages. Water deficits that are applied pre-flowering, particularly in the case of RDI, have the greatest effect on primary shoot length, lateral shoot development and cane thickening (Goodwin & Jerie, 1992; McCarthy, 1998).

When a water deficit is applied between fruit set and veraison there is potential to reduce yield by affecting berry size (Hardie & Considine, 1976; Matthews et al., 1987a; McCarthy, 2000). Hardie & Considine (1976) noted that the most sensitive stage for affecting berry development was during the flowering to fruit set stage, whereas a water deficit applied post-veraison tended to cause a reduction in berry weight. Early and late season water deficits can affect the development of the current season's berries as well as the primordia for the subsequent season's berries (Matthews & Anderson, 1989).

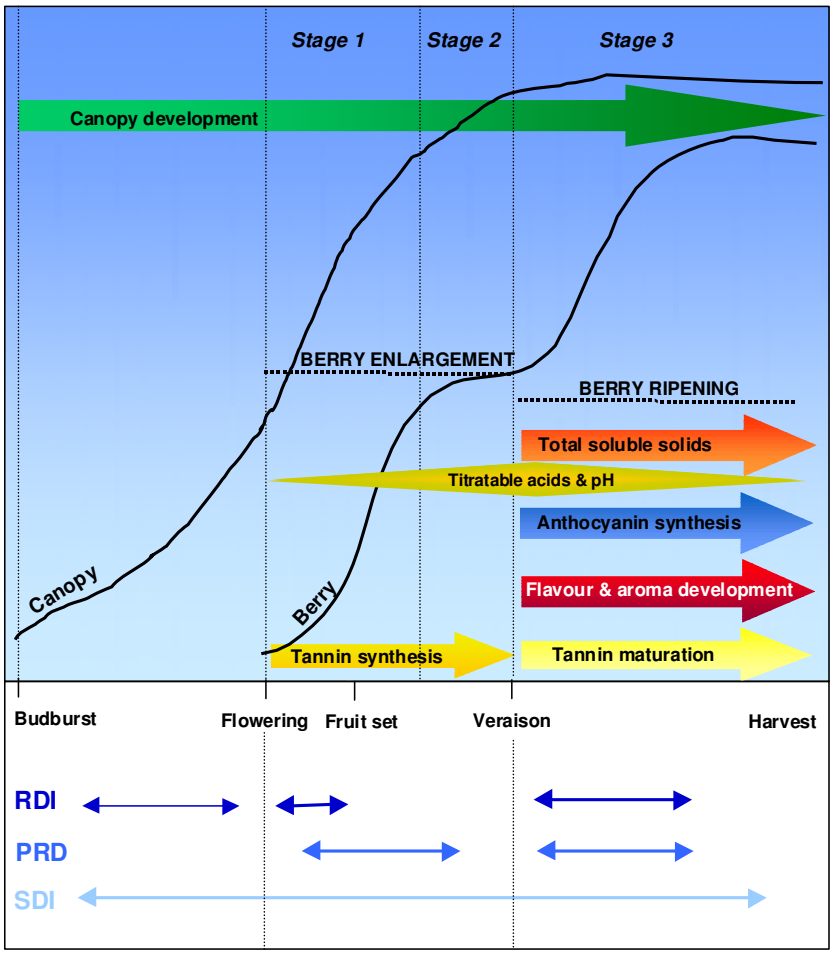


Figure 1.4 Sigmoidal pattern of canopy growth and grape berry development and ripening. The stages of water application are also depicted for the deficit irrigation techniques of regulated deficit irrigation (RDI), partial rootzone drying (PRD) and sustained deficit irrigation (SDI) (adapted from Coombe, 1992; Coombe & McCarthy, 2000).

The trend that appears to be emerging is that deficit irrigation regimes are manageable in semi-arid regions, even though there can be varied effects on berry size and composition. The

general assumption is that smaller berries have better quality and compositional components compared to large berries due to a greater skin:flesh ratio (Hardie & Considine, 1976; Matthews & Anderson, 1988; Sipiora & Gutiérrez Granda, 1998). Differences in berry anthocyanins, phenolics and tannins have been indirectly attributed to berry size which in turn has been influenced by water supply (Hardie & Considine, 1976; Kennedy et al., 2002). However, berry size may not be the only contributing factor to differences in quality attributes. Ojeda et al. (2002) confirmed that water deficit of Shiraz grapes can change phenolic compound concentration either indirectly due to berry size, but also by a direct action on biosynthesis of the phenolic compounds. Other water deficit studies have also found that the effects of irrigation treatment on flavonoid compounds has a greater influence on the final composition than differences in berry size (Roby et al., 2004; Roby & Matthews, 2004; Walker et al., 2005a). Knowing that water deficits can affect yield and berry size, then how does this translate to grape and wine composition?

1.4.2 Water deficit effects on grape and wine composition

Depending on the severity of the soil water deficit, a reduction in photosynthesis and premature leaf senescence can delay fruit ripening (Hardie & Considine, 1976). Conversely, sugar accumulation can be improved by mild water deficits due to a suppression in canopy growth that provides an opportunity for interior leaves to be photosynthetically active, thereby increasing the assimilate level to the developing berries (Smart & Coombe, 1983). Generally, titratable acidity is more affected by water deficits that are applied from fruit set to veraison (van Zyl & Weber, 1977). With respect to grape juice pH, a study by Freeman et al., (1980) noted that in Shiraz exposed to different irrigation regimes, lower juice pH were produced from berries of non-irrigated than irrigated vines. Whilst deficit irrigation can manipulate titratable acidity and juice pH levels, the differences tend to be minimal and probably more related to changes in climatic conditions, regional location and variety (Smart & Coombe, 1983).

Early and late season water deficits can influence skin anthocyanin without any significant effects on ^{o}Brix, titratable acidity or juice pH (Matthews & Anderson, 1988). Conversely, the timing of the water deficit, whether it is post- or pre-veraison has been noted to affect the biosynthesis of flavonoids in the grape berry (Kennedy et al., 2002). Field experiments on

Cabernet Sauvignon and Shiraz indicated that total anthocyanins and total phenolics are increased in PRD treated vines compared to fully irrigated control vines (Dry et al., 1996; Stoll, 2000). This could be attributed to the PRD vines having greater light penetration through the canopy that resulted in an increase in the anthocyanidin constituents of delphinidin, petunidin, peonidin and malvidin (Stoll, 2000). Since light influences the development of various flavonoids in the berry there is a strong possibility for water deficit to increase flavonoid levels in the berry due to increased light exposure resulting from reduced canopy density (Jackson & Lombard, 1993).

Water deficits have also been found to increase the concentration of skin tannins and anthocyanins independent of berry size (Roby et al., 2004). Recent PRD studies by Bindon (2004) postulated that increased anthocyanin concentrations in PRD treated fruit was more likely to be the direct result of the water deficit affecting biochemical signals in the fruit, rather than a berry size factor. Likewise, Roby et al., (2004) and Walker et al., (2005a) reported that improved wine quality produced from smaller berries was due to the treatment effect (pruning or water deficit) rather than differences between the surface area to volume ratio of berries. The changes in berry size as a result of water deficit may also affect juice volume and final wine colour (Matthews & Anderson, 1988; Johnstone et al., 1996).

Some deficit trials have indicated that a post-veraison water stress on Cabernet Sauvignon can increase wine phenolic compounds (anthocyanins and tannins) as a result of changes in berry size (Kennedy et al., 2002). Wines produced from well-watered grapevines can produce lower anthocyanin and tannin levels which result in low wine colour, high pH and low tasting scores (Bravdo et al., 1985; Nadal & Arola, 1995). Deficit irrigation can improve the must and wine by decreasing potassium and juice pH (Freeman & Kliewer, 1983; Hepner et al., 1985) and increasing colour and anthocyanin content (Freeman & Kliewer, 1983; Matthews & Anderson, 1988). In Australia, PRD studies by Stoll (2000) suggested that other factors (i.e. yield , bunch exposure or flavonoid concentrations) besides the irrigation treatments influenced the sensory evaluation of the small-scale ferments wine. Alternatively, there has been some suggestion that wine from PRD treated Shiraz berries may contain free anthocyanins in a copigmentation complex that could potentially improve wine stability during the ageing process (Boulton, 2001; Lambert, 2002; Bindon, 2004).

1.5 General Conclusions and Aims of the Study

Sustained deficit irrigation differs significantly in its management to PRD and RDI and is a technique that could potentially be easily adopted across the winegrape industry if desirable yields and berry compositional attributes could be met. However, deciding how much of a reduction in irrigation volume can be achieved with SDI without causing a detrimental effect on yield, grape composition and wine quality is unknown and has consequently been the subject of this study. With this in mind, an understanding of the physiological behaviour of wine grape cultivars to deficit irrigation techniques would assist in determining the most effective SDI irrigation strategy to maximise both yield and quality in grapes and wine, whilst improving water use efficiency.

The aims of this study were to:

- Explore the differences in physiological responses of *Vitis vinifera* L. Cabernet Sauvignon and Shiraz to SDI and any subsequent impacts on abscisic acid synthesis.
- Determine the effect of SDI on yield components and berry composition relevant to the Australian wine industry
- Examine the effect of SDI on grape phenolic concentration and composition during berry ripening and harvest
- Investigate the composition and sensory attributes of wines made from SDI-treated red winegrapes.

CHAPTER 2: General Materials and Methods

2.1 Field Sites

Field trials were conducted on *Vitis vinifera* L. wine grape cultivars, Cabernet Sauvignon (Dareton, NSW, Australia) and Shiraz (Irymple, Vic, Australia) between November 2003 and December 2006. These cultivars were selected based on their popularity as premium, red wine grape varieties for the Sunraysia region. Furthermore, there have been many anecdotal suggestions that these wine grape varieties respond quite differently under similar irrigation regimes. Both trial sites were situated in the Sunraysia region of the Murray-Darling region (Figure 2.1) that has a warm temperate to semi-arid climate with average annual rainfall of 285 mm that tends to be winter dominant (long-term mean January temperature = 23.6^{o}C, Winkler Degree-Days above 10^{o}C = 2244^{o}C, Class-A Epan = 1542 mm per year) (Gladstone, 1999; Iland, 2004) (Appendix A).

Figure 2.1 Satellite image showing location of Shiraz (Irymple, Vic) and Cabernet Sauvignon (Dareton, NSW) vineyards used for the field trials in the Murray-Darling region, Australia. Image provided by ©Google Earth, NASA, 2006.

Field trials were conducted on seven-year-old Cabernet Sauvignon and Shiraz both grafted to 140 Ruggeri (*V. berlandieri* x *V. rupestris*) (Figure 2.1). The Cabernet Sauvignon was situated on a grower's property in Dareton, NSW (S 34°6'0" E 142°3'0", 42 m elevation) and the Shiraz site was a vineyard at the Department of Primary Industries in Irymple, Vic (S 34°15'0" E 142°10'0", 73 m elevation) (Figure 2.1). The soils in both sites were similar being sandy, clay loam and typical of the aeolian soil of the region with high permeability and deep drainage.

Both varieties were trained onto a two wire vertical trellis, with the Cabernet Sauvignon and Shiraz orientated in an east-west and northeast-southwest direction respectively (Figure 2.2). The Cabernet Sauvignon was planted with 2.4 m between rows and vines, whereas the Shiraz was planted with 3.0 m between rows and 1.8 m between vines. The vines were mechanically pruned and harvested.

Figure 2.2 The field trials were conducted on Cabernet Sauvignon (left) and Shiraz (right) vines that were grafted to 140 Ruggeri rootstock. Each cultivar was trained on a two-wire vertical trellis with 0.40 m spacing between the top and bottom cordons.

2.1.1 Trial design

For both sites the experimental layout was a row-column design with four irrigation treatments each replicated eight times. Treatments comprised four vines per plot with the outer two vines being buffer vines and the inner two vines being measurement vines. Each row consisted of four water treatments: one control (100%) and three graded deficits (Table 2.1, Figure 2.3). These were repeated twice down a row and four times across the trial area, hence eight replicates per irrigation treatment. The rows were repeated in sets of three so as to allow a buffer row on either side of a trial (test) row to provide sufficient hydraulic isolation between different irrigation treatment plots.

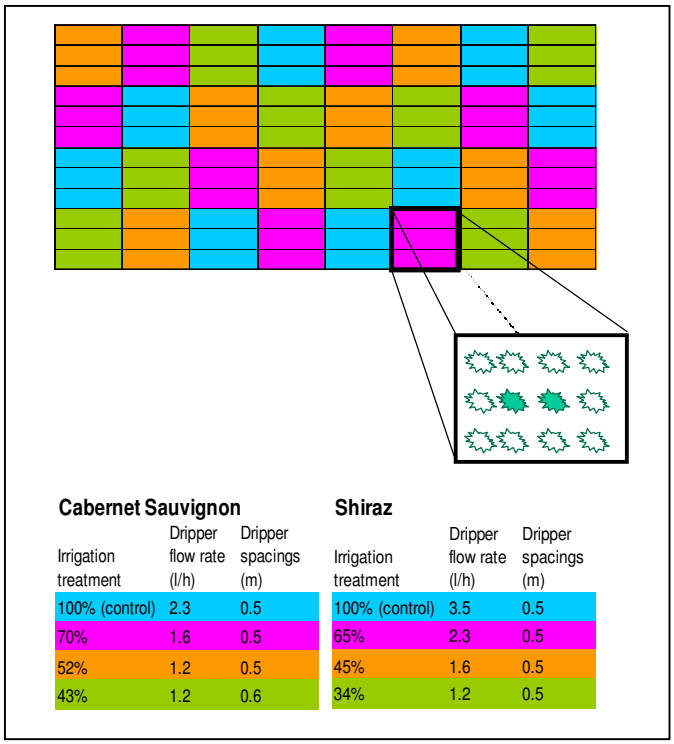
Table 2.1 Irrigation regimes established for the Cabernet Sauvignon and Shiraz trial sites from November 2003 until December 2006.

Variety	Dripper flow rates (L/h) at 0.5 m spacings	Irrigation regimes (% of ET_c)
Cabernet Sauvignon	2.3, 1.6, 1.2#, 1.2#	100%, 70%, 52%, 43%
Shiraz	3.5, 2.3, 1.6, 1.2	100%, 65%, 45%, 34%

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

All treatments were surface drip irrigated using 16 mm polypropylene dripper line (Netafim®, Australia) with pressure compensated, in-line emitters installed at various flow rates so as to create the different water deficit treatments (Table 2.1). The irrigation treatments could not be set-up identically between sites due to differences in the pressure flow rate relationships of the existing irrigation pumps. Soil water content was measured using an EnviroSCAN® logger (Sentek Sensor Technology, Stepney, Australia) that contained eight capacitance probes. Each probe had four sensors at 20, 40, 70 and 100 cm soil depth. Soil matric potential was measured with granular matrix blocks (GBug® logger, Measurement Engineering, Magill, Australia) that were installed at the same depths as the capacitance probes (Figure 2.5). Irrigations were scheduled based on an average soil water potential in the control treatments of up to 40 kPa and determined by weekly readings of volumetric soil moisture and soil matric potential. Determining the volume of water to apply at each irrigation event was dependent on water availability and weather conditions. At the end of the irrigation season the total volume of irrigation water applied (ML/ha) was calculated by adding up the individual irrigation events. From this data, water use efficiency (WUE; t/ML) could be calculated by dividing the yield (t/ha) by irrigation water applied (ML/ha). Since the Cabernet Sauvignon site was on a

grower's property, the Enviroscan unit was already present in the existing vineyard and could provide available soil moisture readings for the control treatment. For the Shiraz site, an Enviroscan unit was installed in the trial area and was able to log available soil moisture for each irrigation treatment. The sustained deficit irrigations (SDI) were applied all season (including post-harvest irrigations and fertiliser applications) and commenced immediately after the trials were established in November 2003.



Cabernet Sauvignon

Irrigation treatment	Dripper flow rate (l/h)	Dripper spacings (m)
100% (control)	2.3	0.5
70%	1.6	0.5
52%	1.2	0.5
43%	1.2	0.6

Shiraz

Irrigation treatment	Dripper flow rate (l/h)	Dripper spacings (m)
100% (control)	3.5	0.5
65%	2.3	0.5
45%	1.6	0.5
34%	1.2	0.5

Figure 2.3 Irrigation design for the Cabernet Sauvignon and Shiraz SDI trial sites during 2003-2006. The insert shows the number of vines per treatment, with the test vines (shaded) in the middle row surrounded by the buffer vines.

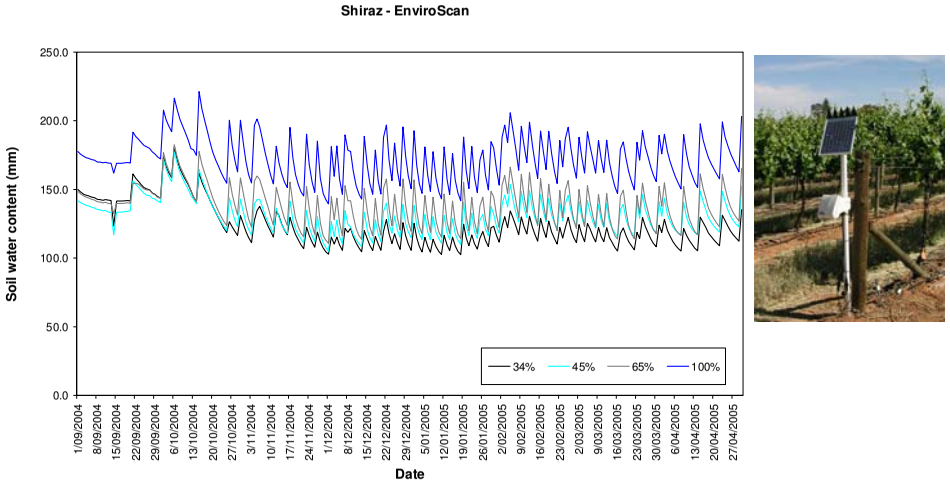


Figure 2.4 Cumulative Enviroscan data showing changes in soil water content (mm) for each irrigation treatment at the Shiraz trial site during 2004-2005.

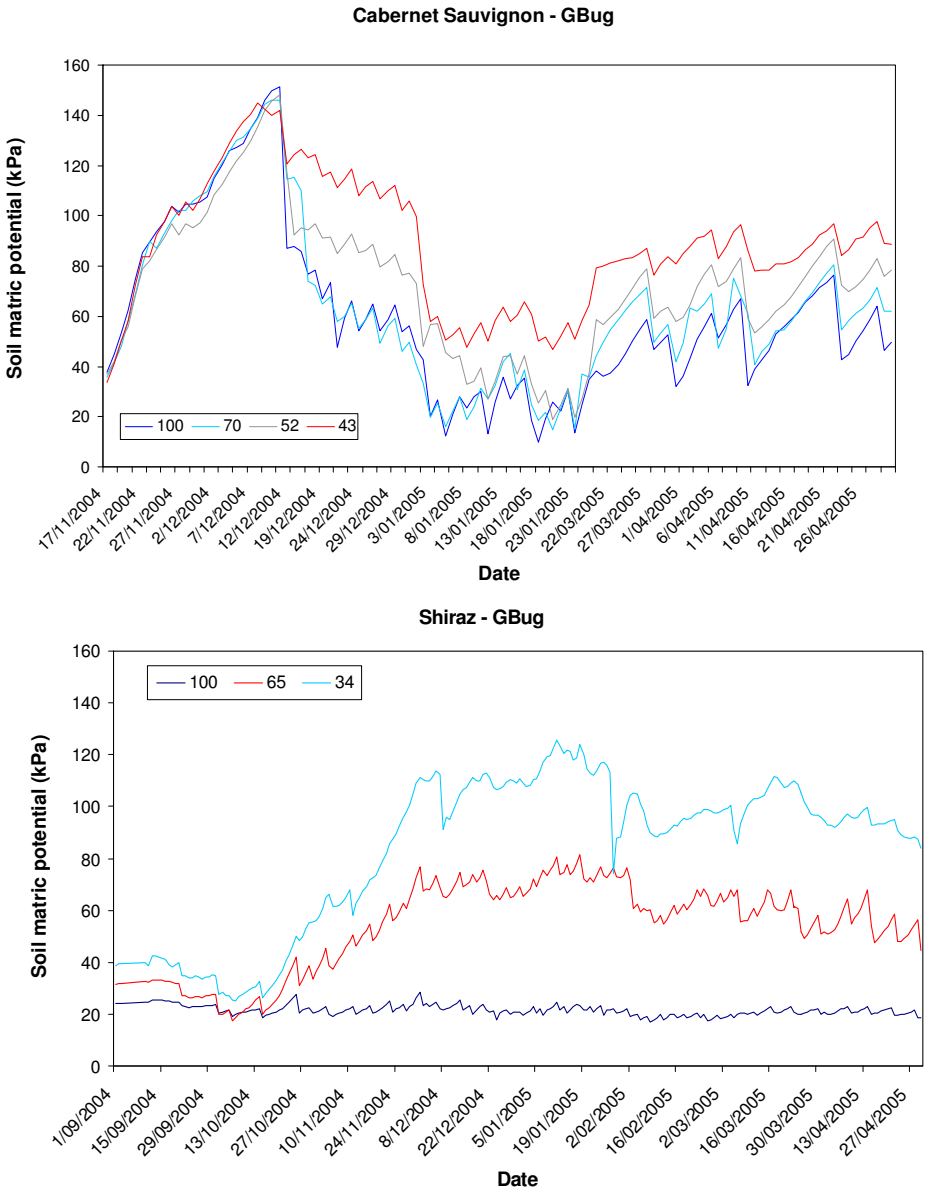


Figure 2.5 Cumulative Gbug data showing changes in soil matric potential (kPa) for Cabernet Sauvignon (100%, 70%, 52% and 43% irrigation treatments) and Shiraz (100%, 65% and 34% irrigation treatments) during 2004-2005.

2.2 Glasshouse Trials

A glasshouse trial was established in August 2004 and 2005 to explore the physiological and hormonal response of grapevine cultivars to water deficit. One-year-old *Vitis vinifera* L. cv.

Shiraz, Cabernet Sauvignon, Tempranillo and Grenache were planted in plastic pots (380 mm diameter x 3400 mm high) (Figure 2.5). These vines were all grown on own-roots to eliminate the possibility of a rootstock influence on physiology and abscisic acid levels (Soar et al., 2004). The potting media contained a mixture of river sand, red loam, peat moss and vermiculite at a ratio of 3:2:3:1 (Gibberd et al., 2001), as well as 0.5 g $FeSO_4$/L soil (Yates Sulphate of Iron) and 2 g/L soil of controlled release (Osmocote®, Scotts, NSW, Australia) plus trace elements for potted plants. All cuttings had been hot water treated at 55°C for 5 min to eradicate any nematode infestation present on the roots (Nicholas et al., 1994).

When the glasshouse trial was first established in August 2004 the vines were grown in a temperature controlled glasshouse set at 18°C (night) and 25°C (day) with two halogen lamps (150 watts each) used for 4 h from 2000 h to 2400 h to simulate longer day length. The vines were trained to two shoots on bamboo stakes (150 cm) with all lateral shoots and inflorescences removed during growth. In March 2005 the vines were shifted to a shade house to commence dormancy and hardening of the canes. For the second season of glasshouse trials, the vines were shifted into a larger glasshouse in August 2005 and trained in the same way as for the previous season.

Figure 2.6 Glasshouse trials established in August 2004 (left) and August 2005 (right) on own-rooted Shiraz, Cabernet Sauvignon, Grenache and Tempranillo at the Department of Primary Industries.

To expedite healthy growth during the growth phase the vines were fertilised with a commercial, liquid soluble fertiliser (Miracle-Gro®) that was applied as directed by the product label and contained 15% Total Nitrogen, 13.1% Total Phosphates and 12.4%

Potassium Chloride. A routine pest and disease program was incorporated to control for powdery mildew and mealy bugs. The chemicals used to control these pests and diseases were either wettable sulphur (preventative fungicide for powdery mildew), Topas (systemic fungicide for powdery mildew) or Confidor® (insecticide for mealy bugs). These were used as required and not during an experimental phase when collecting physiology readings.

The vines were irrigated using Netafim® 4 L/h pressure compensated drippers. There were two irrigation treatments, a control, which was watered to potential ET_c by maintaining soil moisture at field capacity and a deficit irrigation that was watered to simulate deficit irrigation (approximately 50% of the control). The trial design was a randomised complete block with five replicates per variety and irrigation treatment.

2.3 Definitions

Quality; the use of this term in relation to grapes and wine is subjective. Quality can mean different things to individuals and is always changing based on personal experiences. In the context of this thesis it is the degree to which a set of identifiable and measurable grape and wine compositional parameters can be used to provide an indication of potential appearance, aroma, taste or age-ability of the grapes and wine.

Composition; relates to the individual parameters or compounds that can be measured, and quantified, in the berry or wine.

Berry development; this is the period from fruit set to pre-veraison

Berry ripening; this is the period from veraison to harvest

Water use efficiency; tonnes of grapes produced per megalitre of irrigation water applied (t/ML)

Net return per hectare; gross return less the production costs

2.4 Statistical Analyses

All data were analysed statistically using Genstat (8th edition, version 6.1.0.200, Laws Agricultural Trust, UK, ©2002). The harvest measures were analysed using a general analysis

of variance (ANOVA), whereas the berry ripening and leaf area index measures were analysed using repeated measures ANOVA. The significance level is indicated by the P-value. Correlation matrices were performed using Genstat and the confidence limits determined using a table of correlation coefficients. Linear regression analyses were performed using Genstat and Microsoft® Excel 97 software package. The leaf water potential and stomatal conductance figures for Figures 3.15 and 3.16 (Section 3.3.1: Field trials) were adjusted against field effects. The Department of Primary Industries Biometrics unit provided biometrics advice for the experimental designs.

CHAPTER 3: Leaf Water Potential, Stomatal Conductance and ABA Responses of Red Winegrapes to Water Deficit

3.1 Introduction

It is well known that soil water deficits will limit photosynthesis (Kriedemann & Smart, 1971; Liu et al., 1978) with subsequent impacts on vegetative growth (Matthews et al., 1987a; Schultz & Matthews, 1988) and fruit quality (Kliewer et al., 1983; Bravdo et al., 1985; Hepner et al., 1985; Matthews & Anderson, 1989). In the semi-arid viticulture regions of Australia, deficit irrigation techniques are used to manipulate vine growth and fruit composition (Kriedemann & Goodwin, 2003). Anecdotal evidence from the Murray-Darling region in South East Australia indicates that wine grape cultivars, in particular the premium varieties Cabernet Sauvignon and Shiraz, respond differently to hot, dry conditions when managed under similar irrigation regimes. An understanding of the physiological responses of each cultivar to water deficit would assist in designing effective irrigation strategies to maximise both yield and quality, whilst improving water use efficiency.

During a soil water deficit, stomatal closure in grapevines is a dominant factor in minimising transpiration and preventing subsequent damage to a grapevine's hydraulic system (Davies & Gowing, 1999). Generally as a soil dries out, a decrease in stomatal conductance is associated with a reduction in leaf water potential (Smart & Coombe, 1983). Differences in stomatal control of grapevines to water deficit are thought to be due to a combination of hydraulic signals and/or root-sourced chemical signals (Dry & Loveys 1999; Davies et al., 2002; Schultz, 2003; Soar et al., 2006b). It has been suggested that differences in stomatal conductance of grapevine varieties to water deficit could be related to variation in hydraulic conductivity, whereby cultivars with larger xylem conducting vessels are more susceptible to cavitation and therefore stomatal closure (Schultz, 2003). In other instances, reductions in leaf stomatal conductance are regulated by chemical signals (non-hydraulic), predominantly abscisic acid (ABA), that are triggered by root responses to drying soil before being transported to the leaves via the transpiration stream (Loveys & Kriedemann, 1973; Loveys & Düring, 1984; Correia et al., 1995; Davies et al., 2002; Loveys et al., 2005; Soar et al., 2006b).

Various studies of the effects of soil water deficit on grapevine physiology have shown there are differences in water relations and photosynthetic responses between *Vitis vinifera* cultivars (Schultz, 1996; Schultz, 2003; Loveys et al., 2005). Research has shown that the varieties Grenache and Shiraz respond differently to each other when placed under water stress and it was suggested that grapevines might be categorised based on their near-anisohydric (optimistic) or near-isohydric (pessimistic) responses to water stress (Schultz, 2003). Grenache was noted to respond isohydrically to water deficit by controlling stomatal conductance through an interaction between hydraulic and chemical signals (Schultz, 2003). By contrast, Shiraz responded anisohydrically to water deficit due to less rigid stomatal control that allowed a greater fluctuation in leaf water potential with decreasing soil water potential (Schultz, 2003). More recently Loveys et al., (2005) confirmed that Grenache responded to potentially stressful environmental conditions by tightly regulating the stomatal aperture during the day when the evaporative demand was high, thus reducing gas exchange, as opposed to Shiraz, which maintained a higher rate of gas exchange (Loveys et al., 2005).

Furthermore, the differences in stomatal control between varieties may be linked to the presence or absence of an interaction between hydraulic messages and root-to-shoot chemical signals (Davies & Zhang, 1991; Tardieu & Simonneau, 1998). It has been postulated the amount of xylem ABA could be used as an indicator of soil drying, however the concentration of ABA measured from xylem sap cannot solely explain differences in leaf physiology as a result of soil drying (Munns & King, 1988, Davies et al., 2002). Further ABA studies by Tardieu et al. (1996) have suggested that isohydric plants (such as maize) control stomata using a combination of hydraulic and chemical (ABA) signals, whereas anisohydric plants (such as sunflower) control stomatal aperture using hormonal control. Despite differences in the concentration of ABA in xylem sap between Shiraz and Grenache when experiencing conditions of high evaporative demand, it is still unclear whether those differences are due to differences in root responses or differences in ABA synthesis within the canopy itself (Loveys et al., 2005).

The main objective of this present study was to understand the physiological and hormonal responses of four *Vitis vinifera* L. cultivars, including Cabernet Sauvignon, Shiraz, Tempranillo and Grenache, to water deficit. This knowledge could then be used in an attempt

to determine whether certain cultivars are better suited to an induced water stress than others, and how this may ultimately affect grape and wine composition.

3.2 Materials and Methods

The field trials for which physiological and hormonal measurements were taken for this study are described in Chapter 2, section 2.1. The study of glasshouse grown grapevines is described in Chapter 2, section 2.2. Measurements for the glasshouse study were conducted during November and December 2004 and 2005 after the vines were 40 days old and leaves had matured. Since the glasshouse vines were comprised of only two shoots per vine it was important to choose leaves from a similar position along each shoot to reduce any effect of gradients in stomatal conductance and ABA along the shoot (Soar et al., 2004). The field physiology measures were conducted during January to March (veraison to harvest) in 2005 and 2006.

3.2.1 Physiology and hormone measurements

Stomatal conductance:

Stomatal conductance (g_s, $mmol.m^{-2}.s^{-1}$) was measured using a porometer (AP4 series, Delta-T Devices Ltd, Cambridge, UK). Water vapour is released into the porometer cup from the sub-stomatal cavities and the porometer measures the time taken to raise cup humidity by a set amount. This time is dependent on stomatal conductance, which can be derived from a calibration curve generated using the supplied calibration plate. Before every set of readings the instrument was calibrated using the calibration plate to an error tolerance of <10%. To reduce any effect from dirt or chemical residues on the porometer readings, the leaves for the first set of readings of the day were gently cleaned with deionised water and allowed to dry thoroughly.

Fully-expanded, sun-exposed, mature leaves of the field and glasshouse grapevines were used to measure g_s. Field measures were taken for both controls, and 43% and 45% SDI treatments, on the Cabernet Sauvignon and Shiraz vines, respectively. For the field measures eight replicates per treatment were measured. For each field vine, g_s was measured on five, fully

expanded sun-exposed leaves at approximately two hourly intervals between 0900 h and 1700 h. In the glasshouse, g_s was measured on both control and deficit vines between 0900 h and 1700 h. Three leaves per glasshouse vine were measured across five replicates per irrigation treatment.

Leaf water potential:

Leaf water potential (Ψ_l, MPa) was measured in field and glasshouse vines using a pressure chamber (Plant Water Status Console 3000 series, Soil Moisture Equipment Corp., Santa Barbara, CA) at pre-dawn and the same time intervals as for the g_s readings (Schölander et al., 1965). Field measures were taken from the same vines used for the g_s readings. Fully-expanded, sun-exposed (except for pre-dawn measures), mature leaves were selected. For the field measures, three leaves per vine were selected for Ψ_l measures, whereas for the glasshouse vines two leaves per vine were chosen. Immediately before excision, a plastic bag was placed over the leaf laminar. Each leaf was excised from the shoot using a scalpel blade and then placed into the pressure chamber with the petiole protruding from the chamber lid. The chamber was pressurised using industrial grade nitrogen (BOC, Australia) and Ψ_l measured when xylem sap was observed emerging from the cut end of the petiole. For the field and glasshouse trials there were eight and five replicates measured per treatment, respectively at 0500 h, 0900 h, 1130 h, 1400 h and 1630 h on the measurement day.

Xylem sap collection:

To collect the xylem sap, the same leaf that had been used for Ψ_l remained in the pressure chamber and was exposed to an extra 0.1 MPa. The sap expressed from the cut petiole of each leaf was collected using a 200 µL micro-pipette (Gilsen Pipetman®, John Morris Scientific, Melbourne, Australia) and transferred to a separate labelled and pre-weighed (four-decimal places) 1.5 mL microcentrifuge tube. The microcentrifuge tube was stored on dry ice to snap freeze the fresh sap sample and then stored at -80 ^{o}C for later analysis of ABA. None of the sap samples were pooled, but rather analysed on an individual basis for field and glasshouse vines.

Abscisic acid analysis:

Xylem sap samples were analysed individually for abscisic acid (ABA, $[ABA]_{xylem\ sap}(ng.ml^{-1})$) using a stable isotope dilution assay. The xylem sap samples were defrosted in low light

conditions at room temperature before being weighed to four decimal places. To each sample, 100 µL of deuterated ABA [3´, 5´, 5´, 7´, 7´, 7´ - D_6 - ABA] standard (10 ng/100 µL) was added. The samples were dried down using a vacuum centrifuge (Speed Vac®, Savant Instruments Ltd, New York, USA). The dry pellet was resuspended in 50 µL of dry acetone and vortexed (Ratek VM 1, Ratek Instruments, Boronia, Australia). A 100 µL sample of ethereal diazomethane was added to each sample and the tube was closed and incubated at room temperature for 20 min. The tubes were then opened and the sample was allowed to air dry overnight in a fume hood. The remaining residue was resuspended in 100 µL of dry acetone, mixed and centrifuged for 3 min at 10,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia). 80 µL of sample was removed and added to a gas chromatography (GC) vial and either dried down in the vacuum centrifuge or left to dry over-night. At this stage aluminium foil was placed over the top of the GC vials to reduce light infiltration. Each sample was resuspended in 20 µL of dry acetone prior to ABA analysis. ABA quantification was performed using a a gas chromatography/mass spectrometry unit (GC-MS) system (HP 6890 series, J & W Scientific, Folsom, CA) equipped with a mass selective detector (HP 5973, J & W Scientific, Folsom, CA). The GC-MS was operated in selected ion mode as detailed in Soar et al., (2004).

3.2.2 Grapevine growth and canopy development

Leaf area index (LAI):

A non-destructive measure of canopy growth was performed in the field using a handheld ceptometer (AccuPAR LP-80, Decagon Devices Inc., Pullman, USA). Readings were taken at solar noon on clear sky days from budburst until harvest (September 2005 to February 2006). The ceptometer was used to capture photosynthetically active radiation (PAR) in the 400-700 nm waveband. For each vine, the ceptometer captured relative values of PAR in the direction of the solar beam (at solar noon) that were then automatically converted to LAI by the instrument. Measurements were taken from the middle of the row opposite the selected vine and by horizontally guiding the ceptometer approximately 1.0 m from the ground perpendicular into the canopy to ensure the entire probe was capturing the canopy shade. Six measures of PAR were taken per vine by placing the ceptometer in each corner of the vine's canopy shade and then opposite both sides of the trunk. Four replicates per irrigation treatment were measured.

Pruning weights:

Since both vineyards in the field trial were mechanically pruned to a hedge shape it was also necessary to simulate this form of pruning when measuring the pruning weights of the study vines. Canes were hand pruned from a 1.0 m wide segment, randomly positioned over the vine canopy then weighed for pruning weight. The canes were pruned to simulate the normal mechanical pruning practised in the respective vineyards. Eight replicates per irrigation treatment were measured.

3.3 Results

3.3.1 Effect of SDI on grapevine physiology and hormonal responses

Glasshouse scoping study:

The glasshouse vines showed diurnal differences in g_s between varieties under full irrigation (Figure 3.1). The Shiraz vines exhibited significantly higher g_s levels during the day compared to the Cabernet Sauvignon and Grenache vines. The g_s levels of the Shiraz vines gradually increased during the day compared to Grenache where the g_s values remained fairly uniform. The g_s levels of the Tempranillo vines were significantly higher than the Cabernet Sauvignon and Grenache vines at 0900 h, 1030 h, 1200 h and 1330 h.

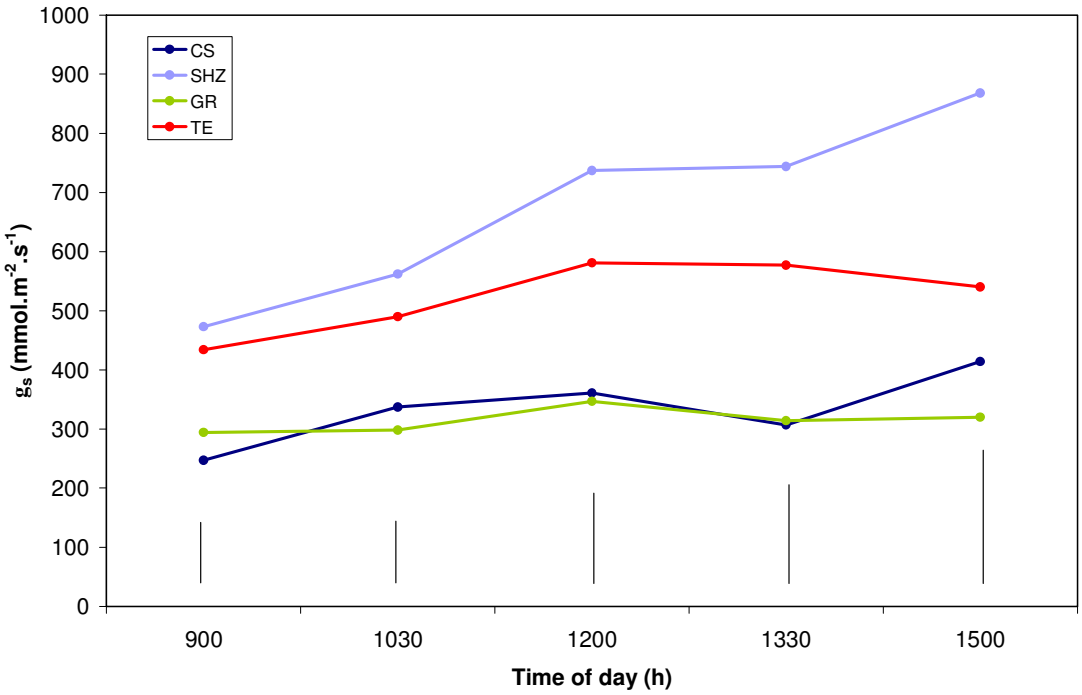


Figure 3.1 Diurnal changes in stomatal conductance (g_s) from Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse control (full irrigation) vines measured on 12 November 2005. LSD bars indicate significance at P=0.05. (n=5).

Irrespective of variety, the control vines at 0500 h (pre-dawn) had higher Ψ_l than the deficit vines (Figure 3.2A). The Cabernet Sauvignon controls had significantly higher Ψ_l than the controls of the other varieties (Figure 3.2A), whereas there were no differences between varieties for deficit vines. By 1400 h the Cabernet Sauvignon control vines had significantly lower Ψ_l than the controls of the other varieties (Figure 3.2B). Furthermore, the Cabernet Sauvignon deficit vines at 1400 h were exhibiting significantly lower Ψ_l than the Tempranillo deficit vines (Figure 3.2B).

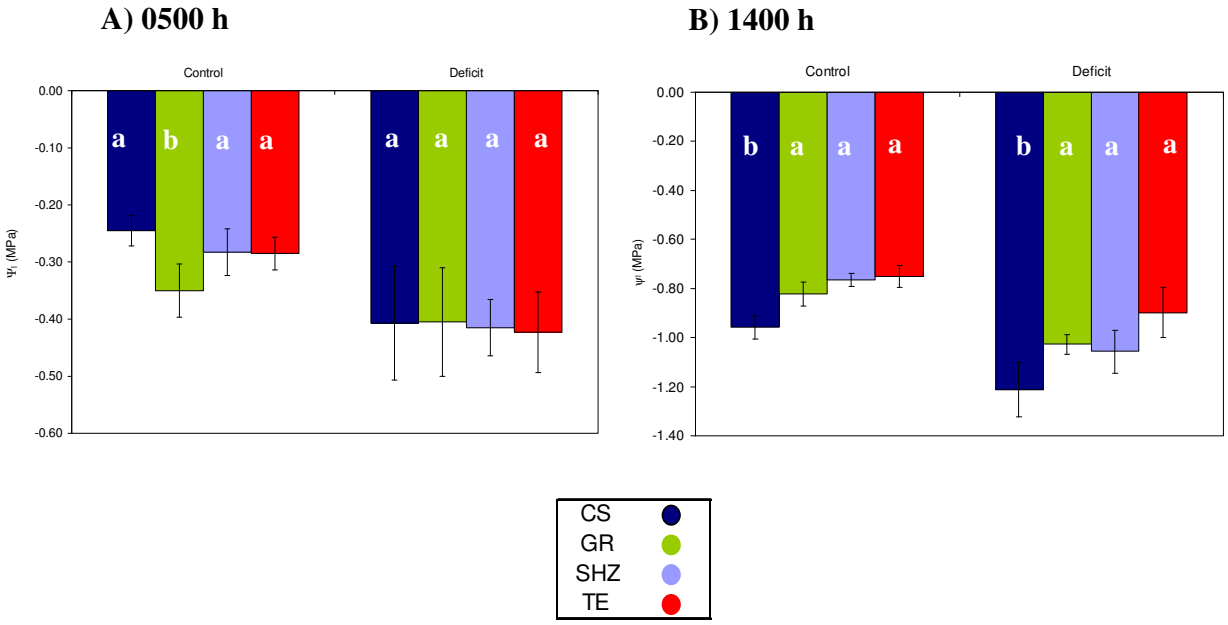


Figure 3.2 Leaf water potential (Ψ_l) of leaves of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005 at A) predawn (0500 h) and B) 1400 h. Means for the control or deficit vines followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10).

At 1400 h, g_s was significantly higher for the Cabernet Sauvignon control than the controls of the other varieties (Figure 3.3). In addition, the Shiraz control had significantly higher g_s values than the Tempranillo control (Figure 3.3). The Grenache deficit vines had lower g_s values than the other deficit varieties but this result was not significant (Figure 3.3).

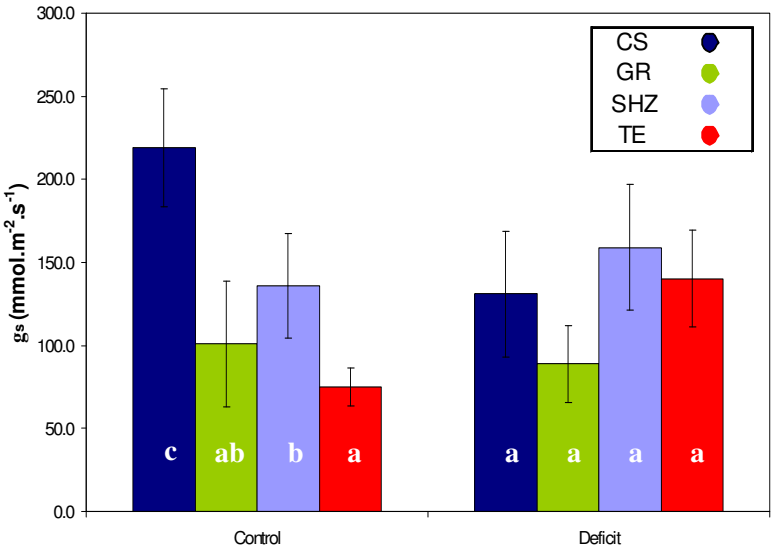


Figure 3.3 Stomatal conductance (g_s) at 1400 h from leaves of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005. Means for the control or deficit vines followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10).

Xylem sap ABA concentrations were lower in the control than the deficit vines regardless of variety (Figure 3.4A). At 0500 h, the Grenache controls had significantly higher xylem sap [ABA] than the other control varieties (Figure 3.4A). In the deficit treatment at 0500 h, the Cabernet Sauvignon and Grenache vines had significantly higher xylem sap [ABA] than the Tempranillo vines (Figure 3.4A). By 1400 h, the xylem sap [ABA] for the control and deficit vines was similar to the 0500h measures (Figure 3.4B). The control Shiraz and Tempranillo

vines had significantly lower xylem sap [ABA] than the Cabernet Sauvignon and Grenache controls (Figure 3.4B). By contrast the xylem sap [ABA] at 1400 h was not significantly different between the varieties that had undergone deficit irrigation.

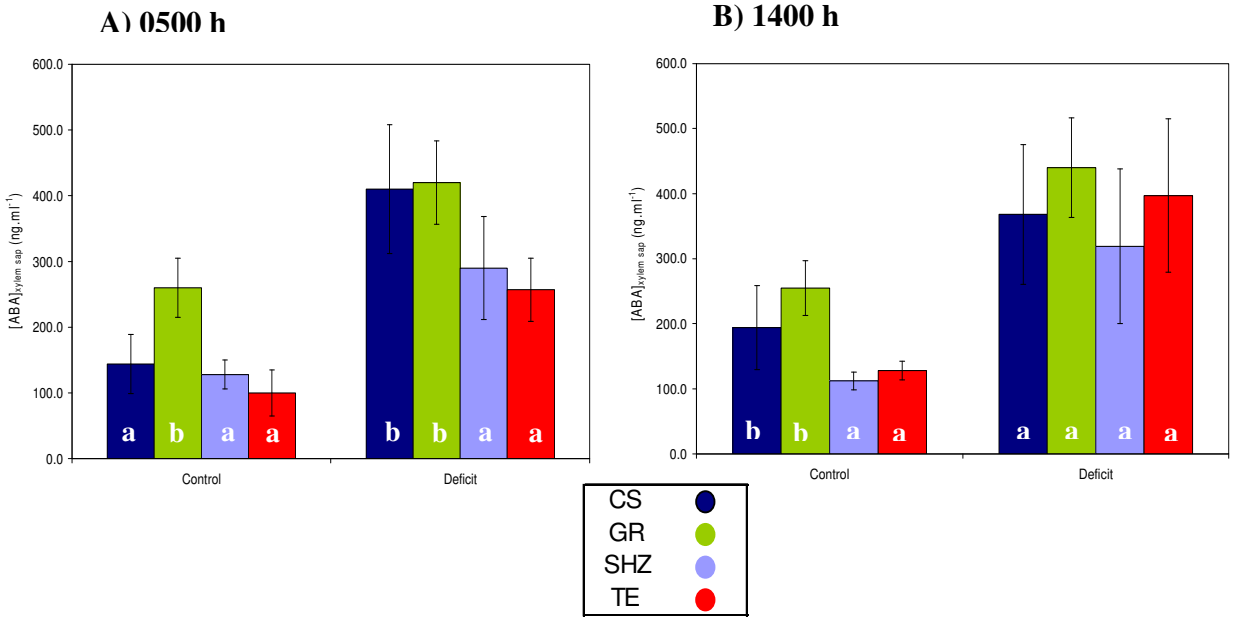


Figure 3.4 Petiole xylem sap abscisic acid [ABA] collected from Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines exposed to a full (control) and deficit irrigation. Measurements were taken on the 22 and 28 November 2005 at A) predawn (0500 h) and B) 1400 h. Means for the control or deficit vines followed by the same letter are not significantly different at P=0.05. Data points represent mean values ± s.e. (n=10).

Figure 3.5 illustrates the relationship between Ψ_l and g_s for each variety at 1400 h. Except for Grenache, the other varieties had a positive relationship where g_s increased with increasing Ψ_l. Overall, the Cabernet Sauvignon (r^2=0.5042) and Tempranillo (r^2=0.4489) vines have strong positive relationships between Ψ_l and g_s, whereas the Grenache (r^2=0.0006) showed little change in Ψ_l as g_s increased.

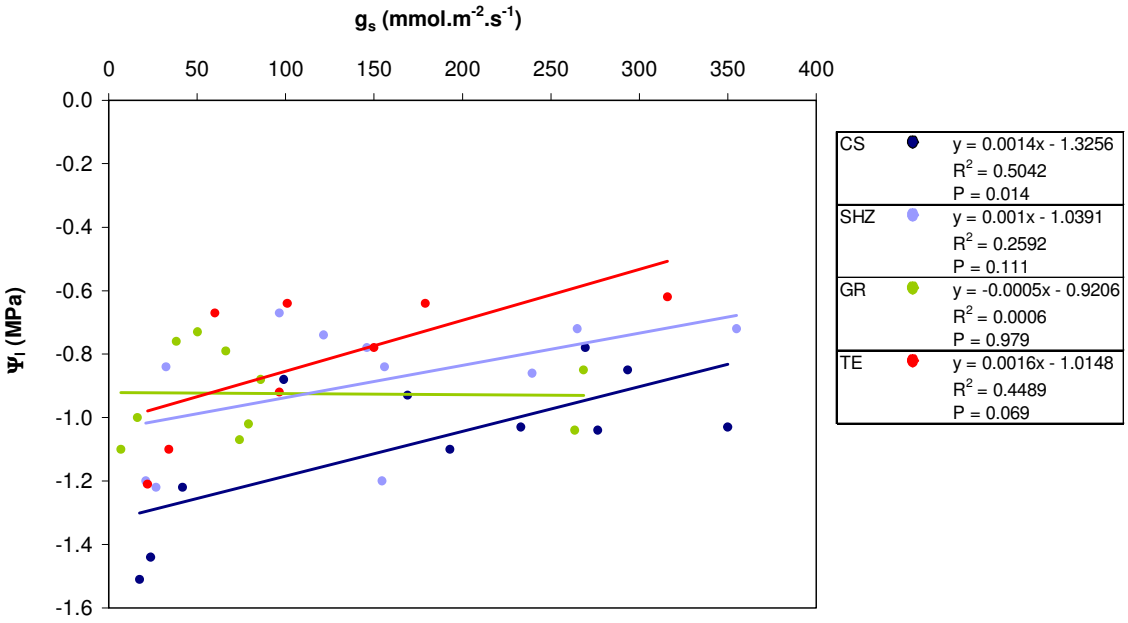


Figure 3.5 Linear regression of leaf water potential (Ψ_l) and stomatal conductance (g_s) of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured at 1400 h on the 22 and 28 November 2005. Regression lines represent both control and deficit treatments, (n=10).

Figure 3.6 illustrates the relationship between Ψ_l and xylem sap [ABA] for each variety at 0500 h (Fig 3.6A) and 1400 h (Fig 3.6B). At both times of the day there was a negative relationship between Ψ_l and xylem sap [ABA] for Cabernet Sauvignon (0500 h: r^2=0.5531, 1400 h: r^2=0.6093), Shiraz (0500 h: r^2=0.3619, 1400 h: r^2=0.4345) and Tempranillo (0500 h: r^2=0.5133, 1400 h: r^2=0.4109). Grenache had no relationship between Ψ_l and xylem sap [ABA] at 0500 h (r^2=0.0146), that changed to a positive relationship by 1400 h (r^2=0.4444).

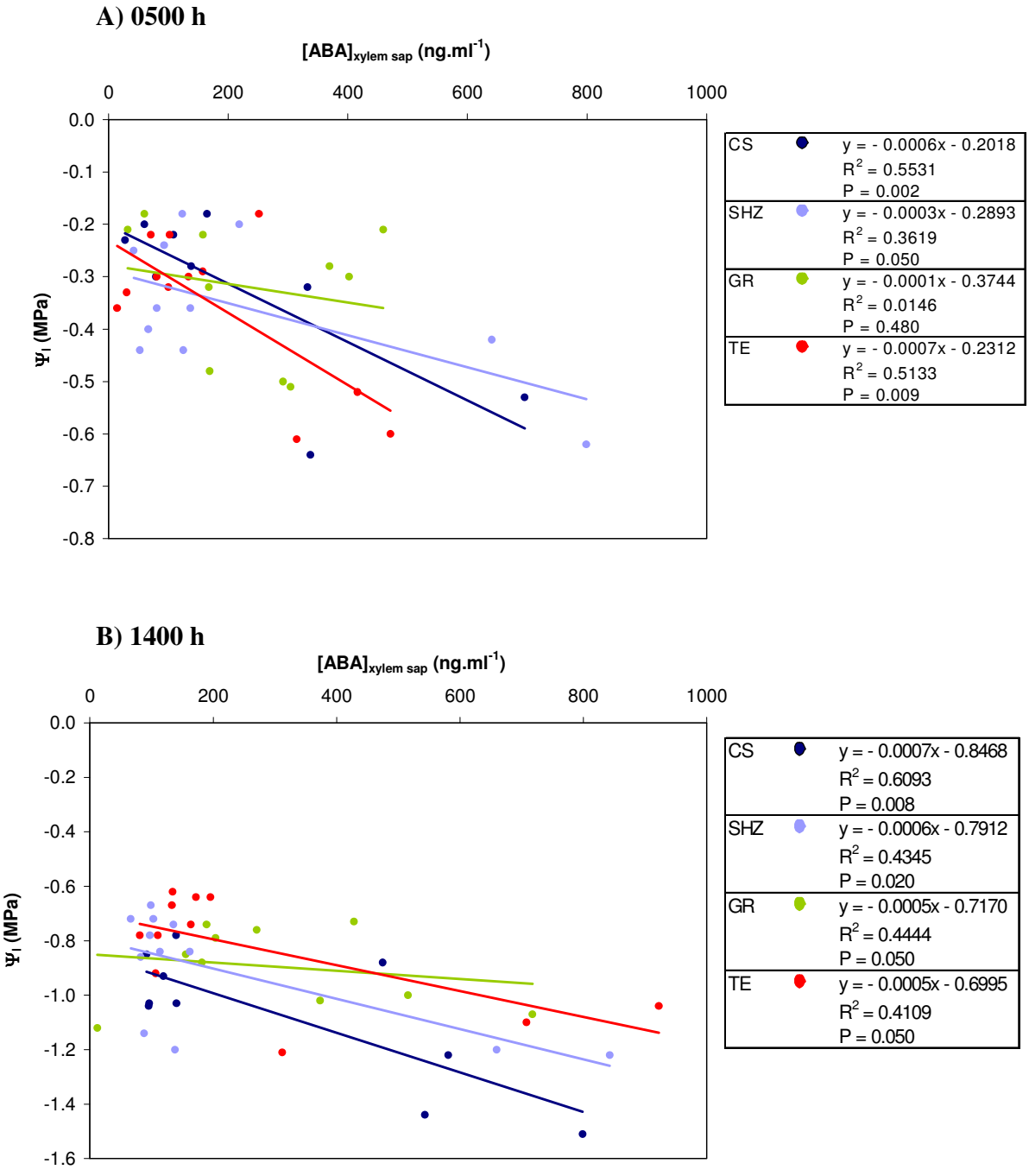


Figure 3.6 Linear regression of leaf water potential (Ψ_l) and xylem sap abscisic acid [ABA] of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured on the 22 and 28 November 2005 at A) 0500 h and B) 1400 h. Regression lines represent both control and deficit treatments, (n=10).

Figure 3.7 illustrates the relationship between g_s and xylem sap ABA at 1400 h. All varieties displayed a negative relationship between g_s and xylem sap [ABA], with higher concentrations of xylem sap ABA in leaves of lower g_s. The Cabernet Sauvignon (r^2=0.861), Shiraz (r^2=0.462) and Grenache (r^2=0.906) vines produced significantly more xylem sap [ABA] as g_s decreased.

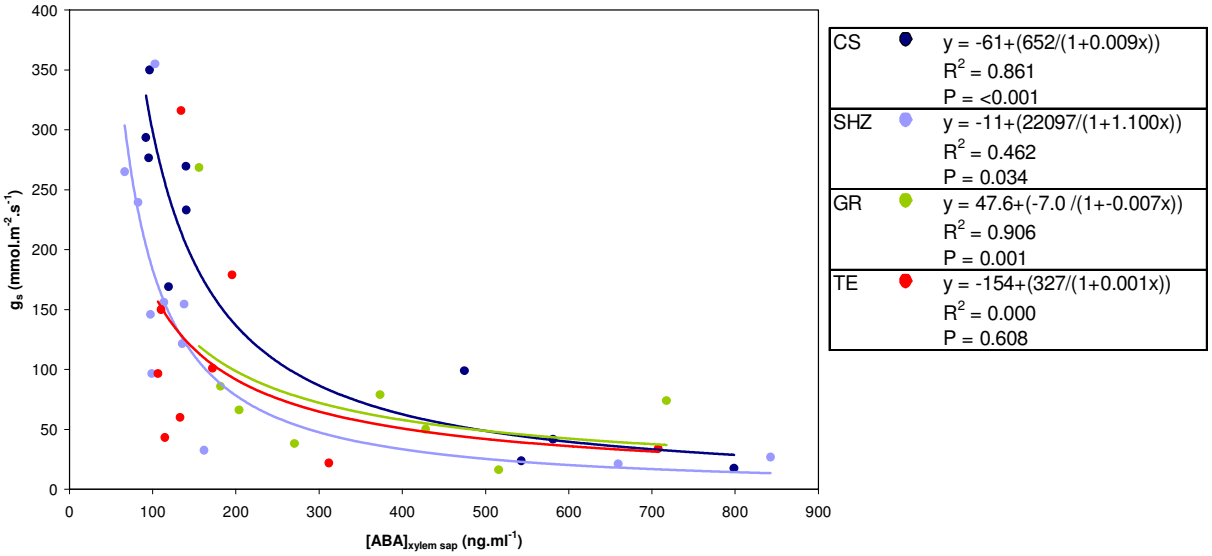


Figure 3.7 Exponential regression of stomatal conductance (g_s) and abscisic acid [ABA] of Cabernet Sauvignon (CS), Shiraz (SHZ), Grenache (GR) and Tempranillo (TE) glasshouse vines measured on the 22 and 28 November 2005 at 1400 h. Regression lines represent both control and deficit treatments, (n=10).

Field trials:

Diurnal changes in Ψ_l of both varieties followed similar patterns, with the SDI treatments having lower Ψ_l than the controls. The Cabernet Sauvignon vines had significantly lower Ψ_l for the 43% SDI-treated vines than the control over the entire day (Figure 3.8A). By contrast, for the Shiraz, whilst the 45% SDI-treated vines tended to have lower Ψ_l than the control, there were only significant differences in Ψ_l between the control and 45% SDI-treated vines after 1400 h (Figure 3.8B).

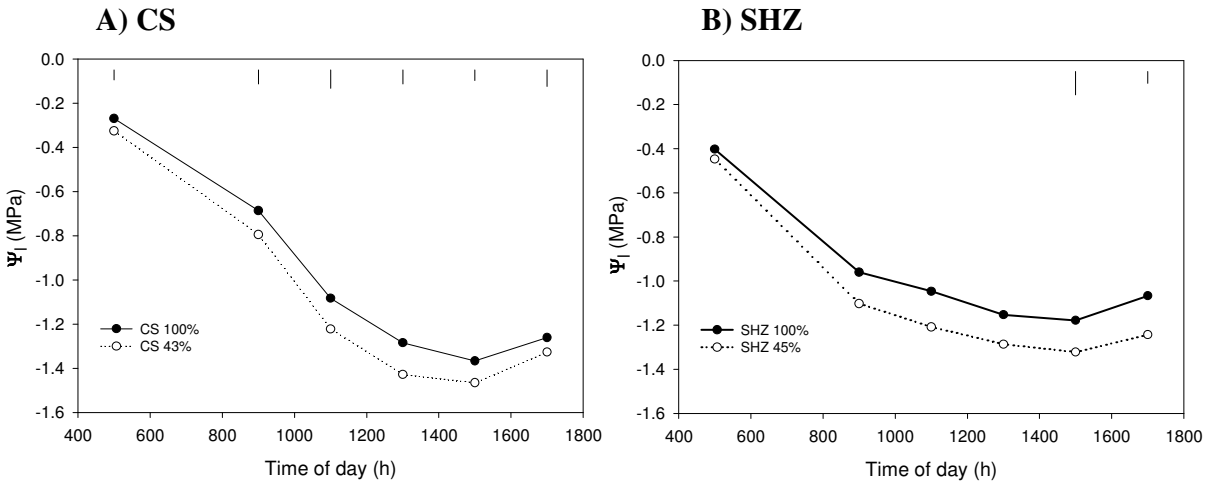


Figure 3.8 Diurnal leaf water potential (Ψ_l) for field-grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) irrigated at a full irrigation (100% control) and a deficit irrigation (43% or 45%). Measurements for Cabernet Sauvignon and Shiraz were taken on the 13 and 18 January 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference (P=0.05) at that time of day, (n=8).

Irrespective of irrigation treatment, Shiraz g_s was generally higher over the day than Cabernet Sauvignon (Fig 3.9A, B). For both varieties, g_s were lower in the SDI-treated vines than the control vines. Furthermore, there tended to be a difference in g_s after 1300 h for the Shiraz compared to the Cabernet Sauvignon, even though there was a minimal difference in Ψ_l between the varieties at the same time.

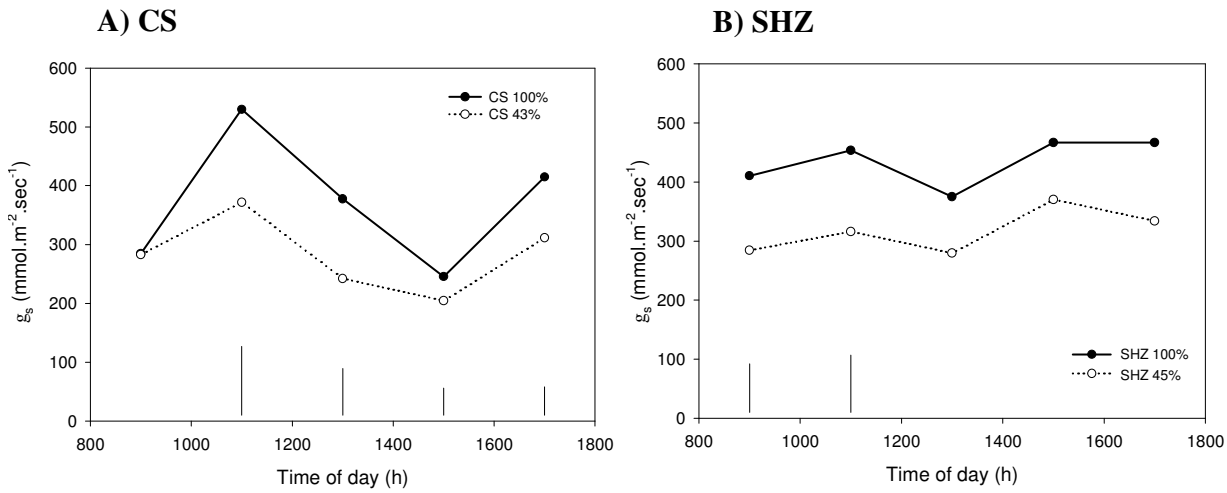


Figure 3.9 Diurnal stomatal conductance (g_s) for field-grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) irrigated at a full irrigation (100% control) and a deficit irrigation (43% or 45%). Measurements for Cabernet Sauvignon and Shiraz were taken on the 13 and 18 January 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference (P=0.05) at that time of day, (n=8).

Figures 3.10, 3.11 and 3.12 are collated from results taken on a different date (10 February 2005 Shiraz: 17 February 2005 Cabernet Sauvignon). The Cabernet Sauvignon control and deficit vines had lower Ψ_l throughout the day than the Shiraz control and SDI-treated vines (Figure 3.10). For both varieties the deficit vines also had significantly lower Ψ_l at most times during the day compared to the control. The g_s levels for both varieties were lower for the SDI-treated vines than the controls (Figure 3.11). After midday the g_s levels for the Cabernet Sauvignon control and SDI-treated vines decreased, compared to the Shiraz control vines.

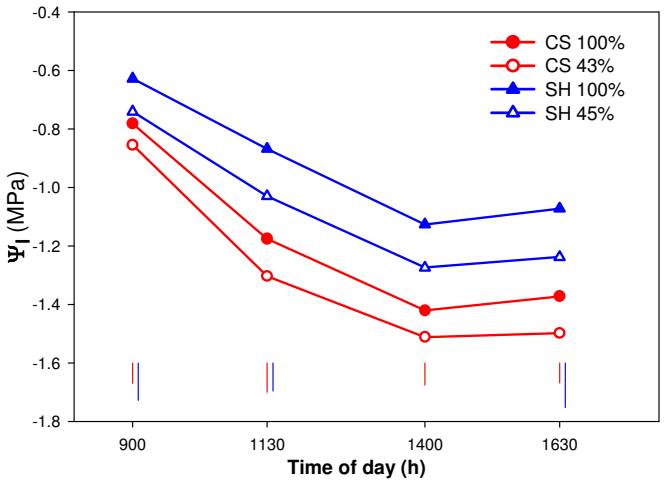


Figure 3.10 Diurnal leaf water potential (Ψ_l) for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference at that time of day (P=0.05), (n=8).

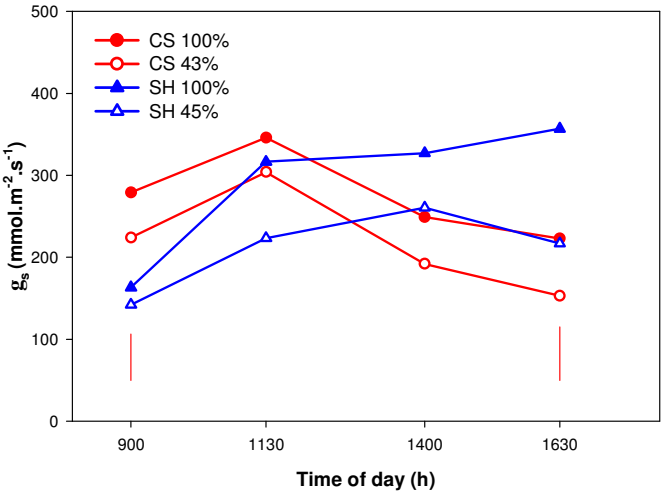


Figure 3.11 Diurnal stomatal conductance (g_s) for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Bars indicate LSD at P=0.05. Absence of a bar indicates no significant difference at that time of day (P=0.05), (n=8).

The quantity of xylem sap extracted from the petioles ranged from 1 to 20 µL and varied in quantity depending on collection time in the day, the diameter of the leaf petiole and the timing of the irrigation cycle. It was observed that less sap could be extracted from the petioles (irrespective of diameter) at pre-dawn and during the afternoon compared to mid morning measures. Also it was easier to extract more sap from the thick rather than thin petioles. Cabernet Sauvignon vines produced higher xylem sap [ABA] throughout the entire day compared to the Shiraz irrespective of the irrigation treatment (Figure 3.12). The Shiraz control vines produced significantly lower xylem sap [ABA] compared to the 45% SDI-treated vines. While the Cabernet Sauvignon 43% SDI-treated vines produced higher xylem sap [ABA] than the control vines, the xylem sap [ABA] was only significantly higher after 1630 h for the 43% SDI-treated vines compared to the control.

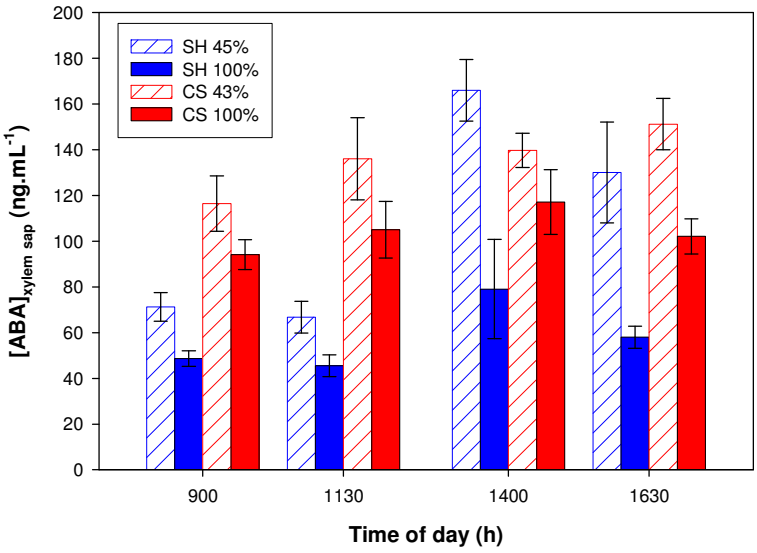


Figure 3.12 Diurnal xylem sap abscisic acid [ABA] levels for field-grown Cabernet Sauvignon (CS) and Shiraz (SH) irrigated at a full irrigation (100% control) and deficit irrigation (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Data points represent mean values ± s.e. (n=8).

The linear regression shows a negative relationship between xylem sap ABA and Ψ_l, for both varieties regardless of irrigation treatment (Figure 3.13). Even though the relationships between Ψ_l and xylem sap [ABA] were very weak, the 43% SDI Cabernet Sauvignon tended to have a more negative relationship (R^2=0.0715) between xylem sap [ABA] and Ψ_l compared to the control (R^2=0.0408) (Figure 3.13A). The Shiraz 45% SDI-treated vines had higher xylem sap [ABA] as Ψ_l decreased (Figure 3.13B).

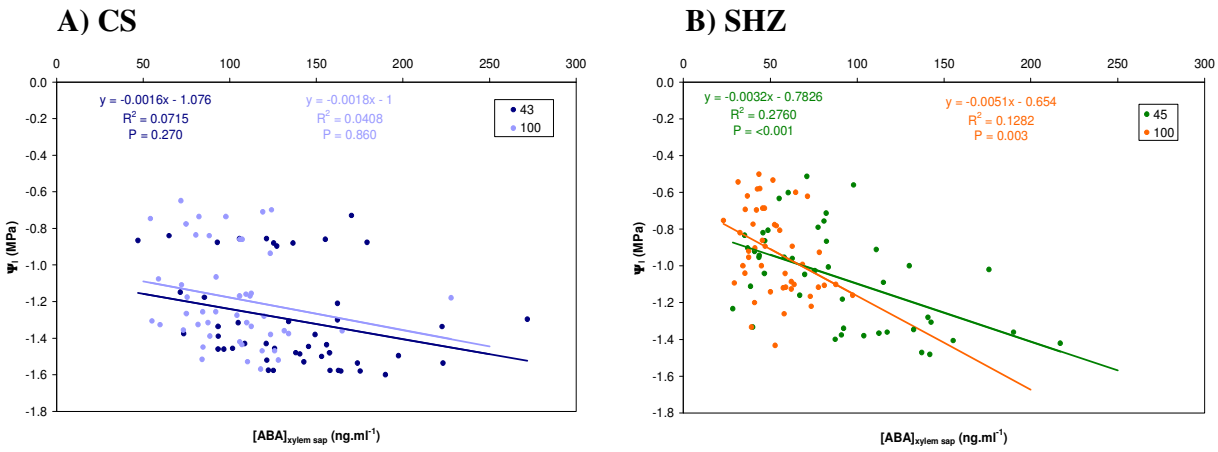


Figure 3.13 Linear regression of leaf water potential (Ψ_l) and xylem sap abscisic acid [ABA] of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control (100%) irrigation or SDI (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8).

Figure 3.14 illustrates the relationship between g_s and xylem sap [ABA] for the Cabernet Sauvignon and Shiraz vines, for the control and SDI (43 and 45%) treatments). The g_s decreased as xylem sap [ABA] increased in both the control and SDI-treated Cabernet Sauvignon vines (Figure 3.14A). The Shiraz control vines produced a range of g_s levels at similar ABA concentrations, whereas the g_s levels tended to increase with higher xylem sap [ABA] for the Shiraz 45% SDI-treated vines (Figure 3.14B).

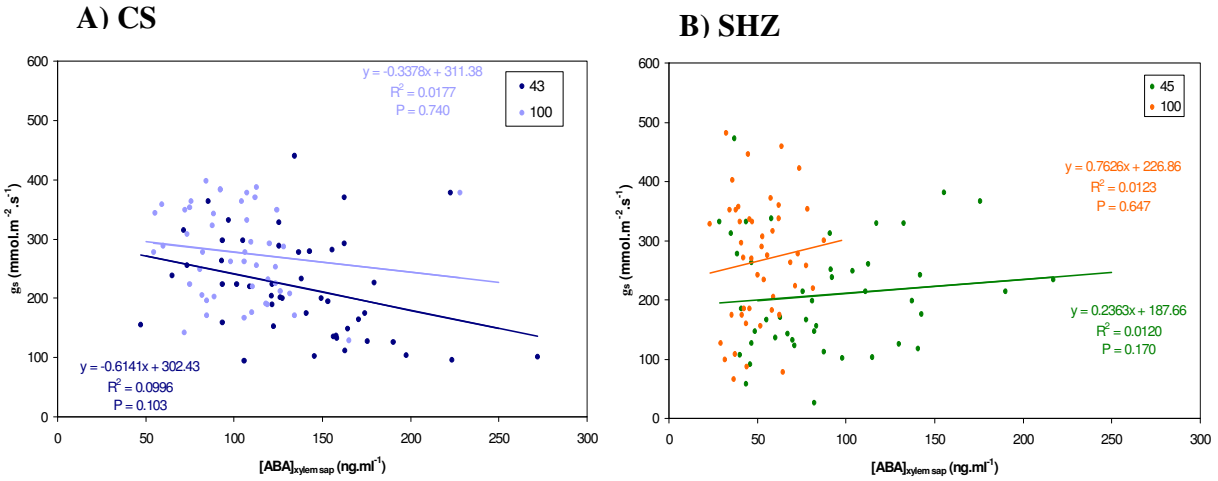


Figure 3.14 Linear regression of stomatal conductance (g_s) and abscisic acid (ABA) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control (100%) irrigation or SDI (43% or 45%). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8).

In the Cabernet Sauvignon, there were strong relationships between Ψ_l and xylem sap [ABA] at all measured times of the day for control and deficit treatments (Figure 3.15A). However, Ψ_l for the Cabernet Sauvignon decreased in the afternoon as xylem sap [ABA] increased, particularly at 1400 h and 1630 h (Figure 3.15A). In the Shiraz vines there was a strong relationship between Ψ_l and xylem sap [ABA] at all measured times of the day (Figure 3.15B). The general trend in the Shiraz was that as xylem sap [ABA] increased the Ψ_l decreased.

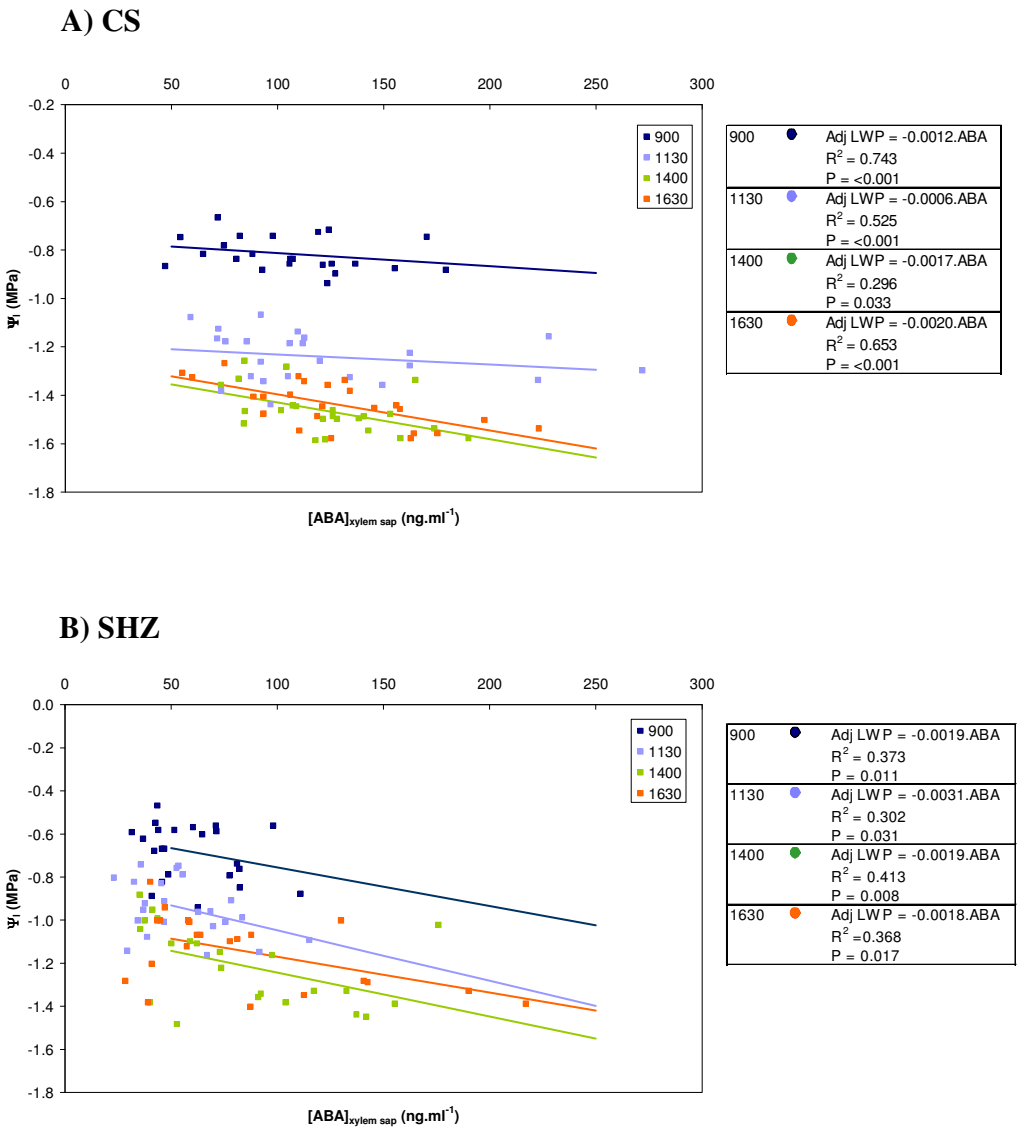


Figure 3.15 Relationship between adjusted leaf water potential (LWP - Ψ_l) and abscisic acid (ABA) measured at different times of the day (0900, 1130, 1400 and 1630 h) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8).

For Cabernet Sauvignon, there was a very weak relationship between g_s and xylem sap [ABA] at 0900 h (Figure 3.16A). However, a negative relationship strengthened with time and by 1630 h the vines that had lower g_s levels had much higher xylem sap [ABA] compared to levels at 0900 h, 1130 h and 1400 h (Figure 3.16A). For Shiraz, there was a very weak relationship at 0900h between g_s and xylem sap [ABA]. However later in the day there were variable responses with the Shiraz vines at 1400 h tending to have higher xylem sap [ABA] as g_s increased, whereas at 1130 h and 1630 h the g_s levels were lower as xylem sap [ABA] increased (Figure 3.16B).

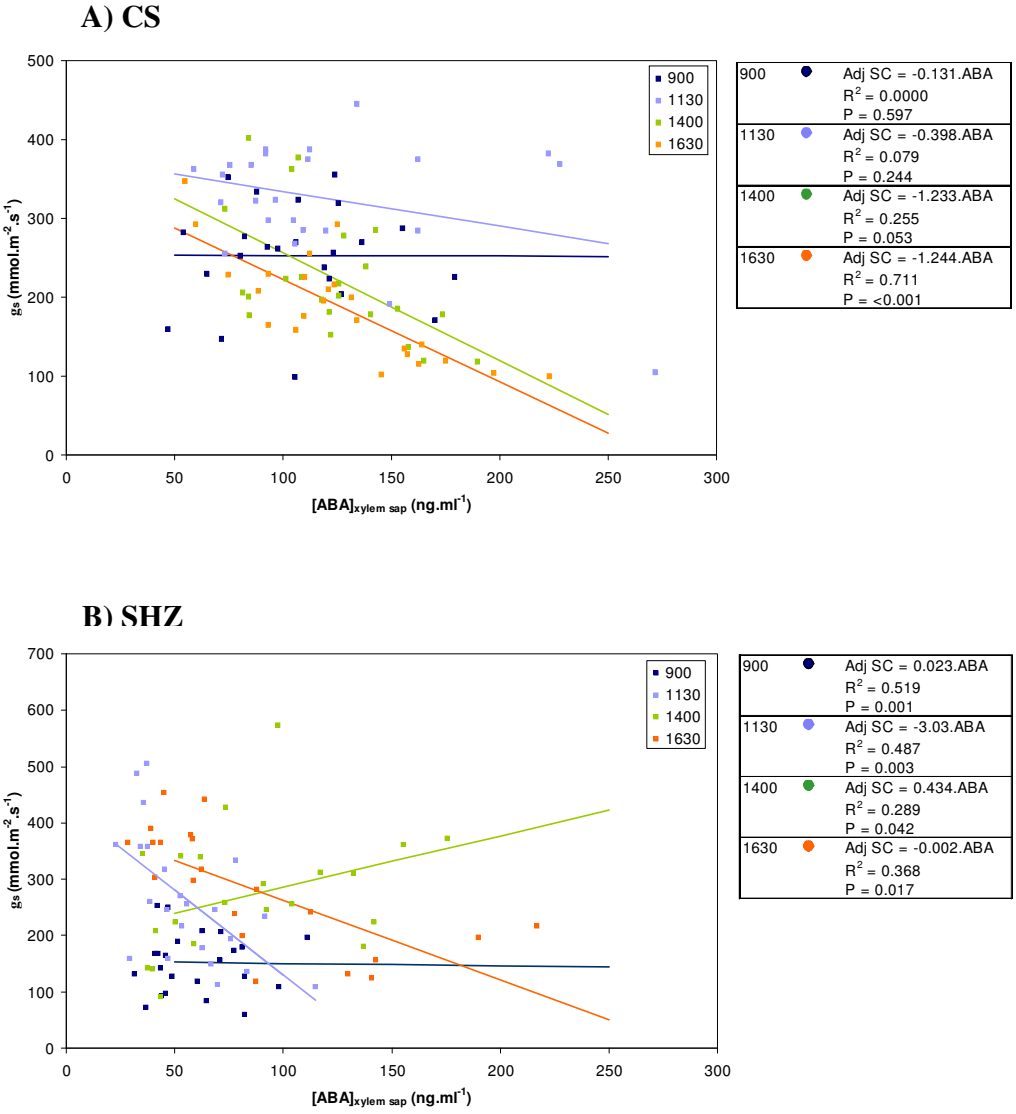


Figure 3.16 Relationship between stomatal conductance (g_s) and abscisic acid [ABA] measured at different times of the day (0900, 1130, 1400 and 1630 h) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ). Measurements for Shiraz and Cabernet Sauvignon were taken on the 10 and 17 February 2005 respectively. Regression lines represent both control and deficit treatments, (n=8).

3.3.2 Leaf area index and pruning weights

Leaf area index (LAI) was not significantly different between treatments for both varieties until after 72 and 155 days after budburst for Cabernet Sauvignon and Shiraz respectively. For the Cabernet Sauvignon at 155 days from budburst the 52% and 43% SDI-treated vines had significantly lower LAI compared to the control and 70% SDI-treated vines (Figure 3.17A). For Shiraz, the 34% SDI-treated vines had a significantly lower LAI than the control vines (Figure 3.17B). The 65% and 45% SDI-treated Shiraz vines also had lower LAI than the control, but this was not significant. The decline in LAI for Cabernet Sauvignon from day 80 to 160 is the result of summer pruning and not the influence of the irrigation treatments increasing leaf senescence.

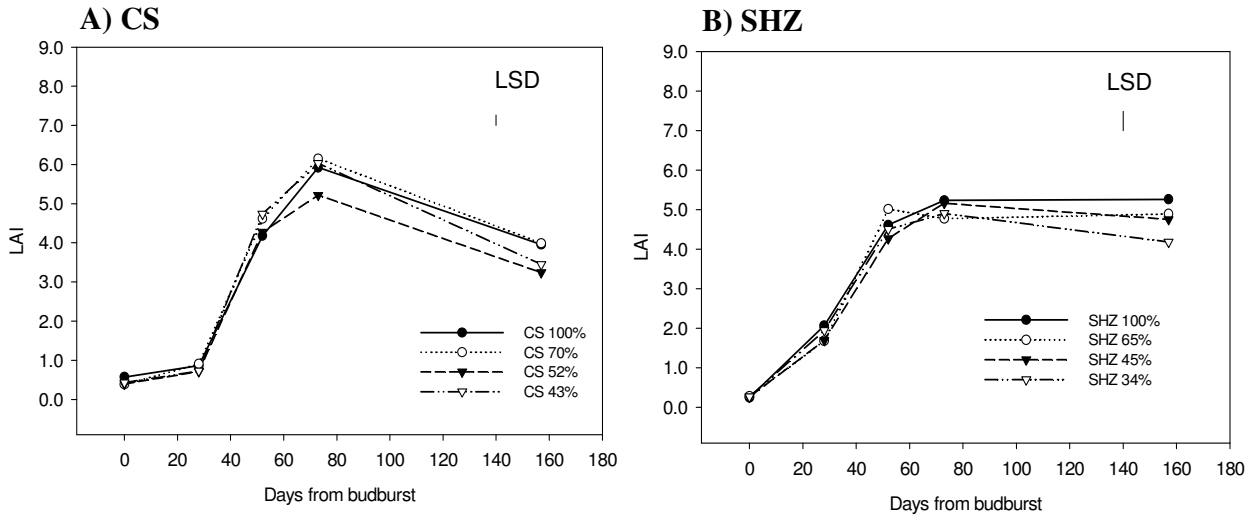


Figure 3.17 Leaf area index (LAI) of field grown A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) exposed to a control and SDI treatments during 2005/2006 (Year 3). Significance indicated by LSD bar for irrigation treatment at P=0.05, (n=4).

Pruning weights for the Cabernet Sauvignon were significantly reduced for the 52% and 45% SDI-treated vines compared to the control and 70% SDI-treated vines in 2004/2005. This was the only season that the SDI treatments caused a reduction in pruning weight for the Cabernet Sauvignon. The Shiraz, while tending to produce lower pruning weights as less water was applied, did not show a significant reduction in pruning weights as a result of the SDI treatments, even after three seasons of receiving deficit irrigation.

Table 3.1 Mean pruning weights for Cabernet Sauvignon and Shiraz taken during 2004-2006. Means followed by the same letter are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row.

Pruning weights (kg/vine)	**Irrigation treatments**			
Cabernet Sauvignon	***100%***	***70%***	***52%***	***43%***
2003/2004	1.56	1.59	1.47	1.17
2004/2005	1.35[b]	1.38[b]	0.78[a]	0.68[a]
2005/2006	1.50	1.70	1.41	1.43
Shiraz	***100%***	***65%***	***45%***	***34%***
2003/2004	1.70	1.72	1.87	1.48
2004/2005	1.52	1.96	1.53	1.33
2005/2006	2.27	2.19	1.82	1.80

3.4 Discussion

To investigate the effects of sustained deficit irrigation (SDI) on physiology and hormonal responses, a series of glasshouse and field measurements were taken during the 2005-2006 seasons. The glasshouse vines were established as a scoping study to explore the physiological responses of certain red winegrape varieties to well-watered and deficit conditions as discussed in Chapter 2, section 2.2. Data have been shown where comparisons could be made

between leaf water potential, stomatal conductance and abscisic acid measures for certain times of the day.

The field trials were conducted to establish whether the SDI treatments were producing differences in physiological response to the water deficit (Chapter 2, section 2.1). Even though the Cabernet Sauvignon and Shiraz varieties were grown in different locations, comparisons were facilitated because the physiological measurements were conducted within the same climatic region and at the same altitude. Furthermore the vines were grown on the same rootstock (140 Ruggeri) of a similar age and in vineyards of similar soil type.

3.4.1 Physiological response of grapevine varieties to water deficit

The differences in grapevine origin and genotype have ultimately influenced their physiological responses to water deficits to the point of potentially grouping grape cultivars based on their stomatal response to water deficits (Schultz & Matthews, 1993). Grapevines may tightly regulate stomatal opening when grown in soils of low water availability to reduce gas exchange and conserve available resources for future growth. This type of physiological response is generally classified as “drought avoiding” or isohydric (pessimistic) (Jones, 1980; Smart & Coombe, 1983). By contrast an anisohydric (optimistic) vine will try to maintain a higher rate of gas exchange for immediate use of available resources irrespective of potential soil deficit conditions (Schultz, 2003; Loveys et al., 2005). Various studies into the effects of soil water deficit on grapevine physiology have shown differences in water relations and photosynthetic responses between *Vitis vinifera* cultivars (Schultz, 1996; Schultz, 2003; Soar et al., 2006b). Research by Schultz (2003) on Syrah (Shiraz) and Grenache has shown these varieties do respond differently to water stress and can potentially be categorised based on their near-anisohydric (Syrah) or near-isohydric (Grenache) responses to water stress. Within the Murray-Darling region, anecdotal evidence suggests certain wine grape cultivars, in particular Shiraz and Cabernet Sauvignon, can respond differently to hot, dry conditions when managed under similar irrigation strategies. Since Shiraz has previously been described as displaying anisohydric responses (Schultz, 2003; Soar et al., 2006b), then it could be hypothesised from these anecdotal observations that Cabernet Sauvignon may be exhibiting more isohydric-like responses when exposed to water deficit.

Glasshouse physiology:

For the purpose of the glasshouse study Shiraz and Grenache were chosen based on the suggestions from current research that these varieties display contrasting physiological responses when exposed to water deficit (Schultz, 2003; Soar et al., 2006b). Shiraz originates from northern France near the Rhône valley, where the climate is typically semi-continental whereas, Grenache originates from Spain around the Mediterranean region of Aragon. Given the contrasting physiological responses of these varieties to soil water deficit it was hypothesised that Cabernet Sauvignon and Tempranillo may potentially exhibit different water use strategies due to their environmental origins. Both varieties have evolved from different climatic regions with Cabernet Sauvignon originating in Bordeaux region of France where the climate is influenced by Atlantic weather and Tempranillo from the continental climate of northern and central Spain.

Initial glasshouse studies showed that under well-watered conditions there was a significant difference in diurnal stomatal conductance levels between the varieties (Figure 3.1). In particular, the stomatal conductance levels of the Shiraz continued to increase during the day, whereas Grenache and Cabernet Sauvignon maintained relatively uniform and lower stomatal conductance levels. In light of the definitions for isohydric and anioshydric responses it could be hypothesised the Cabernet Sauvignon and Grenache are displaying isohydric-like responses compared to the anisohydric-like responses of the Shiraz.

When the glasshouse study was repeated to explore the effect of water deficit on varietal response, the deficit vines irrespective of variety had lower (more negative) leaf water potentials and generally lower stomatal conductance than the control vines (Figure 3.2). For both control and deficit vines the Shiraz and Grenache had similar leaf water potentials and stomatal conductance levels during the afternoon (Figs. 3.2, 3.3). Since Shiraz is considered to be less conservative (anisohydric) it would be expected that the Shiraz deficit vines would display less control over stomatal conductance during the day, therefore allowing greater fluctuations in leaf water potential and transpiration (Soar et al., 2006b). The linear regression of leaf water potential and stomatal conductance for the glasshouse vines suggested that Cabernet Sauvignon, Shiraz and Tempranillo all showed an increase in leaf water potential as stomatal conductance increased - that is more typical of an anisohydric-like response (Figure 3.5). Conversely, the Grenache was the only variety to display more isohydric-like responses

where there was little change in leaf water potential as stomatal conductance levels increased (Figure 3.5). The reason for the inconsistency in physiological responses as hypothesised could be attributed to differences in grapevine behaviour in the glasshouse compared to the field. Evaporative demand is generally much higher in the field than in the glasshouse, therefore potentially favouring root-sourced chemical signals to regulate stomatal conductance (Kramer, 1988). These differences in hydraulic responses are usually influenced by higher vapour pressure deficits (VPD) in the field than the glasshouse conditions (i.e. under field conditions VPDs can be up to 5.68 kPa compared with VPD ~ 1.63 kPa in a glasshouse) (Soar et al., 2006b). Furthermore the potting soils in the glasshouse, whilst being made to simulate a clay-loam soil, most likely experienced more local water deficits, even in the control pots. This would be due to the pots having much smaller rooting depth than field vines as well as lower unsaturated hydraulic conductivities than the field soils that contain more clay/loam and thus have higher water holding capacities (Jones & Tardieu, 1998). Consequently there could have been potential for the control vines to also experience water deficit conditions during the day, thereby influencing the synthesis of root-sourced signals that may have attributed to greater levels of xylem sap [ABA] being produced and thereby regulating stomatal control.

Field physiology:

For both field grown varieties the SDI-treated vines had lower leaf water potential compared to the controls (Figs. 3.8A, B). Leaf water potential for the Cabernet Sauvignon control vines was significantly higher during the day by approximately 8% than for the 43% SDI-treated vines. By contrast, the 45% SDI-treated Shiraz vines, whilst having lower leaf water potentials all day than the control, only had significant reduction in leaf water potential after 1500h (Figure 3.8B). In conjunction with the reduced leaf water potentials, diurnal stomatal conductance levels were reduced for the Cabernet Sauvignon and Shiraz SDI-treated vines than the controls in the field trials (Figure 3.9). These results are comparable to previous deficit irrigation research particularly where a reduction in irrigation water of up to 50% has resulted in reduced leaf water potential and stomatal conductance of water stressed vines (Matthews et al., 1987a; Goodwin & Jerie, 1992; McCarthy, 1997a).

Whilst this current study indicates diurnal differences in stomatal conductance for each variety under approximately 50% water deficit, the diurnal changes in leaf water potential between the varieties are different to what has been found for studies exploring the isohydric and

anisohydric responses of grapevines to water deficit. The water deficit studies by Schultz (2003) found that Syrah (syn. Shiraz) experienced lower (more negative) leaf water potential than the Grenache during the day even with stomatal closure that is more typical of a near-anisohydric vine. By contrast, Grenache maintained higher (more positive) leaf water potential than Syrah with comparable stomatal closure, therefore indicating more near-isohydric behaviour. In my field study the Cabernet Sauvignon tended to have lower (more negative) leaf water potential for the control and deficit vines compared to the Shiraz, despite being grown on the same rootstock. Since the varieties were grown in separate vineyards there is a possibility that differences in soil type and VPD on the day could be influencing the results. If the Cabernet Sauvignon site had lower water holding capacity than the Shiraz site then the soils would dry out faster thereby potentially providing drier conditions across all irrigation treatments and possibly causing greater soil:water deficits than initially expected. However, the transpirational differences observed in the Schultz (2003) study between Syrah and Grenache could be influenced by the different rootstocks that the vines were grafted to rather than a direct isohydric or anisohydric response.

Another observation of my field studies was that the varieties tended to show differences in stomatal conductance after 1300 h, even though leaf water potential values were similar between Cabernet Sauvignon and Shiraz at the same time. Compared to Cabernet Sauvignon, the Shiraz tended to produce higher leaf water potentials in the afternoon as well as having higher stomatal conductance, which potentially indicates a difference in hydraulic conductivity between these varieties. The general finding was that at the beginning of the day when vineyard climatic conditions were cooler the Cabernet Sauvignon and Shiraz had similar values of stomatal conductance. However in the afternoon when vapour pressure deficits, temperature and sunlight exposure were higher, the Cabernet Sauvignon became more conservative by lowering stomatal conductance levels, whereas the Shiraz continued to have similar stomatal conductance levels as in the morning. With this in mind, there is a possibility the Shiraz is displaying an anisohydric-like behaviour compared to the isohydric-like response of the Cabernet Sauvignon and this would agree with other studies (Schultz, 2003; Soar et al., 2006b).

The apparent differences in physiological responses between isohydric and anisohydric vines have been related to either hydraulic or hormonal control of stomatal conductance (Tardieu &

Simonneau, 1998; Schultz, 2003). In the studies by Schultz (2003) the variation between Shiraz and Grenache exposed to a water deficit was attributed to a greater degree of cavitation occurring in the conducting vessels of Grenache, which lead to stomatal closure and thus a variation in hydraulic conductivity between varieties. More recently Soar et al. (2006b) has concluded that differences in stomatal response of field grown Shiraz and Grenache exposed to changes in atmospheric conditions were associated with the production of ABA. Tardieu et al., (1996) found that isohydric plants (ie. maize) may regulate stomatal closure using a combination of hydraulic and ABA signals, whereas stomatal closure for an anisohydric plant (ie. sunflower) could possibly be controlled solely by ABA levels.

3.4.2 Hormonal response of grapevine varieties to water deficit

The role of abscisic acid (ABA) in regulating stomatal aperture and subsequent plant water loss under water deficit conditions has been widely studied in grapevines, both in pot and field experiments (Loveys & Düring, 1984; Correia, et al., 1995; Dry & Loveys, 1999; Stoll et al., 2000b). An understanding of the role of ABA synthesis, as a result of a hormonal response to soil water deficit has been important to the Australian wine industry, particularly in the development of irrigation techniques such as partial rootzone drying (PRD) to improve water use efficiency (Dry & Loveys, 1999; Dry et al., 2000; Stoll, 2000). In this study, both field and glasshouse vines exposed to a water deficit did produce higher xylem sap ABA levels than the control vines (Fig. 3.3, 3.9). Furthermore, higher xylem sap [ABA] of the deficit vines was associated with lower leaf water potential and lower stomatal conductance than the control vines. As roots experience soil water deficits, the synthesis of ABA is increased in the root system and transported to the transpiring canopy to reduce stomatal aperture, thus limiting transpiration (Loveys et al., 2001).

Glasshouse ABA:

Initial observations from the glasshouse trial indicate an increase in xylem sap [ABA] for the deficit vines compared to the well-watered control vines for all varieties, which is similar to what has been found in other studies (Figure 3.4) (Loveys & Düring, 1984; Zhang & Davies, 1989; Stoll et al., 2000b). Since ABA is associated with regulation of stomatal aperture, then higher ABA levels in the glasshouse vines, regardless of variety, should be associated with lower stomatal conductance. This was not the case, as at 1400 h the control varieties had

significantly different stomatal conductance values concomitantly with similar xylem sap ABA concentrations (Fig. 3.3, 3.4B). For instance, the Cabernet Sauvignon control vines, with significantly higher stomatal conductance than the other varieties, did not have the lowest xylem sap [ABA] (Fig. 3.3, 3.4B). Perhaps the differences in stomatal control between these varieties were due to differences in root responses and/or changes in ABA synthesis within the canopy itself (Loveys et al., 2005). Soar et al. (2004) showed that regulation of stomatal conductance could occur from bulk leaf ABA independently of root-derived ABA in own-rooted Shiraz grown at field capacity (low stress). It is interesting to note that the glasshouse grown Cabernet Sauvignon in this study had a strong negative relationship of stomatal conductance and the concentration of xylem sap ABA, at the same time that the other varieties had weak relationships (Figure 3.7). This suggests that control of stomatal aperture in the Cabernet Sauvignon is more likely to be associated with ABA, but whether this ABA is derived from the roots or canopy is unable to be explained by this study.

Field ABA:

For the field trials, the SDI-treated vines for both varieties always had more ABA than the control vines in conjunction with lower diurnal leaf water potentials and lower stomatal conductance (Figure 3.12). Previous PRD field trials on Shiraz and Cabernet Sauvignon have shown that by drying part of the root system, the levels of root-sourced ABA can be increased which consequently reduces stomatal conductance without any change in leaf water potential (Loveys et al., 1998; Stoll et al., 2000b). In the case of my study the increase in xylem sap [ABA] may be attributed to the root system experiencing a soil water deficit. This was also associated with lowered leaf water potential which is more typical of a hydraulic response (Williams & Matthews, 1990).

Even though the relationships were weak, more negative leaf water potentials were associated with higher levels of xylem sap [ABA] for both varieties (Figure 3.13). Contrasting relationships existed between stomatal conductance and xylem sap [ABA] for both varieties. Cabernet Sauvignon had lower stomatal conductance associated with higher xylem sap [ABA], whereas the Shiraz, particularly the SDI-treated vines, displayed a weak relationship with higher stomatal conductance levels associated with higher xylem sap [ABA] (Figure 3.14). Such trends in leaf water potential and stomatal conductance could be associated with fluctuations in diurnal temperatures (Hennessey & Field, 1991), light intensity (Hennessey &

Field, 1991; Tardieu & Simonneau, 1998) and soil moisture (Loveys & Düring, 1984; Dry & Loveys, 1999). However there is a strong possibility that either xylem sap [ABA] or total leaf ABA is influencing diurnal trends in leaf water potential and stomatal conductance. Pot experiments by Soar et al., (2006b) investigating the levels of ABA from roots, leaves and xylem sap found that Shiraz had higher leaf [ABA] levels than Grenache, but lower xylem sap [ABA]. Grenache roots were also found to produce much lower concentrations of [ABA] than what were detected in the xylem sap, whereas Shiraz roots produced a similar concentration level of ABA as to what was present in the xylem sap. With this in mind the differences in stomatal conductance between Shiraz and Cabernet Sauvignon could possibily be driven by the variation in ABA concentration depending on whether it is sourced more from leaves (local synthesis) or root-derived (imported synthesis). It also appears that changes in diurnal leaf water potential may be influencing what is happening to the level of ABA synthesis more than the water deficits.

The pattern of xylem sap ABA production during the day for both varieties was different (Figure 3.12). The xylem sap [ABA] in the Shiraz was consistently lower in the morning than the afternoon irrespective of irrigation treatment. By contrast, the Cabernet Sauvignon appeared to have elevated levels of xylem sap [ABA] all day for each irrigation treatment even though the deficit vines produced lower leaf water potentials than the controls during the day. These results suggest the varieties have different sensitivities to xylem sap [ABA], which may relate to a combination of root to shoot signals and canopy derived-ABA (Soar et al., 2006b). The significant increase in xylem sap [ABA] for the Shiraz deficit vines in the afternoon compared to the morning could be due to a greater hormonal response as previously postulated for anisohydric plants (Tardieu et al., 1996; Tardieu & Simonneau, 1998). However, for the Cabernet Sauvignon the relatively consistent xylem sap [ABA] over the day for the control and deficit vines could be attributed to a combination of hydraulic responses and root-sourced (chemical) and/or canopy leaf ABA, levels that tend to be more typical of an isohydric response (Tardieu et al., 1996; Tardieu & Simonneau, 1998).

Whilst it has been discussed that deficit irrigation can manipulate root-derived ABA levels and subsequent xylem sap [ABA], rootstock genotypes may vary in sensitivity to soil drying and consequent ABA production. Recent rootstock studies by Soar et al., (2006a) using the scion Shiraz grafted to various rootstocks concluded that rootstocks alone do not appear to differ in

their ability to synthesise ABA. Rather it was suggested the rootstock/scion combination could possibly influence the degree of water stress by the vine, particularly when less-vigorous rootstocks are grafted to scions that are more anisohydric (optimistic). This may provide an opportunity for the scion to potentially develop a larger canopy that has higher total vine transpiration than a smaller canopy, especially when exposed to a soil water deficit or climatic stress (high temperatures). In the study by Soar et al., (2006a) the combination of Shiraz grafted to 140 Ruggeri resulted in a greater degree of water stress for the scion, than Shiraz on 5C Teleki, Ramsey or own-roots.

For my study, both field varieties were grafted to 140 Ruggeri. In light of what has been already discussed this rootstock may be affecting the typical anisohydric and isohydric responses of these varieties. Since anisohydric plants utilise hormonal signals to regulate stomatal conductance, then Shiraz grafted to 140 Ruggeri may be disadvantaged by this rootstock. This could be due to the structure and pattern of root distribution of 140 Ruggeri either providing inadequate water to the Shiraz canopy or insufficient root-derived ABA for sufficient stomatal closure to prevent excessive water loss (Williams & Smith, 1991; Soar et al., 2006a). By contrast Cabernet Sauvignon, being isohydric, has a dual control mechanism of hormonal and hydraulic responses that irrespective of the xylem sap [ABA] produced by 140 Ruggeri, the hydraulic system may be able to buffer any severe water loss by also reducing stomatal conductance in response to soil water deficit.

In the Soar et al., (2006a) study it was suggested that rootstock effects on scion response to water deficit was initiated by the rootstock's ability to extract soil water that in turn changed leaf water potential thereby inducing xylem sap ABA synthesis that mediated stomatal closure. This suggests that under water deficit conditions, xylem sap [ABA] is probably more indicative of leaf water stress rather than an indication of root-derived synthesis. Irrespective of the rootstock influence, the changes in leaf water potential of the Cabernet Sauvignon may be influencing what happens in the leaf with respect to ABA synthesis more so than the Shiraz. Such responses may assist in explaining the anecdotal observations that Cabernet Sauvignon appears to be more conservative compared to the vigorous growth response of Shiraz when both varieties are exposed to similar irrigation practices and climatic conditions.

3.4.3 LAI and pruning weights of SDI-treated vines

Soil water deficits are known to reduce shoot growth and canopy leaf area (Williams & Matthews, 1990). For both varieties, leaf area index (LAI) was reduced as less water was applied, but this was only apparent near harvest. The Cabernet Sauvignon was more sensitive to a water reduction of 50% or more compared to the Shiraz, which did not show any significant changes in LAI until exposed to a 34% water deficit. Since the SDI treatments were applied from the beginning of the irrigation season (pre-flowering) it was anticipated the developing shoots would have been affected by the water deficits. In previous RDI studies, application of a mild water stress pre-veraison has been successful in controlling vegetative growth (Poni et al., 1993; McCarthy, 1997a). Since the vines in this study were mature, with well-established root systems, there could have been potential for the vines to extract water from deeper soil depths, thereby buffering the effect of the water deficits. This could also explain why there were no canopy differences between irrigation treatments from budburst to veraison, as during the first half of the growing season the availability of sufficient moisture in the soil profile would have resulted in minimal irrigation being applied.

Likewise it was expected that pruning weights would be reduced for the SDI treatments, particularly after three seasons of SDI. This however was not the case, and it was only in the second season for the Cabernet Sauvignon that pruning weights showed a significant reduction when exposed to water deficits less than approximately 50% compared to the control. These results are in contrast to PRD studies where vines that also received a 50% water reduction were found to produce a consistent reduction in total pruning weight (Dry et al., 1999; Stoll, 2000). Since both varieties did produce physiological responses to the water deficits, differences in the canopy sizes may be attributed to their isohydric-like or anisohydric-like responses. The optimistic (anisohydric-like) response of Shiraz could potentially allow it to continue growing during soil water deficits as opposed to the more pessimistic (isohydric-like) response of the Cabernet Sauvignon. Tighter stomatal control for the Cabernet Sauvignon during soil water deficits could potentially lead to a greater reduction in photosynthesis resulting in reduced canopy growth. When water stress inhibits photosynthesis there can be re-mobilisation of carbohydrate reserves from woody tissues and roots to bunches for ripening and preservation of the seed (Rühl & Alleweldt, 1990; Candolfi-Vasconcelos et al., 1994). These changes in carbohydrate dynamics could be related to the way in which isohydric-like

and anisohydric-like varieties accumulate and allocate carbon during water deficit conditions. This in turn could then be related to the sustainability issue of long-term deficit irrigation practices that could potentially alter nitrogen and carbohydrate reserves required for vine viability and successful berry ripening.

3.5 Conclusions

Water deficits affected the physiological and hormonal responses of the field and glasshouse vines. Generally, as less water was applied stomatal conductance and leaf water potential were reduced and xylem sap [ABA] increased.

- Leaf water potential and stomatal conductance levels tended to be significantly reduced in the field grown Cabernet Sauvignon and Shiraz when exposed to approximately 50% or more sustained water deficit.

- Xylem sap [ABA] levels were significantly higher when field grown vines were exposed to a soil water deficit. The difference in xylem sap [ABA] between Cabernet Sauvignon and Shiraz may be related to whether the ABA is derived from the roots or canopy, or both. Furthermore the type of rootstock may also be a pre-determining factor to the degree to which an isohydric or anisohydric vine may tolerate a particular level of water deficit.

- The glasshouse study demonstrated varietal differences in stomatal conductance and the transport and utilisation of root-sourced chemical signals such as ABA to abiotic stress responses.

- Under field situations, Cabernet Sauvignon appeared to display physiological responses typical of an isohydric-like vine, compared to the anisohydric-like responses of the Shiraz. These responses may also be supported by the pattern of xylem sap [ABA] production.

- Leaf area index was significantly lower from veraison to harvest for the Cabernet Sauvignon vines that had approximately 50% or less water. The SDI treatments also reduced pruning weights in Cabernet Sauvignon but not Shiraz. These differences in canopy development may be a reflection of the isohydric-like and anisohydric-like responses of the Cabernet Sauvignon and Shiraz vines respectively that in turn could influence carbohydrate dynamics and long-term viability of vine growth and berry ripening under deficit irrigation.

CHAPTER 4: Changes in Yield Components and Berry Composition of Red Wine Grapes Exposed to SDI

4.1 Introduction

Across the Murray-Darling region wine grape production is reliant on irrigation for consistent production and viability. One difficulty in optimising grape yield and quality in this semi-arid climate is balancing conservation of irrigation water with the need to maintain healthy grapevine function. Historically, irrigated grapevines have a tendency to be vigorous, producing large canopies, which tend to shade fruit and lead to potential problems such as pest and disease outbreaks, delayed fruit ripening and variability in fruit quality. Careful control of irrigation inputs is widely regarded as the Australian wine industry's best management tool for manipulating vegetative vigour and grape quality, while conserving irrigation water.

The adoption of strategic irrigation management techniques such as regulated deficit irrigation (RDI) (Goodwin & Macrae, 1990; McCarthy, 1998) and partial root-zone drying (PRD) (Dry et al., 1996; Loveys et al., 2001) have improved irrigation efficiency (WUE-tonnes of fruit per megalitre of water applied), canopy structure and fruit composition, particularly for red wine grape varieties. These water deficit strategies use irrigation management as a tool during various stages of grapevine growth and development to manipulate final wine composition.

The effect of water deficit on grapevine production differs depending on the stage of canopy growth and berry development when the water deficit is applied. For instance, RDI applied to Shiraz during early canopy development (between budburst and flowering) and early berry formation (flowering to setting) can reduce shoot growth and affect berry cell division leading to smaller berries and potentially reduced yield (McCarthy, 1997a; McCarthy, 2000). By contrast, winegrapes exposed to PRD generally maintain berry size and yields despite receiving a reduction in irrigation water (Kriedemann & Goodwin, 2003). Studies on field-grown Sultanina vines demonstrated that water deficit applied during early berry development had a more negative effect on yield than when the water deficit was applied after veraison (Myburg, 2003). Water deficits applied after veraison during berry ripening may also influence the development of berry components such as sugars, organic acids and juice pH, as

well as the composition of various colour and flavour compounds (Freeman & Kliewer, 1983; Jackson & Lombard, 1993).

Traditionally, sugar concentration (TSS; ^{o}Brix) and titratable acidity (TA) have been the main indicators for determining harvest date (Krstic et al., 2003). Within the Australian wine industry, the most commonly used parameters for assessing grape quality at the winery weighbridge include total soluble solids (TSS), titratable acidity (TA) and juice pH (Gishen et al., 2002; Iland et al., 2004). In addition, phenolic compounds (such as anthocyanins and tannins) are important criteria, particularly when assessing quality of red wine grapes. Also, berry size and berry total anthocyanins (colour) are sometimes considered important parameters in defining potential berry composition.

To remain sustainable in the current global wine market, production of winegrapes from semi-arid regions, like the Murray-Darling (Vic/NSW, Australia), will need to improve vineyard water use efficiency by adopting efficient irrigation practices that can maintain the production of desirable grape and wine quality parameters. In general, the slightly reduced yields that can occur as a result of deficit irrigation tend to be offset by improved grape quality. When the improvement in grape quality (in particular with red varieties) is recognised in contractual arrangements, financial returns may be higher depending on the winery purchasing the fruit. RDI and PRD are irrigation practices with the potential to improve WUE, but which also have anecdotally produced variable responses in grape quality, even when applied under similar management and environmental conditions. These observations point to a need to explore alternative deficit irrigation strategies that can be easily implemented and still provide improvements in WUE and grape quality. Sustained deficit irrigation (SDI) is an alternative irrigation technique that is explained in the general introduction (Chapter 1) and is the focus of this research. The aim of this study was to determine the effect of SDI on yield components and berry composition relevant to the Australian wine industry. Furthermore, there is a need to try to understand how much of a SDI reduction can be tolerated by Cabernet Sauvignon and Shiraz without jeopardising yield components and berry quality parameters.

4.2 Materials and Methods

To investigate the effects of sustained deficit irrigation (SDI) on yield components and berry composition parameters, a series of measurements between berry ripening and harvest were taken during the 2004-2006 seasons. Berries were harvested as closely as possible to 24^{o}Brix. Bunches were collected at different stages of development between veraison and harvest from the Shiraz and Cabernet Sauvignon (*Vitis vinifera* L.) field trial sites (Chapter 2; section 2.1) during 2003/2004, 2004/2005 and 2005/2006 seasons. Both varieties were grafted to 140 Ruggeri (*V. berlandieri* x *V. rupestris*) and trained onto a two wire vertical trellis (Chapter 2, Figure 2.2). The experimental design for the irrigation treatments is explained in Section 2.1.1 (Chapter 2).

4.2.1 Determination of yield components and berry composition parameters. .

Yield was determined from a random 1.0 m wide longitudinal segment, randomly positioned over the vine canopy for each treatment plot. Bunch number per vine and total weight of bunches was recorded to determine average bunch weight and yield per vine. A sub-sample of ten bunches were randomly selected for berry quality analyses and stored in plastic bags, in a cool room at 3.0^{o}C until analysis could be conducted. During berry ripening (veraison to harvest) in 2004/2005 and 2005/2006, only four of the eight replicates were used for collecting bunch samples for berry composition analyses. Within each plot, one randomly selected vine was used for regular bunch sampling throughout berry ripening. From each sample vine, three bunches were randomly selected along a diagonal cross-section across the canopy.

For a single replicate, all bunches were hand-picked and the fruit mixed before randomly selecting a sample of 100 berries to determine average berry weight, ^{o}Brix, juice pH and TA. A further 30 berry sample was collected, weighed and stored at 3.0^{o}C for HPLC analysis of berry skins for anthocyanin profiles and total tannins respectively (Chapter 5). The remaining berries were frozen at -20^{o}C for spectral analysis of total anthocyanins (colour) and total phenolics using the methods described by Iland et al. (2004) (Chapter 5). The measures for total soluble solids (^{o}Brix), juice pH and TA (g/L tartaric acid equivalents) were performed on the collection day for the berry ripening samples, whereas for the harvest (yield) samples these berries were processed within a 24 h period after sampling.

The 100-berry sample that was used to determine average berry weight was pressed with a mortar and pestle and the juice strained through a 0.07 mm sieve into a separate container for the ^{o}Brix, TA and juice pH readings. The juice was transferred to a 10.0 mL plastic centrifuge tube and spun for 10 min at 3,500 x g (Rotofix 32, Zentrifugen, Hettich, Germany). The titration was performed by diluting 10.0 mL of the supernatant with 20 mL of distilled water. Titratable acidity and juice pH samples were analysed and recorded using an autotitrator (Schott titroLine alpha, Analytical Equipment Company, Adelaide, Australia). A 500 µL sample of the remaining juice was used to measure ^{o}Brix using a digital refractometer (Palette PR-101, Atago, Tokyo, Japan) with deionised water as the reference. The skin weight to berry weight ratio was determined by dividing the average fresh, skin weight per berry by the average berry weight. The average fresh skin weight per berry was determined from the fresh berry skins that were removed from the 30 berry sample designated for HPLC analysis (section 5.2.2).

4.3 Results

4.3.1 Effect of SDI on Cabernet Sauvignon

Yield and berry composition for the 2004, 2005 and 2006 vintages for Cabernet Sauvignon are shown in Table 4.1. Yields were similar across the different irrigation treatments in 2004, but tended to decrease as less water was applied in 2005 and 2006. Yield was significantly reduced in 2005 and 2006, respectively, for the 43% SDI-treatment compared to the control. Bunches per vine showed no significant differences between any of the SDI irrigation treatments and control for any vintage. However, during 2005 and 2006, the 52% and 43% SDI-treated vines produced significantly lighter bunches that contained berries of lower mass compared to the control bunches. As less water was applied the trend was for berry weight to be reduced. In particular, the 43% SDI-treated vines produced significantly lighter berries than the control and 70% SDI-treated vines in 2005 and 2006. Juice pH tended to increase and TA decrease as less water was applied

.

Table 4.1 Yield components and berry composition parameters from Cabernet Sauvignon bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (70%, 52% and 43% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row.

CABERNET SAUVIGNON	**Irrigation treatments**			
2004 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (kg/vine)	10.5	11.8	10.9	10.6
Bunches per vine	215	241	226	216
Bunch wt. (g)	48.6	49.5	48.0	48.7
Berry wt. (g)	0.81^{c}	0.79^{bc}	0.73^{a}	0.75^{ab}
Total Soluble Solids (^{o}Brix)	26.3	26.7	26.9	26.9
Juice pH	3.76	3.75	3.84	3.81
Titratable Acidity	4.5	4.4	4.3	4.1
2005 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (kg/vine)	16.1^{b}	14.5^{b}	12.3^{a}	10.6^{a}
Bunches per vine	250	243	234	223
Bunch wt. (g)	64.7^{b}	60.4^{b}	52.6^{a}	47.7^{a}
Berry wt. (g)	0.93^{c}	0.88^{bc}	0.82^{ab}	0.77^{a}
Skin wt / berry wt ratio	0.34	0.32	0.33	0.32
Total Soluble Solids (^{o}Brix)	24.2^{a}	24.4^{ab}	24.7^{bc}	25.0^{c}
Juice pH	3.75	3.75	3.78	3.79
Titratable Acidity	4.8	4.7	4.7	4.5
2006 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (kg/vine)	14.6^{b}	15.0^{b}	14.8^{b}	11.2^{a}
Bunches per vine	245	245	262	228
Bunch wt. (g)	59.3^{b}	61.2^{b}	56.3^{b}	49.2^{a}
Berry wt. (g)	1.09^{c}	1.04^{bc}	0.96^{ab}	0.95^{a}
Skin wt / berry wt ratio	0.26	0.26	0.28	0.25
Total Soluble Solids (^{o}Brix)	23.9	23.9	23.9	24.6
Juice pH	3.67	3.69	3.74	3.74
Titratable Acidity	5.0	5.0	5.0	4.4

The effect of SDI on berry ripening from veraison to harvest for Cabernet Sauvignon was monitored over the 2004/2005 and 2005/2006 (Figure 4.1) seasons. Overall, there were no significant differences between TSS for the irrigation treatments (Figs. 4.1A, B), even though

berry weight was different between the treatments. In 2005, berry weight was significantly lower for the 52% and 43% SDI treatments than the irrigation treatments that received more water (Figure 4.1C) for most of the berry ripening period. However in 2006, there were no significant differences in berry weight during the berry ripening period between irrigation treatments, yet the 52% and 43% SDI-treated vines tended to produce lighter berries than the control (Figure 4.1D). TA decreased as juice pH increased, but showed no significant differences between irrigation treatments (Figs. 4.1E, F). Generally, the 52% and 43% SDI-treated vines had lower TA levels during berry ripening than the 100% and 70% irrigation treatments.

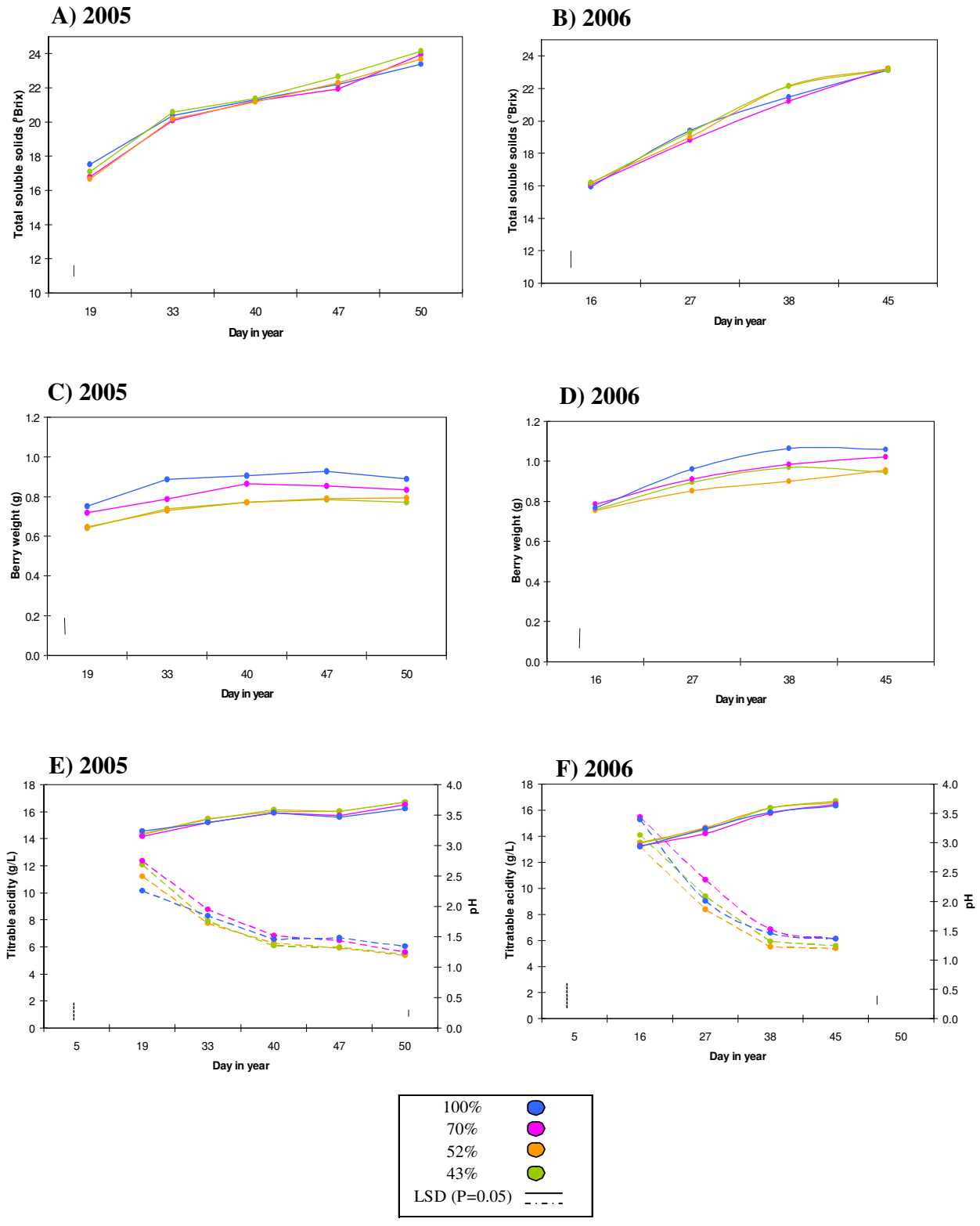


Figure 4.1 Effect of SDI on 2005 and 2006 mean berry ripening parameters for Cabernet Sauvignon exposed to a control and SDI irrigations 70%, 52% and 43%. A + B) total soluble solids, C + D) berry weight, and D + E) titratable acidity (dotted lines) and juice pH. LSD bars indicate treatment significance at P=0.05, (n=4).

The 43% and 52% SDI-treatments resulted in significantly lower yields (tonnes/ha) in 2005 and 2006 compared to the control and 70% SDI-treated vines. However, vines exposed to SDI produced more fruit per ML of water compared to the control, indicating greater efficiency of water use (Table 4.2).

Table 4.2 Water applied and WUE for the different irrigation treatments for Cabernet Sauvignon from 2004-2006. Figures followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row.

CABERNET SAUVIGNON	**Irrigation treatments**			
2004 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (t/ha)	18.7	21.1	19.4	18.9
Irrigation water applied (ML/ha)	6.0	4.2	3.1	2.6
WUE (t/ML)	3.1[a]	5.0[b]	6.2[bc]	7.3[c]
2005 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (t/ha)	28.7[b]	25.9[b]	21.9[a]	18.9[a]
Irrigation water applied (ML/ha)	6.6	4.6	3.4	2.8
WUE (t/ML)	4.4[a]	5.6[b]	6.5[c]	6.8[c]
2006 Vintage	**100%**	**70%**	**52%**	**43%**
Yield (t/ha)	26.0[b]	26.8[b]	26.4[b]	20.0[a]
Irrigation water applied (ML/ha)	7.5	5.3	3.9	3.2
WUE (t/ML)	3.5[a]	5.1[b]	6.8[c]	6.2[c]

4.3.2 Effect of SDI on Shiraz

Yield and berry composition for the 2004, 2005 and 2006 vintages for the Shiraz are shown in Table 4.3. In general, the SDI-treated Shiraz vines tended to mature earlier (approximately 5-7 days) than the control irrigated vines and this required harvesting the treatments on different days. Yields tended to be lower as less water was applied in 2004 but did not differ significantly to the control. In 2005 and 2006, yields were significantly lower for the 34% SDI-treated vines compared to the control. Bunches per vine showed no significant differences between any of the SDI irrigation treatments and control for any vintage. Average bunch weight and average berry weight were significantly lower for the 34% SDI-treated vines

compared to the control in 2005 and 2006. Juice pH and TA showed no significant differences between irrigation treatments in 2004. In 2005 and 2006, juice pH was significantly higher for the 34% SDI-treated vines compared to the control, whereas the TA levels tended to reduce as less water was applied but did not differ significantly.

Table 4.3 Yield component and berry composition parameters from Shiraz bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (65%, 45% and 34% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row.

SHIRAZ	**Irrigation treatments**			
2004 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (kg/vine)	15.8	15.8	13.5	12.7
Bunches per vine	247	247	248	233
Bunch wt. (g)	64.2	64.8	58.3	56.0
Berry wt. (g)	0.87	0.86	0.84	0.79
Total soluble solids (^{o}Brix)	23.5	23.1	24.1	24.2
Juice pH	4.30	4.22	4.31	4.20
Titratable acidity	3.5	3.8	3.7	3.9
2005 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (kg/vine)	17.7^{b}	17.0^{b}	15.8^{b}	11.4^{a}
Bunches per vine	208	200	216	195
Bunch wt. (g)	84.2^{b}	85.7^{b}	72.0^{ab}	66.5^{a}
Berry wt. (g)	0.95^{b}	0.91^{b}	0.88^{b}	0.79^{a}
Skin wt / berry wt. ratio	0.23	0.22	0.22	0.25
Total soluble solids (^{o}Brix)	23.8^{a}	23.6^{a}	24.9^{b}	25.4^{b}
Juice pH	3.89^{ab}	3.88^{a}	3.97^{bc}	4.03^{c}
Titratable acidity	4.7	4.7	4.6	4.3
2006 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (kg/vine)	18.1^{c}	16.4^{bc}	14.5^{ab}	12.3^{a}
Bunches per vine	212	191	186	203
Bunch wt. (g)	87.4^{b}	93.0^{b}	81.2^{b}	60.9^{a}
Berry wt. (g)	1.08^{c}	0.99^{bc}	0.95^{b}	0.82^{a}
Skin wt / berry wt. ratio	0.19	0.20	0.19	0.19
Total soluble solids (^{o}Brix)	24.7	23.7	23.6	23.5
Juice pH	4.26^{b}	4.26^{b}	4.09^{a}	4.34^{c}
Titratable acidity	3.3^{a}	3.4^{a}	3.9^{b}	3.3^{a}

The effect of SDI on berry ripening parameters for Shiraz was monitored over the 2005 and 2006 (Figure 4.2) seasons. In each season the development of TSS levels was similar across irrigation treatments (Figs. 4.2A, B). In 2005, TSS levels for the control vines (100%) although not significantly different tended to be lower than the SDI-treated vines, causing those vines to reach the target sugar levels at a later date. Berry weight was significantly lower at most sampling dates for the 45% and 34% SDI treatments than the control throughout ripening (Figs. 4.2C, D). In 2005, the 34% SDI-treated vines produced significantly lighter berries than the control and 65% SDI-treated vines throughout the entire berry ripening period (Figure 4.1C). In 2006, the irrigation treatments caused larger differences between berry weights with the 34% and 45% SDI-treated vines producing significantly lighter berries at the beginning of veraison than the control (Figure 4.1D). As expected, TA decreased as juice pH increased, and followed similar trends across irrigation treatments (Figs. 4.2E, F).

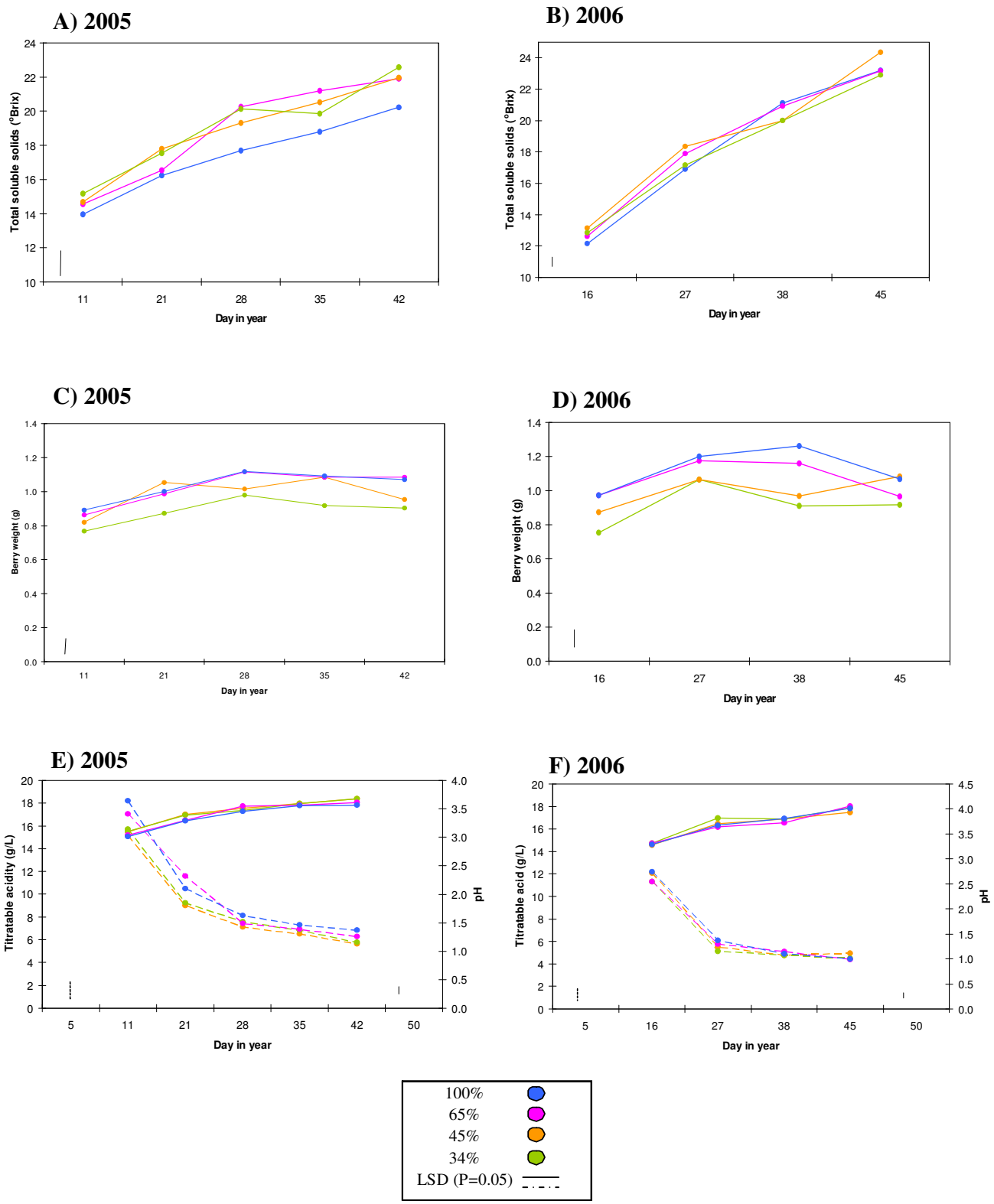


Figure 4.2 Effect of SDI on 2005 and 2006 mean berry ripening parameters for Shiraz exposed to control and SDI irrigations (65%, 45% and 34%). A + B) total soluble solids, C + D) berry weight, and D + E) titratable acidity (dotted lines) and juice pH. LSD bars indicate treatment significance at P=0.05, (n=4).

Irrigation applied and the influence of SDI on WUE of Shiraz is shown in Table 4.4. As for the Cabernet Sauvignon, yields of the Shiraz vines were lower as less water was applied. After two seasons irrigation with reduced water, the 45% and 34% SDI-treated vines were showing significant improvements in WUE (approximately double) compared to the control.

Table 4.4 Water applied and WUE for the different irrigation treatments for Shiraz from 2004-2006. Means followed by the same letter within a row are not significantly different at P=0.05, (n=8). No letters signify ns between irrigation treatments within a row.

SHIRAZ	**Irrigation treatments**			
2004 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (t/ha)	29.3	29.3	25.0	23.5
Irrigation water applied (ML/ha)	3.4	2.2	1.5	1.2
WUE (t/ML)	8.6^{a}	13.3^{b}	16.7^{bc}	19.6^{c}
2005 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (t/ha)	32.7^{b}	31.5^{b}	29.2^{b}	24.8^{a}
Irrigation water applied (ML/ha)	5.7	3.7	2.6	1.9
WUE (t/ML)	5.7^{a}	8.5^{ab}	11.2^{bc}	12.6^{c}
2006 Vintage	**100%**	**65%**	**45%**	**34%**
Yield (t/ha)	33.5^{c}	30.3^{bc}	26.8^{ab}	22.8^{a}
Irrigation water applied (ML/ha)	5.9	3.8	2.6	2.0
WUE (t/ML)	5.7^{a}	8.0^{ab}	10.3^{bc}	11.4^{c}

4.3.3 Interaction between SDI, yield components and berry composition parameters

Correlation matrices were used to determine whether there were any relationships between various berry composition parameters at each irrigation level. The data were analysed for each irrigation treatment across all three seasons from 2004-2006 for the Cabernet Sauvignon (Table 4.5) and the Shiraz (Table 4.6). Correlation coefficients (r values) greater than 0.576 or 0.708 indicate a significant relationship (positive or negative) at the 95% or 99% confidence level respectively.

The Cabernet Sauvignon data (Table 4.5) show a strong positive correlation between bunch numbers and yield for the 100% (r = 0.777), 52% (r = 0.826) and 43% (r = 0.780) irrigation treatments. Bunch weight was also positively correlated with yield for all the irrigation treatments. This indicates that higher yields would be associated with heavier bunch weights per vines possibly as a result of heavier total berry mass. TSS (^{o}Brix) was strongly negatively correlated with berry weight for the 100% (r = -0.743) and 70% (r = -0.678).

The Shiraz data in Table 4.6 show a strong positive correlation between yield and berry weight for all the irrigation treatments (100%: r = 0.766, 65%: r = 0.594, 45%: r = 0.668, 34%: r = 0.789). As less water was applied the juice pH was negatively correlated with yield levels for the 45% (r = -0.689) and 34% (r = -0.588) SDI treatments. Bunch weights were strongly positively correlated with berry weight for all the irrigation treatments (100%: r = 0.894, 65%: r = 0.780, 45%: r = 0.899, 34%: r = 0.905).

Table 4.5 Correlation matrices of berry composition for Cabernet Sauvignon treated with A) 100% (control) irrigation, B) 70%, C) 52% and D) 43% SDI irrigation. Data were pooled over three seasons from 2004 to 2006. Since the df = 10, then P<0.05 when r>0.576 (no shade); P<0.01 when r>0.708 (grey shade).

A) 100% Control	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.777	1					
Bunch wt.	0.826	0.300	1				
Berry wt. (g)	0.503	0.326	0.491	1			
°Brix	-0.516	-0.393	-0.465	-0.743	1		
Juice pH	-0.245	-0.279	-0.142	-0.456	0.397	1	
TA (g/L)	0.296	0.377	0.177	0.351	-0.528	-0.627	1

B) 70% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.404	1					
Bunch wt.	0.751	-0.294	1				
Berry wt. (g)	0.527	0.174	0.408	1			
°Brix	-0.559	-0.147	-0.472	-0.678	1		
Juice pH	-0.366	-0.043	-0.334	-0.376	0.268	1	
TA (g/L)	0.501	0.049	0.476	0.406	-0.692	-0.584	1

C) 52% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.826	1					
Bunch wt.	0.731	0.231	1				
Berry wt. (g)	0.758	0.493	0.692	1			
°Brix	-0.400	-0.284	-0.354	-0.416	1		
Juice pH	-0.184	0.067	-0.434	-0.400	0.524	1	
TA (g/L)	0.063	0.030	0.089	-0.065	-0.654	-0.521	1

D) 43% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.780	1					
Bunch wt.	0.613	-0.012	1				
Berry wt. (g)	0.309	-0.184	0.257	1			
°Brix	-0.272	-0.255	-0.136	-0.432	1		
Juice pH	-0.110	-0.156	-0.361	-0.488	0.444	1	
TA (g/L)	-0.067	-0.132	0.065	-0.197	-0.418	-0.727	1

Table 4.6 Correlation matrices of berry composition for Shiraz treated with A) 100% (control) irrigation, B) 65%, C) 45% and D) 34% SDI irrigation. Data were pooled over three seasons from 2004 to 2006. Since the df = 10, then P<0.05 when r>0.576 (no shade); P<0.01 when r >0.708 (grey shade).

A) 100% Control	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.500	1					
Bunch wt.	0.666	-0.290	1				
Berry wt. (g)	0.766	-0.057	0.894	1			
°Brix	0.104	-0.333	0.407	0.322	1		
Juice pH	-0.409	0.099	-0.450	-0.348	-0.098	1	
TA (g/L)	0.382	-0.016	0.380	0.236	0.154	-0.891	1

B) 65% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.269	1					
Bunch wt.	0.479	-0.659	1				
Berry wt. (g)	0.594	-0.420	0.780	1			
°Brix	-0.399	-0.119	-0.207	-0.248	1		
Juice pH	-0.484	-0.196	-0.451	-0.413	0.287	1	
TA (g/L)	0.284	-0.142	0.263	0.197	-0.355	-0.865	1

C) 45% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.375	1					
Bunch wt.	0.582	-0.473	1				
Berry wt. (g)	0.668	-0.276	0.899	1			
°Brix	-0.350	-0.310	-0.122	-0.340	1		
Juice pH	-0.687	-0.153	-0.675	-0.675	0.243	1	
TA (g/L)	0.378	0.056	0.167	0.231	0.140	-0.539	1

D) 34% SDI	Yield (kg/vine)	Bunch no.	Bunch wt. (g)	Berry wt. (g)	°Brix	Juice pH	TA (g/L)
Yield (kg/vine)	1						
Bunch no.	0.559	1					
Bunch wt.	0.780	-0.058	1				
Berry wt. (g)	0.789	-0.097	0.905	1			
°Brix	-0.142	-0.184	0.005	-0.129	1		
Juice pH	-0.587	-0.042	-0.643	-0.643	0.053	1	
TA (g/L)	-0.520	0.102	0.517	0.399	0.051	-0.863	1

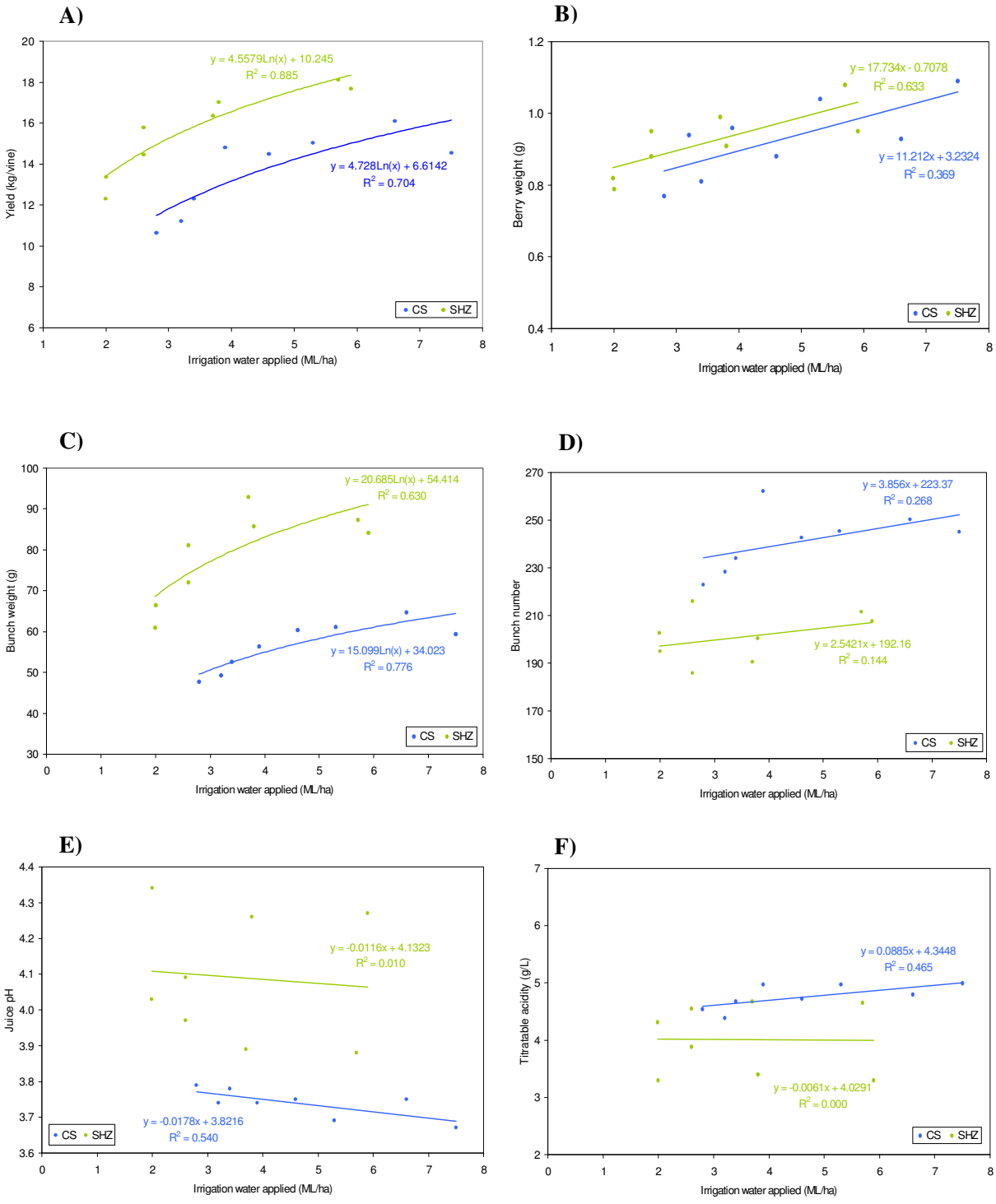


Figure 4.3 Regression analyses of the quantity of irrigation water applied (ML/ha) and A) yield, B) berry weight, C) average bunch weight, D) bunch number, E) juice pH and F) titratable acidity for Cabernet Sauvignon (CS) and Shiraz (SHZ). Data points represent the treatment means for 2005 and 2006 seasons, (n=8).

Figure 4.3 illustrates the relationship between the volume of irrigation water applied (ML/ha) and various yield components and berry composition during the 2005 and 2006 seasons. The data for 2004 were not included as it was considered preliminary data due to the irrigation treatments only being implemented two months before the 2004 harvest. Both varieties produced lower yields and lighter berries when less water was applied (Figs. 4.3A, B). Likewise, bunch weights and bunch numbers decreased as less water was applied for each variety (Figs. 4.3C, D). For both varieties, the low R^2 values for bunch numbers (CS, R^2=0.268; SHZ, R^2=0.144) compared to the larger R^2 values for berry weight (CS, R^2=0.369; SHZ, R^2=0.633) shows that a possible driver of the yield responses to the irrigation treatments could be berry weight. At similar irrigation volumes, the Shiraz vines tended to produce higher yields due to a combination of the individual bunch and berry weights being heavier. Conversely the Cabernet Sauvignon vines, whilst producing lower yields than the Shiraz, tended to have more bunches per vine that weighed less as a result of lower berry mass. Juice pH showed a tight, negative relationship against irrigation water applied for the Cabernet Sauvignon, but not for the Shiraz (Figure 4.3E). Similarly for TA, the Cabernet Sauvignon berries tended to produce slightly higher TA levels when exposed to more irrigation, whereas the Shiraz vines showed no relationship between TA and the volume of irrigation water applied (Figure 4.3F).

4.4 Discussion

4.4.1 Effect of SDI on yield and its components

Yield:

Grapevine water deficits can affect yield and berry weight with the potential to change grape and wine quality (Bravdo et al., 1985; Hepner et al., 1985; Kennedy et al., 2002). For both varieties significant differences in yield between the irrigation treatments were not apparent until 2005. This delay could be attributed to the perennial nature of grapevines, whereby a stress caused by water deficit in one season can have a 'carry-over' effect on productivity in the following season (Petrie et al., 2004). Since the cycle of yield development in a grapevine typically extends over 14 to 18 months or more, factors that may influence the critical points during reproduction (inflorescence initiation and differentiation) may not be expressed until the following season (Krstic et al., 2005). Regardless of the SDI treatments, Cabernet Sauvignon produced higher yields across all treatments in 2006 compared to 2004, whereas the Shiraz maintained comparable yields across all years. A yield decrease of 20% to 30% can be expected when a vine is irrigated with 50% less water than a fully irrigated control (Grimes & Williams, 1990). Similarly, after three irrigation seasons, the 43% SDI-treated Cabernet Sauvignon vines in this study experienced a 23% reduction in yield, whereas the yields of the 52% and 70% SDI-treated vines were not significantly different compared to the control. For Shiraz in 2006, the 45% and 34% SDI-treated vines experienced a 20% and 32% yield reduction respectively relative to the control. Compared to the district average of 20-25 t/ha for the same varieties (Jeff Milne pers. comm.), these vines still produced comparable yields per hectare even after being exposed to a sustained water deficit of 50% or less all irrigation season. Whilst yield was expected to decrease as less water was applied, it was not anticipated that the vines exposed to the SDI treatments receiving the least amount of water (43% and 34%) would still produce yields that were comparable to the district average of 20-25 t/ha for the same red winegrape varieties.

Bunch number and bunch weight:

The continued application of SDI to Cabernet Sauvignon and Shiraz did not cause a significant reduction in bunch number per vine and the reduction in yield was primarily due to a decrease

in berry weight and thus bunch weight. PRD studies have also noted that the number of bunches or the number of berries per bunch, which are factors determining yield, remain unaffected by 50% PRD irrigation when applied over several seasons (Dry et al., 1996; Stoll et al., 2000a). However, other papers have noted that continued application of reduced irrigation can modify key initiation and development stages of the following season's inflorescences, resulting in a reduction of bunch number per vine (Krstic et al., 2005). Perhaps the number of bunches per vine remained unaffected in this study due to the availability of carbohydrate reserves present in the actively photosynthesising canopy at flowering, which in turn adequately supplied the developing bunches (Petrie et al., 2004). Furthermore the SDI treatments may not be affecting bud initiation, but potentially influencing inflorescence development during the fruit set stage that may lead to changes in berry size and berry number per bunch.

From the correlation matrices, the Cabernet Sauvignon yields for each irrigation treatment were sometimes related to bunch number per vine, but were always strongly associated with bunch weight (Table 4.5). By contrast, Shiraz yields appear to be more associated with changes in berry weights than bunch weight (Table 4.6). These relationship differences could be due to the inherent bunch architecture of each variety at the particular trial site (Kerridge & Antcliff, 1996). Whilst no quantitative measures were made, it was observed that the Cabernet Sauvignon vines tended to produce more open bunches comprised of uniformly sized berries for each irrigation treatment. Conversely, the general observation for the Shiraz was of longer, heavier bunches that had a higher proportion of large berries in the treatments receiving more water (100% and 65%), whereas the Shiraz vines treated with less water (45% and 34% SDI treatments) tended to produce more open bunches with smaller berries. These differences in bunch architecture between the varieties and irrigation treatments may explain why the differences in yield were more positively correlated with bunch weight for Cabernet Sauvignon and berry weight for Shiraz.

Berry weight:

Berry weight or size has widely been accepted, in the past, as a major factor determining grape and wine quality, particularly as the general assumption is for small berries (lower berry mass) to have improved compositional parameters than larger berries. At harvest in 2005 and 2006, both varieties had significantly lower berry weights as less water was applied. This is

comparable to earlier water deficit studies, where a reduction in both yield and berry weight has been attributed to reduced soil water availability (Matthews et al., 1987a; Goodwin & Jerie, 1992; McCarthy, 2000; Kennedy et al., 2002). However, many of these studies have applied a soil water deficit at a particular stage of vine growth or berry development. Water deficits applied between the early stages of berry formation, such as anthesis to setting (Stage 1, E-L 27-33), can irreversibly affect berry size (Ojeda et al., 2002). Matthews and Anderson (1989) found that pre-veraison water deficits inhibited yield, berry weight and bud development more so than post-veraison water deficits. RDI studies that imposed water deficits by withholding irrigation during stages of berry development have found that berry weight was most sensitive to water deficits during the post-flowering period to pre-veraison (McCarthy, 2000). While grape berries are extremely sensitive to water deficits applied immediately after flowering, there is evidence that berries experience a more resistant phase approximately four weeks after flowering (Alexander, 1965). During the early stages of growth, berries experience changes in pericarp growth due to cell division and expansion that is more sensitive to water stress than the cell expansion phase after veraison (Harris et al., 1968; Coombe & Iland, 2004).

In this study, water deficits were applied all season from the first irrigation through to post-harvest. As previously explained, the sustained water deficit was not created by withholding water, but rather, by applying a lesser volume of water for the entire irrigation season. This is in contrast to previous water deficit studies on RDI that withheld irrigation during certain stages of vine growth, or PRD which creates discrete wet and dry zones around the root system by application of alternate irrigations (Dry et al, 1996; McCarthy, 1998). Consequently the reduction in berry weight in this study is probably due to the SDI treatments producing a soil:water deficit during berry formation that resulted in the reduction in the number of cells per berry, which has been suggested in other studies (Matthews et al., 1987b; McCarthy, 1997b). In 2005 at the beginning of berry ripening, the Cabernet Sauvignon vines exposed to the 52% and 43% SDI treatments were already showing signs of berry weight reduction compared to the control (Figure 4.1C). In the following season, 2006, there were no significant differences in berry weight during berry ripening between the irrigation treatments (Figure 4.1D). However, differences in berry weight did occur at harvest in 2006, with those vines receiving less water having significantly lower berry weights. This variation between seasons

in the development of the berry weights could be due to greater soil water deficits in some seasons during berry formation (pre-veraison).

Spring rainfall that occurred at the Cabernet Sauvignon trial site in 2005 may provide an alternative explanation as to why no berry weight differences were observed between the SDI treatments at the beginning of veraison in 2006. In this instance, berry cell division may not have been retarded by water deficit during pre-veraison due to a buffering effect provided by the supplementary rainfall events. Consequently, the development of differences in berry weights during the later developmental stages could be the result of a solute reduction in the pulp for vines receiving less water. Conversely for the Shiraz during 2005 and 2006, the 45% and 34% SDI-treated vines tended to have lighter berries than the control during veraison to harvest, suggesting these SDI treatments did affect berry development from as early as the pre-veraison period irrespective of any supplementary water from rainfall events.

Overall, by harvest, berry weights were generally significantly lower for those SDI treatments receiving 50% or less water than the control for both the Cabernet Sauvignon and Shiraz. Whilst there were fluctuations between seasons, these reductions in berry weight are comparable to other water deficit studies that have applied similar levels of water deficits (Ginestar et al., 1998; Ojeda et al., 2002).

4.4.2 Effect of SDI on berry composition

The balance of juice pH and TA is important in the wine fermentation process and in determining final wine colour and flavour. Generally grape juice of lower pH values will be less prone to microbial oxidation and spoilage reactions during fermentation as well as tending to produce a better balanced wine with respect to wine colour and expression of final fruit characters. Furthermore the overall concentration of TA in the must is required to balance alcohol and residual sugar levels of the wine, thus juice of higher TA levels will tend to produce more acidic tasting wine (Iland & Gago, 2002). Generally during the berry ripening period as TSS levels increase, TA levels decrease and juice pH increases (Iland & Gago, 2002). For this study it was also found that, irrespective of irrigation treatment, the TSS levels and juice pH increased, whilst TA decreased during veraison to harvest. Overall, during veraison to harvest, the SDI treatments did not influence the developing pH or TA levels

compared to the control. At harvest the general trend for the Cabernet Sauvignon was that with less water applied the juice pH was higher and the TA levels lower. This result is similar to findings by Bravdo et al. (1985) where field grown Cabernet Sauvignon vines experienced a moderate decrease in TA when exposed to a water deficit, but no change in juice pH was observed. For the Shiraz vines at harvest, similar trends in juice pH and TA emerged as for the Cabernet Sauvignon with the 34% SDI-treated vines having significantly higher juice pH compared to the control for both 2005 and 2006 harvests.

Overall, the results for both varieties are comparable to other Australian studies exploring the effect of grapevine water deficits on the balance of juice pH and TA. For instance, Cabernet Sauvignon berries from PRD treated vines have been found to produce significantly lower juice pH and higher TA than the control (Dry et al., 1999). Conversely, post-veraison water deficit trials on Shiraz grown in the Murray-Darling region have not shown any significant effects on juice pH and TA levels of berries exposed to different water regimes (McCarthy, 1997a; Petrie et al., 2004).

The differences between vintages for the juice pH and TA in this study, particularly for Shiraz, are difficult to explain. Generally, vines grown in hot climates tend to produce high berry sugar with low acid levels, compared to vines from cool climates. Furthermore there is a tendency for exposed berries to have higher juice pH and lower TA levels than shaded berries, with water stress also increasing juice pH (Freeman & Kliewer, 1983; Bravdo et al., 1985; Williams & Matthews, 1990). Thus, under the irrigation and climatic conditions of this study there appears to be potential to manipulate TA levels with SDI application, whereas the juice pH, whilst at times being sensitive to SDI, is more variable possibly due to seasonal conditions, such as extreme day or night temperature changes. Furthermore the reductions in canopy development for the SDI treatments to the control may have increased light exposure on the bunches and subsequent berry temperatures that is known to accelerate malate respiration thereby decreasing TA and increasing pH levels (Iland & Gago, 2002).

4.4.3 Management of SDI in the vineyard

The actual amount of water applied to the control vines was anticipated to be comparable to the district average for irrigated winegrapes in the Murray-Darling region (approximately 6.0-

7.0 ML/ha). Due to climatic variation, most likely from the prevailing drought conditions, water applied to the control vines tended to differ from the district average. The Cabernet Sauvignon site consistently received more water each season than the previous irrigation season, which would have affected the final volume of water received by the SDI vines for any given vintage. In particular, by 2006 the Cabernet Sauvignon control vines were receiving 25% (1.5 ML/ha) more water than in 2004. This means the 43% SDI vines in 2006, despite receiving less water than the control, could potentially have been responding in a similar manner to the 52% SDI vines in 2004 that received a comparable volume of water.

By comparison the Shiraz site was particularly difficult to irrigate due to variability across the vineyard with respect to soil depth and consequent water holding capacity (discussed in Chapter 7). In the first season (2004), the Shiraz vines were irrigated when the average soil water tension (to a soil depth of 100cm) for the control vines reached 60 kPa. The maximum water requirement (ET_c) was estimated using the FAO56 methodology (Allen et al., 1998) that uses a reference evapotranspiration (ET_o) calculation, which was multiplied by a crop coefficient based on effective canopy cover (Kriedemann & Goodwin, 2003) obtained for different stages of vine growth (Masoud Edraki pers. comm.). Under this form of irrigation scheduling, all the treatments experienced drier soil moisture conditions than anticipated due to an underestimation of the irrigation volume required to refill the soil profile (Appendix B, Figure B.1). To overcome this in 2005 and 2006, the Shiraz vines were irrigated when the average soil water tension of the control vines reached 40 kPa. To achieve this the vines were irrigated with shorter (reduced hours), but more frequent irrigations that were based only on the refill points for the Enviroscan data. This resulted in an increase in water applied to all the vines such that it was just under the district average (~ 6.0 ML/ha) (Appendix B).

Water use efficiency (WUE) increased in the SDI treatments compared to the control. Generally, gains in WUE are not due to any significant yield reductions, even when there has been a substantial reduction in irrigation volume (Dry et al., 1999; Stoll et al., 2000a). After three seasons of exposure to SDI, the 52% and 43% SDI-treated Cabernet Sauvignon vines had increased WUE of 46% and 44%, respectively, compared to the 100% control. Similarly by 2006 the 45% and 34% SDI-treated Shiraz also had higher WUE of 45% and 50%, respectively. The yield reduction in Shiraz may have been associated with a larger carry-over effect from the previous season, compared to the Cabernet Sauvignon, which may have

inhibited carbohydrate reserves for the following season's developing crop. Water deficits have been noted to decrease starch concentration of potted vines (Becker & Zimmermann, 1984), but in field-grown vines have caused significant improvements in total carbohydrate reserves (Petrie et al., 2004). Either way the SDI treatments have the potential to influence the physiological and hormonal responses of these varieties as previously discussed in Chapter 3.

4.5 Conclusions

Overall, the SDI treatments after three seasons had significantly lower yield, due primarily to a reduction in berry weight, with no significant changes in bunch numbers. Berry weight was sensitive to the reduced irrigation, with those SDI treatments receiving less water than the control producing smaller berries than the fully irrigated vines at harvest.

- SDI reduced yield by up to 30% in Cabernet Sauvignon and Shiraz when applied at approximately 50% of ET_c.
- Irrespective of the yield reductions, water use efficiency was improved between 40-50% for the SDI-treated Cabernet Sauvignon and Shiraz compared to the control.
- Berry weight was reduced for SDI-treated vines in Cabernet Sauvignon and Shiraz vines and the response was greater as less water was applied. The lighter berries from SDI-treated vines tended to have increased pH and decreased TA levels than the control.
- Berry composition components (^{o}Brix, juice pH and TA) were different between varieties and years. There was a tendency to increase juice pH and decrease TA with increasing intensity of SDI, which could possibly be associated with seasonal conditions and physiological responses to the various levels of water deficit.
- An SDI of 50-45% water deficit could be applied over one or two seasons to achieve improvements in WUE and berry composition. However, the response to continued application of SDI would need to be further explored to determine whether this irrigation technique would be sustainable in the long term without detrimental effects to vine viability or soil structure.

CHAPTER 5: Composition of Phenolic Compounds in Berries from SDI-Treated Vines

5.1 Introduction

For many years phenolic compounds in grape berries have been recognised as an indicator of grape maturity and vintage potential in red wines (Somers & Pocock, 1986; Gonzalez-San Jose et al., 1991). The major phenolic compounds present in grape berries that are of interest in red wine production are the anthocyanins, flavonols and proanthocyanidins (condensed tannins) (Chapter 1, Figure 1.3). These phenolic compounds contribute to the colour, mouth-feel and astringency of red wines.

Anthocyanins are primarily responsible for the colour of red wine (Rankine et al., 1958) and are mainly extracted from the skin tissue of red wine grapes (Burns et al., 2002). Grape anthocyanins include derivatives of the anthocyanidins i.e. malvidin, petunidin, peonidin, delphinidin and cyanidin (Robinson & Davis, 2000), with malvidin derivatives being the most abundant in *Vinifera* wine grape cultivars (Bakker et al., 1985; Robinson & Davies, 2000). Flavonols are colourless and are only present in the skin tissue and are thought to act as UV protectants for the grape berries. Flavonols are effective cofactors in wine copigmentation complexes (Levengood & Boulton, 2004). The main grape flavonols are quercitin-3-*O*-glucoside and -3-*O*-glucuronide (Cheynier & Rigaud, 1986; Price et al., 1995), which during wine production have been found to form stable copigment associations with the main anthocyanin, malvidin-3-*O*-glucoside to enhance wine colour during the ageing process (Lambert, 2002). Proanthocyanidins or condensed tannins are oligomers or polymers of flavan-3-ols (Cheynier et al., 1997) and are present in the skin, pulp, stem and seeds of grapes (Downey et al., 2003a; Kennedy et al., 2002; do Ó-Marques et al., 2005). These compounds are responsible for the astringent and bitter mouth-feel associated with red wines (Gawel, 1998). Overall, tannins are the most abundant in grape berries followed by anthocyanins and lastly flavonols (Souquet et al., 1996).

Anthocyanins and tannins are formed at different times during berry development and ripening, with anthocyanin synthesis occurring in early veraison (Boss et al., 1996; Haselgrove

et al., 2000; Spayd et al., 2002; Downey et al., 2004), compared to tannin synthesis from flowering to post-veraison (Kennedy et al., 2000; Downey et al., 2003a; Robinson, 2006). Flavonol synthesis is triggered by visible and UV light signals and they can accumulate in berry skins from bloom until as long as the berry skin is exposed to direct sunlight (Stafford, 1990; Price et al., 1995; Downey et al., 2003b). During berry ripening the accumulation of flavonoid compounds is influenced by variables such as viticultural management, climate and grape variety (Pirie & Mullins, 1980; Price et al., 1995; Brossaud et al., 1999).

Of the many viticultural and environmental variables that can affect grape flavonoid composition, vine water status, particularly under water deficit conditions, is regarded as one of the major influences (Kennedy et al., 2000; Kennedy et al., 2002; Roby et al., 2004). While there is consensus in the literature that water deficits do affect flavonoid synthesis (Dry et al., 2000; Esteban et al., 2001; Ojeda et al., 2002), there are differing reports as to whether the changes are due to a direct stimulation of flavonoid biosynthesis or to differences in berry size and/or thickness of the berry skin and inner mesocarp tissue (Roby et al., 2004; Walker et al., 2005a). Another major influence on anthocyanin and phenolic accumulation as a result of water deficit is bunch exposure (Archer & Strauss, 1989; Jackson & Lombard, 1993). PRD trials have shown that increases in the anthocyanidin (delphinidin, cyanidin, petunidin, peonidin and malvidin) derivatives can be related to increased light infiltration through the canopy (Dry et al., 2000; Stoll, 2000). In the case of flavonols that require sunlight for biosynthesis (Downey et al., 2003b), water deficits that alter canopy structure could have the potential to increase grape flavonol levels. Changes in canopy structure might affect the canopy microclimate which in turn could lead to greater light exposure of the bunches and subsequent temperature increases that affect anthocyanin accumulation (Bergquist et al., 2001; Spayd et al. 2002; Downey et al., 2004).

With this in mind, the main objective of this study was to examine the effect of sustained deficit irrigation (SDI) on grape phenolic concentration and composition during berry ripening and harvest of some premium wine grape varieties (*Vitis vinifera* L. Cabernet Sauvignon and Shiraz) grown in the Murray-Darling region.

5.2 Materials and Methods

The measures for the various phenolic compounds were performed on whole berries or berry skins that were collected from the field trial sites described in Chapter 2. The berry samples were prepared and stored as outlined in Chapter 4 (Section 4.2.2).

5.2.1 Determination of total anthocyanins and total phenolics

Total anthocyanins (colour) and total phenolics were determined for berry ripening and harvest samples using the UV-vis spectral procedure developed by Iland et al., (2004). Berry samples were removed from the -20^{o}C freezer and defrosted overnight at 3.0^{o}C. The defrosted berries were homogenised for 30 sec using a Grindo Mix (GM 200, Retsch, Tokyo, Japan) to macerate the flesh, skins and seeds into a smooth, paste-like mixture and ensure that no large pieces of skins or seeds were present. A 1.0 g (+/-0.05 g) sample of homogenate was placed in a 10.0 mL plastic centrifuge tube and 10.0 mL of 50% (v/v) aqueous ethanol (pH 2.0 adjusted with HCl) was added. The tube was capped and periodically mixed for 1 hour and then centrifuged for 10 min at 3,500 x g (Rotofix 32, Zentrifugen, Hettich, Germany). In a glass test tube, 1.0 mL of the supernatant was added to 10.0 mL of 1M HCl and mixed thoroughly. The diluted HCl extract was incubated at room temperature for 3-4 hours. The remaining volume of supernatant was recorded for the final extract volume value required for the final calculations. Absorbency readings for total anthocyanins (colour; A_{520}) and total phenolics (A_{280}) were performed using a spectrophotometer (GBC UV-Visible Cintra 10e, GBC, Sydney, Australia).

Calculations for total anthocyanins and total phenolics of the grape berries were performed using formulae from Iland et al., (2004). Abbreviations in the formulae include:

A = mean berry fresh weight (g)
B = weight of homogenate taken for extraction (g)
C = final extract volume (mL)
Dilution factor = 11, being the dilution of 1.0 mL of extract plus 10 mL of HCl

- *Anthocyanins per berry (mg / berry)*

$$= \frac{A_{520}}{500} \times \text{dilution factor} \times \frac{C}{100} \times \frac{A}{B} \times 1000$$

Simplified to;

$$= A_{520} \times 0.22 \times C \times \frac{A}{B}$$

- *Anthocyanins per gram berry weight (mg / g)*

$$= \frac{\text{Anthocyanins per berry (mg)}}{\text{mean berry weight (g)}}$$

- *Total Phenolics per berry (au / berry)*

$$= A_{280} \times \text{dilution factor} \times \frac{C}{100} \times \frac{A}{B}$$

Simplified to;

$$= A_{280} \times 0.11 \times C \times \frac{A}{B}$$

- *Total phenolics per gram berry weight (au / g)*

$$= \frac{\text{total phenolics per berry (au units)}}{\text{mean berry weight (g)}}$$

5.2.2 Extraction of anthocyanins and flavonols from berry skins for HPLC analysis

A sample of 30 berries was weighed to determine mean berry weight prior to removing the skins. Fresh skins were weighed before being snap frozen in liquid nitrogen and stored at minus 80^{o}C. The frozen skins were ground into a fine powder using a mortar and pestle and liquid nitrogen to keep the sample frozen. The ground sample was stored at -80^{o}C. Prior to analysis, a 0.1 g skin sample for each field replicate was weighed and kept frozen at -80^{o}C. For HPLC analysis, 1.0 mL methanol/water (1:1) was added to the pre-weighed skin sample, vortexed (Ratek VM1, Ratek Instruments, Boronia, Australia) and then sonicated (EXP 10M, Unisonics, Sydney, Australia) for 20 min. The sample was vortexed again and centrifuged for 10 min at 13,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia) to pellet any solids. A 200 µL aliquot of supernatant was transferred to a HPLC autosampler vial for analysis.

5.2.3 HPLC analysis of anthocyanin and flavonol profiles

High-Performance Liquid Chromatography (HPLC) was used to determine the anthocyanin and flavonol content and composition of the grapes from each irrigation treatment during berry ripening and harvest. Figures 5.1A and 5.1B illustrate the separation and identification of the main anthocyanins and flavonols measured in both wine grape varieties during the analyses. The anthocyanin and flavonol peaks were compared against the elution order from published literature (Wulf & Nagel, 1978; Haselgrove, 1997; Downey et al., 2004). The elution times and absorbance spectra for the anthocyanins and flavonols were compared with a commercial standard of malvidin-3-O-glucoside (Extrasynthese, Ganay, France) and quercitin-3-O-glucoside (Extrasynthese, Ganay, France) respectively

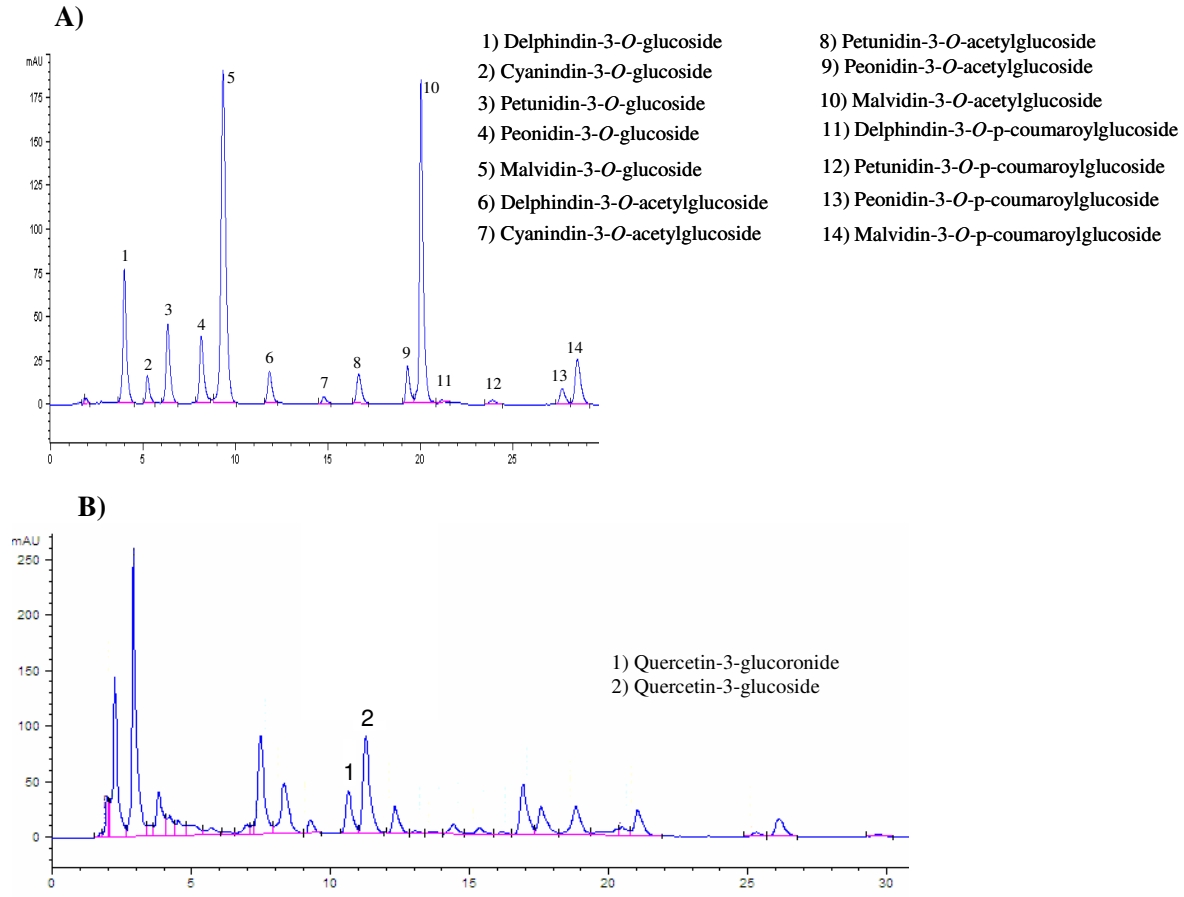


Figure 5.1 HPLC chromatogram of a Cabernet Sauvignon grape skin extract, recorded at 520nm and illustrating the separation and identification of the A) anthocyanins and B) flavonols measured during the analyses.

Samples were analysed on a HPLC system (Agilent HP1100, Agilent, Melbourne, Australia) HPLC system installed with a Wakosil analytical column (150 mm x 4.6 mm, 3µm particle size; SGE, Australia). A binary solvent gradient was used, where Solvent A was 10% formic acid (v/v water) and Solvent B was 10% formic acid with methanol and the gradient conditions as indicated in Table 5.1. The column was maintained at 40ºC and the flow rate was 1.0 mL/minute.

Table 5.1 Solvent gradient for HPLC to separate anthocyanin profiles in grapes using 10% formic acid (solvent A) and 10% formic acid:methanol (solvent B).

Time (min)	% Solvent A	% Solvent B
0	82	18
14	71	29
16	68	32
18	59	41
18.1	70	30
29	59	41
32	50	50
34.5	0	100
35	82	18

The anthocyanin concentrations in grape skins were expressed as malvidin-3-*O*-glucoside equivalents. The anthocyanin concentration was determined using a standard curve prepared from malvidin-3-*O*-glucoside standard (Extrasynthese, Ganay, France) and expressed as mg of malvidin-3-*O*-glucoside equivalents per g of fresh berry skin (Figure 5.2). Similarly, the flavonol concentration per grape skin was expressed as mg of quercetin-3-*O*-glucoside and determined using the standard curve in Figure 5.3.

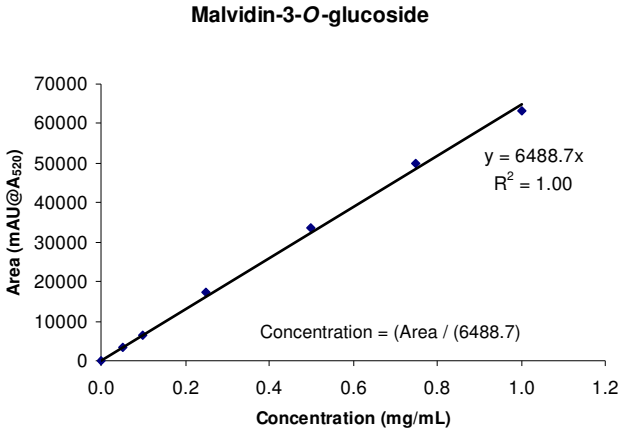


Figure 5.2 Standard curve for calculating concentration of mg of malvidin-3-*O*-glucoside per mL of grape skin extract.

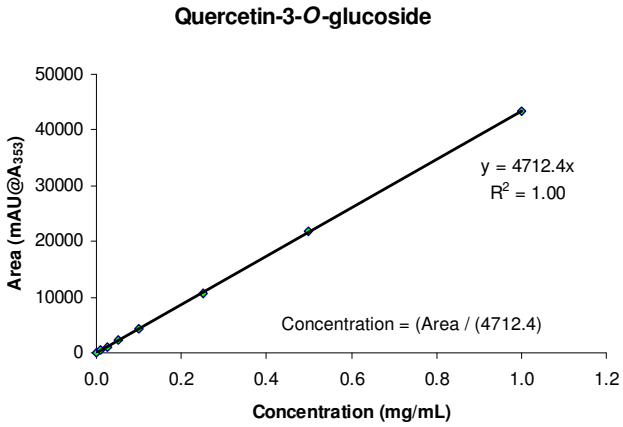


Figure 5.3 Standard curve for calculating concentration of mg of quercitin-3-*O*-glucoside per mL of grape skin extract.

5.2.4 Total tannin analysis methods for berry skins

The following method was adapted from Harbertson et al. (2002) and Harbertson et al. (2003) that uses protein precipitation to determine skin tannin concentration (Downey & Adams, 2005). Solution recipes for the tannin buffers and reagents are described in Appendix C, Table C.1.

To an eppendorf tube, 0.1 g of finely ground skin from each field replicate was weighed and 1.0 mL of wine-like buffer (Buffer A) added. The samples were sonicated (EXP 10M, Unisonics, Sydney, Australia) for 20 min before vortexing and centrifuging for 10 min at 13,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia). After centrifugation, a 500 µL sample of supernatant was removed to a microfuge tube and 1.0 mL of protein solution added. The mixture was vortexed before centrifugation for 10 min at 13,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia) to pellet the tannin-protein precipitate. The supernatant was carefully removed and the pellet washed with 250 µL of washing buffer (Buffer B) then centrifuged for 5 min at 13,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia). The supernatant was removed and 875 µL of resuspension buffer (Buffer C) added and incubated at room temperature for 15 min. The tubes were then vortexed and incubated for a further 10 min at room temperature. Samples were transferred to 10 mm disposable cuvettes and the background tannin absorbance read at 510 nm. To the same cuvettes, a 125 µL aliquot of ferric chloride was then added, vortexed and incubated for 10 min. Tannin absorbance was measured at 510 nm using a spectrophotometer (GBC UV-Visible Cintra 10e, GBC, Sydney, Australia). The zero solution contained 875 µL resuspension buffer and 125 µL of ferric chloride. The tannin concentration was expressed as catechin equivalents (CE) and determined using the following equation (Downey & Adams, 2005);

Total Tannin (mg CE / g fresh weight skin)
= (Final tannin absorbance – Background tannin absorbance) x 3.656

5.3 Results

5.3.1 Changes in phenolic concentration during berry ripening

Cabernet Sauvignon:

For Cabernet Sauvignon, berry total anthocyanin concentration (mg/g) increased from the start of berry ripening through to harvest during the 2005 and 2006 seasons (Figs. 5.4A, B). For 2005 and 2006, there were no significant differences in berry total anthocyanin concentration of the different irrigation treatments during berry ripening Figure 5.4A, B).

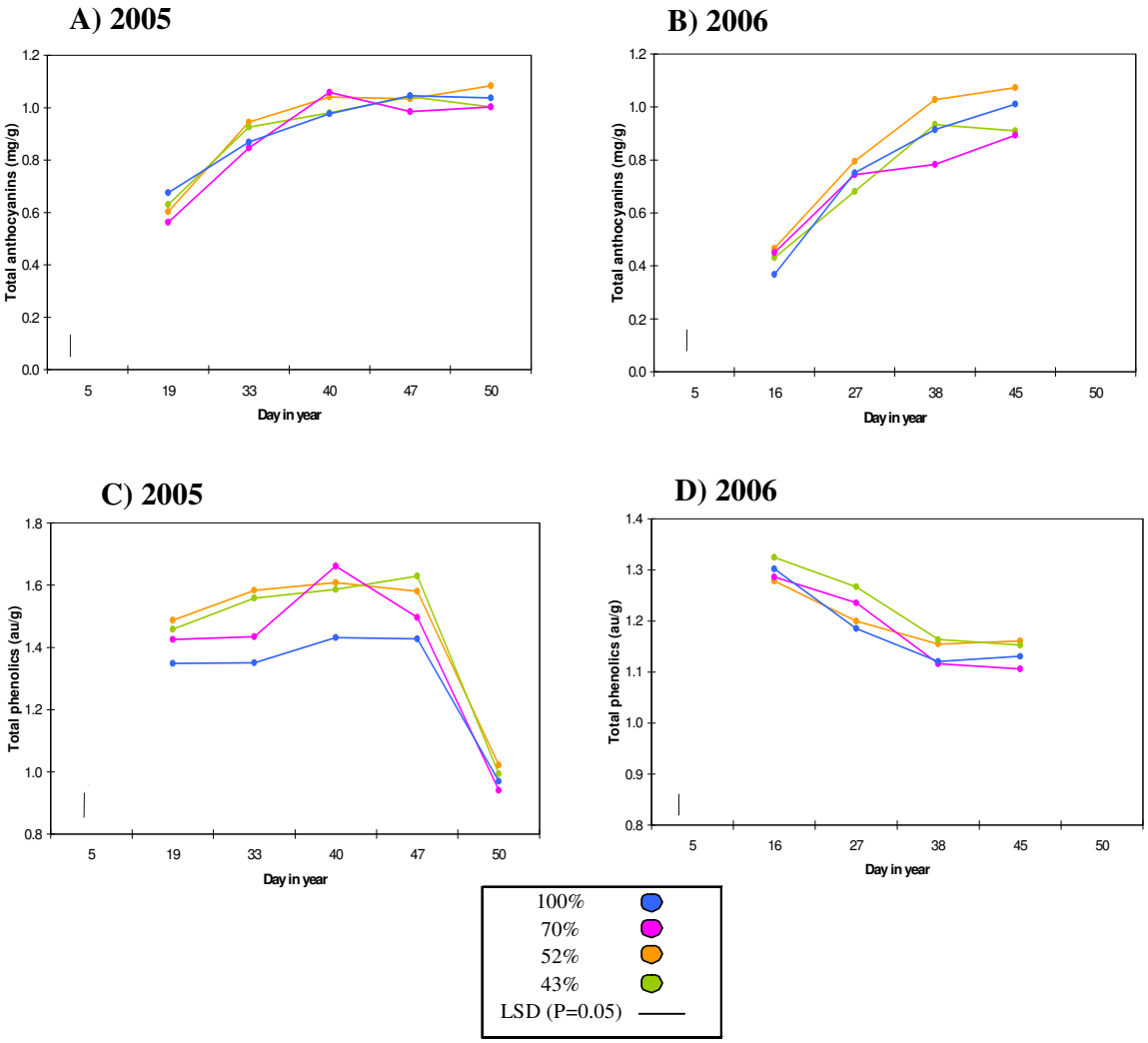


Figure 5.4 Effect of SDI during berry ripening on berry total anthocyanin concentration (mg/g) and berry total phenolic concentration (au/g) for Cabernet Sauvignon exposed to a control and SDI irrigations 70%, 52% and 43%. A) 2005 berry total anthocyanin concentration (mg/g), B) 2006 berry total anthocyanin concentration (mg/g), C) 2005 berry total phenolic concentration (au/g) and D) 2006 berry total phenolic concentration (au/g). LSD bar indicates treatment significance at P=0.05, (n=4).

Berry total phenolic concentration (au/g) in Cabernet Sauvignon showed different trends with time in 2005 compared to 2006 (Figs. 5.4C, D). In 2005, the control (100%) irrigation treatment produced significantly lower total phenolic concentrations than the 43% SDI-treated vines during most of berry ripening (Figure 5.4C). Also, for each irrigation treatment the berry phenolic concentrations increased during berry ripening before decreasing just prior to harvest in 2005 (Figure 5.4C). By contrast, in 2006 there was no significant difference between berry total phenolic concentration for any irrigation treatment, with berry total phenolic concentrations decreasing throughout the ripening period (Figure 5.4D).

Shiraz:

In both seasons, berry total anthocyanin concentration for Shiraz in each irrigation treatment increased from the beginning of berry ripening until harvest (Figs. 5.5A, B). Although not significant, the SDI treatments in 2005 tended to produce higher berry total anthocyanin concentration (mg/g) compared to the control (Figure 5.5A). In 2006, this trend was reversed for the last two measurements prior to harvest, with the control treatment tending to produce higher berry total anthocyanin concentrations than the SDI treatments (Figure 5.5B) but was not significant.

In 2005, the berry total phenolic concentrations for each irrigation treatment increased during berry ripening before decreasing between days 35 to 42 (Figure 5.5C). In 2006, the berry total phenolic concentrations for each irrigation treatment increased during berry ripening until harvest. In both seasons, the 65% SDI-treated vines, whilst not being significantly different, tended to produce higher berry total phenolic concentrations than the other irrigation treatments.

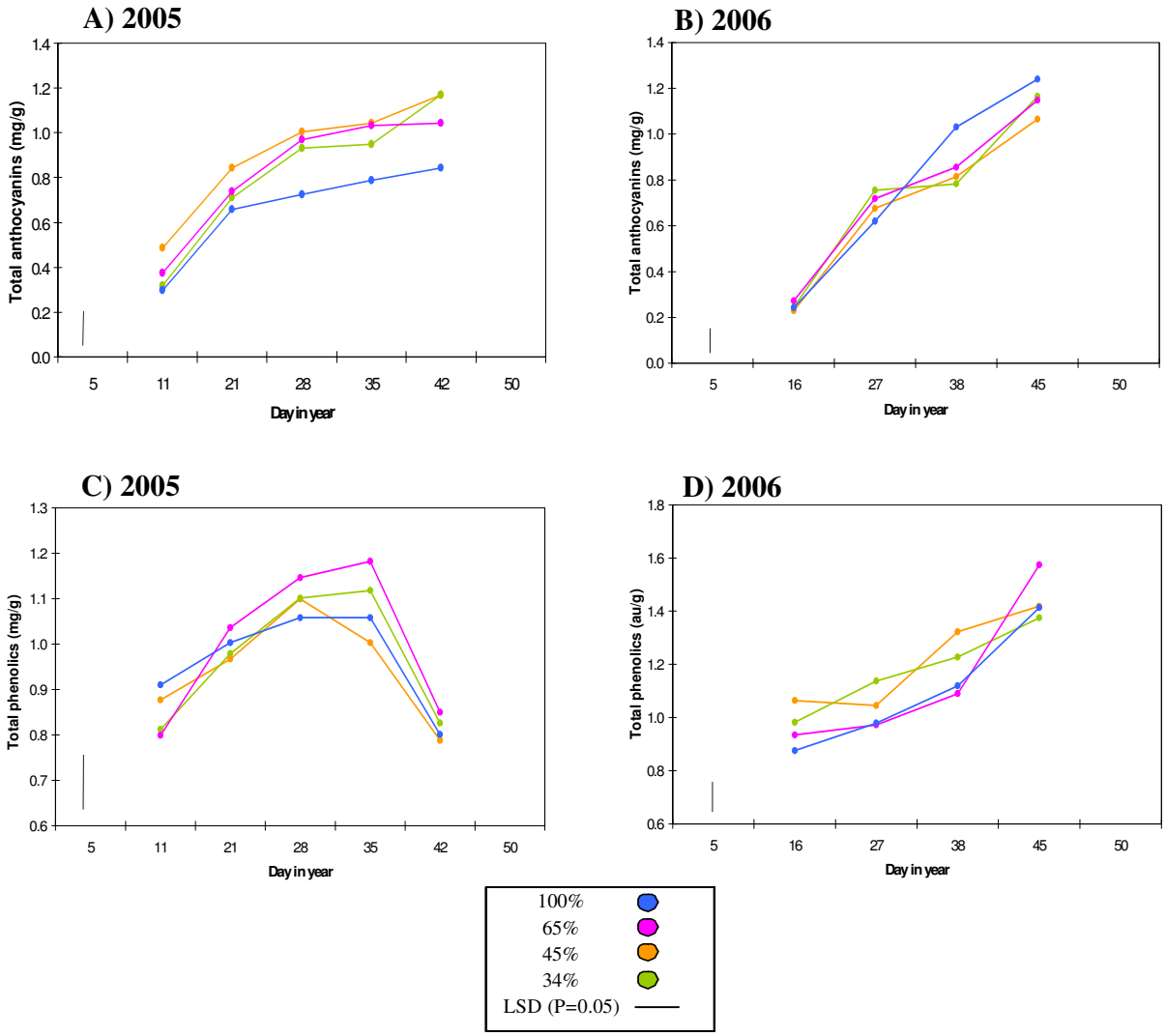


Figure 5.5 Effect of SDI during berry ripening on berry total anthocyanin concentration (mg/g) and berry total phenolic concentration (au/g) for Shiraz exposed to a control and SDI irrigations 65%, 45% and 34%. A) 2005 berry total anthocyanin concentration (mg/g), B) 2006 berry total anthocyanin concentration (mg/g), C) 2005 berry total phenolic concentration (au/g) and D) 2006 berry total phenolic concentration (au/g). LSD bar indicates treatment significance at P=0.05, (n=4).

5.3.2 Changes in tannin concentration during berry ripening

Total skin tannin concentration (mg CE/g), whilst showing no significant differences between irrigation treatments at P=0.05, did decrease during berry ripening for all irrigation treatments for both Cabernet Sauvignon and Shiraz (Figure 5.6). The Cabernet Sauvignon (Figure 5.6A) control tended to produce higher skin tannin concentrations than the SDI treatments near harvest. The Shiraz skin tannin concentrations were similar for irrigation treatments during berry ripening (Figure 5.6B).

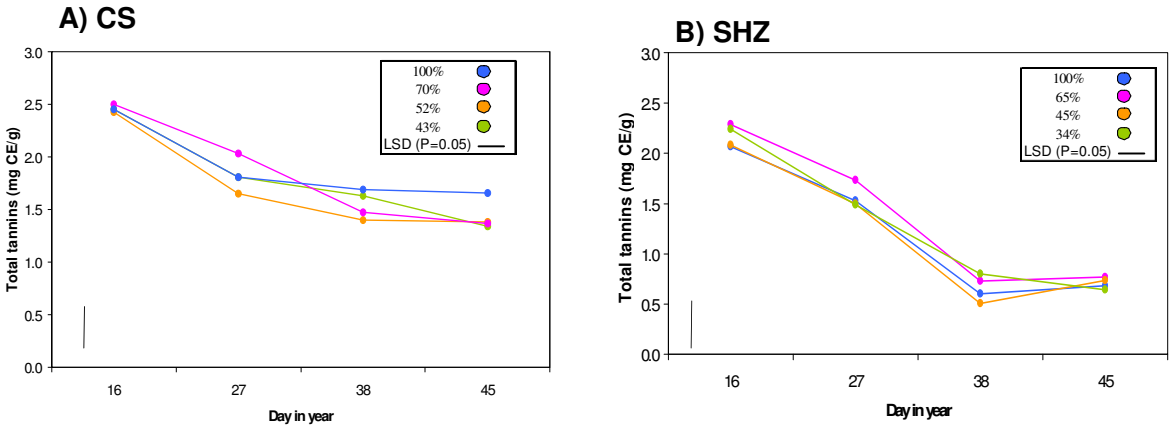


Figure 5.6 Effect of SDI during berry ripening on the development of total skin tannin concentration (mg catechin equivalent/g skin) from A) Cabernet Sauvignon (CS) and B) Shiraz (SHZ) grape skins exposed to a control and SDI irrigations. Berries were collected between veraison and harvest in 2006. LSD bar indicates treatment significance at P=0.05, (n=4).

5.3.3 Differences in anthocyanin and phenolic composition at harvest

Spectral results:

For Cabernet Sauvignon, there was no clear effect of irrigation treatment on the berry total anthocyanin and total phenolic content or concentration in any season at harvest (Table 5.2). The trend for berry total anthocyanin concentration per gram of berry weight (mg/g) was to increase as less water was applied. In 2004 and 2006, the 43% SDI-treated berries had 15-22%

higher berry total anthocyanin concentration per gram of berry weight (mg/g) than the control berries. Berry total phenolic content (au/berry) showed no significant differences between irrigation treatments for any vintage. However, in 2005 and 2006 berry total phenolic concentration per gram of berry weight (au/g) was significantly higher (10-12%) in the 43% SDI-treated berries than the control. Generally the berry total content and concentration of anthocyanins and phenolics, decreased from 2004 to 2005 for all irrigation treatments.

Table 5.2 Berry total anthocyanin and berry total phenolic content and concentration for Cabernet Sauvignon berries harvested in 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (70%, 52% and 43% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row.

Cabernet Sauvignon	**Irrigation treatments**			
2004 Vintage	**100%**	**70%**	**52%**	**43%**
Total anthocyanins (mg/berry)	1.23[ab]	1.21[ab]	1.17[a]	1.31[b]
Total anthocyanins (mg/g)	1.53[a]	1.53[a]	1.60[a]	1.75[b]
Total phenolics (au/berry)	1.64	1.57	1.49	1.61
Total phenolics (au/g)	2.03	1.99	2.05	2.15
2005 Vintage	**100%**	**70%**	**52%**	**43%**
Total anthocyanins (mg/berry)	1.10	1.05	1.00	0.97
Total anthocyanins (mg/g)	1.18	1.19	1.22	1.27
Total phenolics (au/berry)	1.42	1.38	1.34	1.30
Total phenolics (au/g)	1.53[a]	1.57[a]	1.65[ab]	1.71[b]
2006 Vintage	**100%**	**70%**	**52%**	**43%**
Total anthocyanins (mg/berry)	1.03	0.98	0.96	1.10
Total anthocyanins (mg/g)	0.95[a]	0.94[a]	0.98[a]	1.16[b]
Total phenolics (au/berry)	1.53	1.48	1.41	1.46
Total phenolics (au/g)	1.41[a]	1.42[a]	1.46[a]	1.55[b]

For Shiraz, berry total anthocyanin concentration (mg/g) increased as less water was applied in 2005 and 2006 (Table 5.3). In 2005, berry total anthocyanin concentration (mg/g) was significantly higher for the 45% and 34% SDI-treated Shiraz berries than the control (Table 5.3). The content and concentration of berry total phenolics showed no consistent pattern between the irrigation treatments for any vintage. In 2006, the SDI-treated vines produced significantly lower berry total phenolic content (au/berry) (11–14% reduction) than the control

vines. In 2005, berry total phenolic concentrations (au/g) were significantly higher for the 34% SDI-treated vines than the control vines.

Table 5.3 Berry total anthocyanin and berry total phenolic content and concentration from Shiraz bunches harvested between 2004-2006 and exposed to a full irrigation (100% control) and SDI irrigations (65%, 45% and 34% of the control). Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row.

SHIRAZ	**Irrigation treatments**			
2004 Vintage	**100%**	**65%**	**45%**	**34%**
Total anthocyanins per berry (mg/berry)	1.10	1.04	0.97	1.00
Total anthocyanins per gram berry (mg/g)	1.33	1.29	1.24	1.38
Total phenolics per berry (au/berry)	1.11	1.09	1.06	1.04
Total phenolics per gram berry (au/g)	1.36	1.34	1.35	1.48
2005 Vintage	**100%**	**65%**	**45%**	**34%**
Total anthocyanins per berry (mg/berry)	1.10	1.07	1.18	1.12
Total anthocyanins per gram berry (mg/g)	1.20[a]	1.20[a]	1.39[b]	1.48[b]
Total phenolics per berry (au/berry)	0.99	0.94	1.02	0.96
Total phenolics per gram berry (au/g)	1.06[a]	1.05[a]	1.19[ab]	1.25[b]
2006 Vintage	**100%**	**65%**	**45%**	**34%**
Total anthocyanins per berry (mg/berry)	1.08	1.03	1.04	0.93
Total anthocyanins per gram berry (mg/g)	0.99	1.08	1.14	1.11
Total phenolics per berry (au/berry)	1.52[b]	1.36[a]	1.31[a]	1.32[a]
Total phenolics per gram berry (au/g)	1.41	1.24	1.43	1.42

HPLC results:

Tables 5.4 and 5.5 detail the percent composition of skin anthocyanins of Cabernet Sauvignon berries in 2005 and 2006 at harvest. Since there were no significant differences (P=0.05) the standard errors for each mean have been listed to indicate the degree of variability within the data. Although not significant, the skin total anthocyanin concentration (mg/g) of the SDI-treated Cabernet Sauvignon tended to be higher for the SDI treatments compared to the control by 10-17% in 2005, and 5-25% in 2006 (Tables 5.4, 5.5). Furthermore the highest concentration of skin total anthocyanins was recorded in the 43% SDI-treated vines. Of the parent anthocyanidins, malvidin was the most abundant for all irrigation treatments. Skin total

anthocyanin concentrations (mg/g) were generally higher for all irrigation treatments in 2006 than in 2005; this could be attributed to a shift in the proportions of various anthocyanidins. From 2005 to 2006 there appears to have been a decrease in 3-*O*-glucosides and an increase in 3-*O*-acetylglucosides that may be contributing to the change in skin total anthocyanin concentrations. Within each season the parent anthocyanidins generally in greatest abundance were malvidin, followed by delphinidin, peonidin, petunidin and cyanidin.

Table 5.4 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Cabernet Sauvignon berry skins collected at the 2005 harvest from Control (100%) and SDI treatments 70%, 52% and 43%. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8).

2005 Vintage	**Irrigation Treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
Total Delphinidin	9.2 ± 0.27	9.3 ± 0.38	9.2 ± 0.30	9.5 ± 0.15
Total Cyanidin	1.2 ± 0.08	1.4 ± 0.08	1.3 ± 0.08	1.6 ± 0.12
Total Petunidin	7.3 ± 0.21	7.8 ± 0.22	8.3 ± 0.47	8.2 ± 0.17
Total Peonidin	8.6 ± 0.35	8.8 ± 0.63	9.3 ± 0.44	10.2 ± 0.48
Total Malvidin	73.7 ± 0.73	72.7 ± 0.90	71.9 ± 0.87	70.5 ± 0.67
Total Dihydroxylated (Cy + Peo)	9.8 ± 0.41	10.2 ± 0.66	10.6 ± 0.50	11.8 ± 0.58
Total Trihydroxylated (Del + Pet + Mal)	90.2 ± 0.41	89.8 ± 0.66	89.4 ± 0.50	88.2 ± 0.58
Total -3-*O*-glucoside	58.9 ± 0.91	59.3 ± 0.99	58.6 ± 0.78	59.1 ± 0.75
Total -3-*O*-acetylglucoside	30.8 ± 0.71	30.2 ± 0.78	30.0 ± 0.58	30.0 ± 0.52
Total -3-*O*-p-coumaroylglucoside	10.3 ± 0.20	10.5 ± 0.24	11.4 ± 0.51	10.9 ± 0.27
Total Anthocyanins (mg/g)	1.07 ± 0.07	1.22 ± 0.06	1.18 ± 0.11	1.25 ± 0.07

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

Table 5.5 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Cabernet Sauvignon berry skins collected at the 2006 harvest from Control (100%) and SDI treatments 70%, 52% and 43%. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8).

2006 Vintage	**Irrigation Treatments**			
Cabernet Sauvignon (mg/g)	**100%**	**70%**	**52%**	**43%**
Total Delphinidin	9.2 ± 0.10	8.7 ± 0.28	8.1 ± 0.35	8.4 ± 0.44
Total Cyanidin	1.0 ± 0.09	1.1 ± 0.06	1.1 ± 0.09	0.89 ± 0.09
Total Petunidin	6.7 ± 0.22	7.1 ± 0.21	6.8 ± 0.30	7.0 ± 0.27
Total Peonidin	8.3 ± 0.55	8.4 ± 0.74	9.3 ± 0.56	8.4 ± 0.38
Total Malvidin	74.8 ± 0.84	74.6 ± 0.47	74.7 ± 0.87	75.4 ± 1.04
Total Dihydroxylated (Cy + Peo)	9.3 ± 0.57	9.5 ± 0.79	10.4 ± 0.65	9.3 ± 0.46
Total Trihydroxylated (Del + Pet + Mal)	90.7 ± 0.57	90.5 ± 0.79	89.6 ± 0.65	90.7 ± 0.46
Total -3-*O*-glucoside	55.8 ± 0.87	57.3 ± 0.25	57.05 ± 0.87	57.3 ± 0.53
Total -3-*O*-acetylglucoside	34.6 ± 0.93	33.0 ± 0.16	32.8 ± 0.86	32.5 ± 0.39
Total -3-*O*-p-coumaroylglucoside	9.6 ± 0.33	9.8 ± 0.23	10.2 ± 0.21	10.2 ± 0.24
Total Anthocyanins (mg/g)	2.8 ± 0.22	3.1 ± 0.28	2.7 ± 0.23	3.6 ± 0.20

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

As for the Cabernet Sauvignon, there were no significant differences for Shiraz (Tables 5.6, 5.7) between the irrigation treatments for each anthocyanidin or skin total anthocyanin concentration. Skin total anthocyanin concentration for Shiraz tended to be higher as less water was applied and were higher for all treatments in 2006 (Table 5.7) compared to 2005 (Table 5.6). Although not significantly different, there tended to be higher percent composition of each anthocyanidin constituent in the 65% and 45% SDI treatments than the control in 2005. Alternatively in 2006 the skin total anthocyanin concentrations, while not being significant, were higher in the 34% SDI-treated vines than the other irrigation treatments. Within each season the parent anthocyanidins generally in greatest abundance were malvidin, followed by peonidin, petunidin, delphinidin and cyanidin. Malvidin levels were higher in 2006 than 2005 for all irrigation treatments and were higher for the 34% SDI-treated vines in 2006. From 2005 to 2006, the percent composition of 3-*O*-acetylglucoside and 3-*O*-p-coumaroylglucoside increased from 2005 to 2006, whereas total -3-*O*-glucoside decreased. Overall, Shiraz berries from the 2006 vintage produced higher concentrations of skin total anthocyanins than in 2005, particularly the 34% SDI-treated vines that produced 2.3 times more skin total anthocyanins in 2006 compared to 2005.

Table 5.6 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Shiraz berry skins collected at 2005 harvest from Control (100%) and SDI treatments 65%, 45% and 34% expressed as mg/g of skin. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8).

2005 Vintage	**Irrigation Treatments**			
Shiraz	**100%**	**65%**	**45%**	**34%**
Total Delphinidin	6.1 ± 0.08	6.1 ± 0.41	6.2 ± 0.39	6.4 ± 0.22
Total Cyanidin	0.74 ± 0.09	0.8 ± 0.12	0.8 ± 0.16	0.8 ± 0.06
Total Petunidin	7.0 ± 0.34	7.1 ± 0.54	7.5 ± 0.53	7.5 ± 0.36
Total Peonidin	15.9 ± 1.67	19.9 ± 3.07	14.4 ± 0.86	17.5 ± 1.98
Total Malvidin	70.2 ± 1.44	66.2 ± 2.64	71.1 ± 0.92	67.9 ± 2.02
Total Dihydroxylated (Cy + Peo)	16.7 ± 1.72	20.6 ± 3.07	15.2 ± 0.84	18.2 ± 2.04
Total Trihydroxylated (Del + Pet + Mal)	82.3 ± 1.72	79.4 ± 3.07	84.8 ± 0.84	81.8 ± 2.04
Total -3-*O*-glucoside	44.1 ± 2.12	41.4 ± 4.69	47.2 ± 1.80	43.6 ± 3.5
Total -3-*O*-acetylglucoside	24.6 ± 0.73	20.9 ± 2.28	23.5 ± 0.65	22.8 ± 2.28
Total -3-*O*-p-coumaroylglucoside	31.4 ± 2.61	37.7 ± 3.23	29.3 ± 2.13	33.6 ± 2.39
Total Anthocyanins (mg/g)	1.77 ± 0.18	2.00 ± 0.18	2.25 ± 0.16	1.80 ± 0.17

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

Table 5.7 Percent composition of parent anthocyanidin (delphinidin, cyanidin, petunidin, peonidin, malvidin); di- and trihydroxylated anthocyanidin; and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidin of Shiraz berry skins collected at 2006 harvest from Control (100%) and SDI treatments 65%, 45% and 34% expressed as mg/g of skin. Total anthocyanins (mg/g) from harvested berry skins are also included. Means ± s.e. (n = 8).

2006 Vintage	**Irrigation Treatments**			
Shiraz (mg/g)	**100%**	**65%**	**45%**	**34%**
Total Delphinidin	5.7 ± 0.42	5.9 ± 0.50	5.4 ± 0.48	4.8 ± 0.64
Total Cyanidin	0.7 ± 0.19	0.4 ± 0.02	0.4 ± 0.04	0.4 ± 0.02
Total Petunidin	5.4 ± 0.12	5.3 ± 0.16	5.3 ± 0.38	5.0 ± 0.17
Total Peonidin	14.7 ± 0.52	15.3 ± 0.90	14.4 ± 1.30	11.9 ± 1.01
Total Malvidin	73.5 ± 0.75	73.2 ± 1.31	74.6 ± 1.70	77.9 ± 1.41
Total Dihydroxylated (Cy + Peo)	15.4 ± 0.55	15.7 ± 0.93	14.8 ± 1.32	12.3 ± 1.02
Total Trihydroxylated (Del + Pet + Mal)	84.6 ± 0.55	84.3 ± 0.93	85.2 ± 1.32	87.8 ± 1.02
Total -3-*O*-glucoside	33.7 ± 0.73	29.1 ± 2.43	43.5 ± 1.70	35.9 ± 1.74
Total -3-*O*-acetylglucoside	27.1 ± 0.35	28.3 ± 1.19	24.9 ± 2.31	26.7 ± 0.71
Total -3-*O*-p-coumaroylglucoside	38.9 ± 0.78	42.6 ± 1.55	41.5 ± 2.11	37.2 ± 2.13
Total Anthocyanins (mg/g)	3.80 ± 0.36	3.37 ± 0.20	3.66 ± 0.20	4.14 ± 0.26

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

5.3.4 Differences in total tannin at harvest

Skin total tannin concentrations (mg CE/g) at harvest showed no significant differences between irrigation treatments for either variety during 2005 and 2006 (Table 5.8). For Cabernet Sauvignon, more tannin was produced for all irrigation treatments in 2006 than in 2005. The Shiraz berry skins produced similar tannin levels in both seasons for all irrigation treatments. Overall, the Cabernet Sauvignon control (100%) vines produced approximately 42% more tannin than the Shiraz control berries in 2006.

Table 5.8 Concentration of total tannins from Cabernet Sauvignon and Shiraz berry skins collected at the 2005 and 2006 harvest from Control (100%) and SDI treatments expressed as mg of catechin equivalent/g of skin. Means ± s.e. (n=8).

Total Skin Tannin (mg CE/g)	**Irrigation Treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
2005	0.85 ± 0.07	1.00 ± 0.10	0.86 ± 0.12	1.05 ± 0.11
2006	1.08 ± 0.16	1.10 ± 0.07	0.94 ± 0.15	1.24 ± 0.11
Shiraz	**100%**	**65%**	**45%**	**34%**
2005	0.83 ± 0.14	0.78 ± 0.13	0.83 ± 0.12	0.87 ± 0.10
2006	0.76 ± 0.13	0.77 ± 0.10	0.76 ± 0.15	0.81 ± 0.14

5.3.5 Differences in flavonol content at harvest

Skin total flavonols were also measured for each variety (Section 5.2.3) at the 2005 and 2006 harvests to determine whether the SDI treatments were having an affect on the synthesis of these light-dependent molecules (Table 5.9). For Cabernet Sauvignon there was a significant increase in skin flavonol concentration for the 52% and 43% SDI treatments compared to the control in 2005. No significant differences in skin flavonol concentration were observed for Cabernet Sauvignon in 2006, even though the 43% SDI-treated vines produced approximately 35% higher skin flavonol concentration than the other irrigation treatments (Table 5.9). For Shiraz, there were no significant differences in skin flavonol concentration between the SDI treatments and the control in 2005 (Table 5.9). However, in 2006, the 65% and 45% SDI-treated Shiraz vines had significantly lower skin flavonol concentration than the control and 34% SDI vines (Table 5.9)

Table 5.9 Concentration of skin total flavonols from Cabernet Sauvignon and Shiraz berry skins collected at the 2005 and 2006 harvest from Control (100%) and SDI treatments expressed as mg/g of skin. Significance indicated by different letters. Means followed by the same letter within a row are not significantly different at P=0.05, (n = 8). No letters signify ns between irrigation treatments within a row.

Total Skin Flavonols (mg/g)	**Irrigation Treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
2005	0.16[a]	0.23[ab]	0.26[bc]	0.32[c]
2006	0.16	0.17	0.17	0.23
Shiraz	**100%**	**65%**	**45%**	**34%**
2005	0.30	0.25	0.27	0.35
2006	0.38[c]	0.34[a]	0.36[ab]	0.49[c]

In Cabernet Sauvignon, a reduction in leaf area index across all three growing seasons from 2004 to 2006, was associated with an increase in skin flavonol concentration (Figure 5.7).

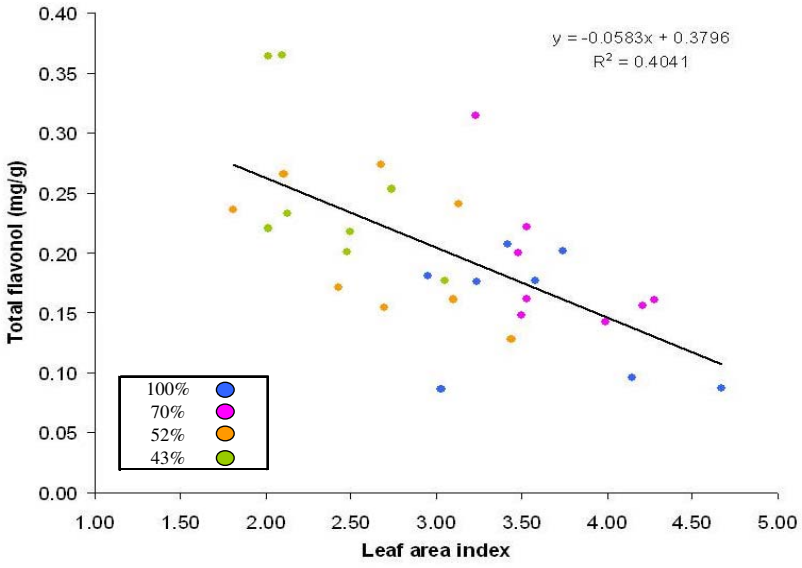


Figure 5.7 Linear regression of total flavonols (mg/g of skin) vs leaf area index (leaf area:soil area) for Cabernet Sauvignon berries exposed to a control and SDI irrigations 70%, 52% and 43% during 2004-2006, (n=8).

5.3.6 Interaction between SDI, berry weight and phenolic composition

Figure 5.8 A) and B) illustrates the relationships between berry total anthocyanin and phenolic concentration and berry weight during the 2005 and 2006 seasons. For both varieties, the concentration of anthocyanins decreased as berry weight increased (Figure 5.8A). Phenolics of Cabernet Sauvignon were more dependent on berry weight than for the Shiraz (Figure 5.8B). When compared against the volume of irrigation applied, both varieties had higher concentrations of anthocyanins and phenolics as less water was applied, but the relationship was weak except for the phenolic concentrations of the Cabernet Sauvignon (Figs. 5.8 C, D). As yield per vine increased, both varieties had lower concentrations of anthocyanins and phenolics (Figs. 5.8 E, F), with Cabernet Sauvignon displaying a stronger relationship between yield and anthocyanin or phenolic concentration than Shiraz.

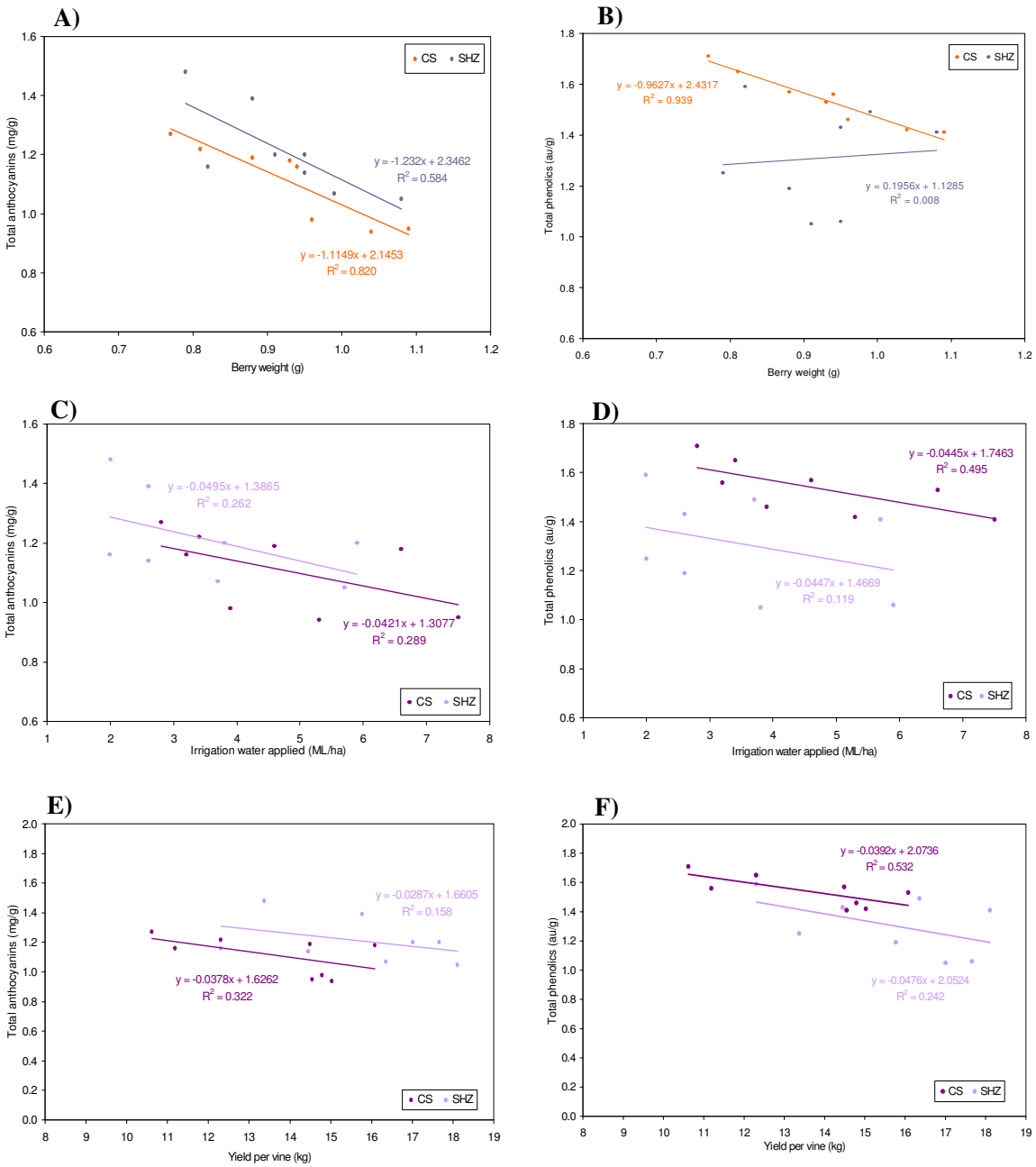


Figure 5.8 Regression analyses of A), C) and E) total anthocyanins (mg/g) and B), D) and F) total phenolics (au/g) against berry weight, amount of irrigation water applied (ML/ha) and yield per vine (kg) for Cabernet Sauvignon (CS) and Shiraz (SHZ). Data was pooled over 2005 and 2006 seasons, (n=8).

5.4 Discussion

5.4.1 Effect of SDI on anthocyanins and total phenolics

During berry ripening:

In this study, while anthocyanin concentration on most of the berry ripening sampling dates for Cabernet Sauvignon and Shiraz were not consistently affected by reduced irrigation, the vines irrigated with less water tended to produce higher concentrations of total anthocyanins and total phenolics per berry weight than the control vines at various stages during berry ripening. Other findings have noted that berry total anthocyanins and other phenolic compounds to increase from the onset of veraison until harvest (Ojeda et al., 2002; Downey et al., 2004). Petrie et al. (2004) showed that in Shiraz berries the anthocyanin concentration did not differ significantly between deficit and fully irrigated vines until the vines had been exposed to the water deficits for several seasons. In this same study it was found that in the first season the anthocyanin concentrations were unaffected, however by the second season those vines receiving the lowest amount of water had smaller berries and higher anthocyanin concentrations than the controls (Petrie et al., 2004). It is possible that significant increases in anthocyanin concentrations may also have been observed with time had this study been extended for more years. However this still does not completely explain why after three seasons of exposure to deficit irrigation the SDI-treated vines did not consistently produce more total anthocyanins or phenolics per unit berry weight than the control during berry ripening. Perhaps there was some other factor, such as light exposure of bunches, affecting the accumulation of these phenolic compounds that buffered any potential water deficit effect. Ristic (2004) suggested that the accumulation of anthocyanins was dependent on the level of sunlight on bunches, with the optimum range of sunlight interception for maximum anthocyanin accumulation to be around 50% of full sun. However, others have found that anthocyanin accumulation in Shiraz and Cabernet Sauvignon is not very dependent of light exposure and that temperature has a greater influence on anthocyanin biosynthesis (Bergqvist et al., 2001; Spayd et al., 2002; Downey et al, 2004).

The level of total phenolic accumulation was different for each variety and year. In 2005, total phenolic concentrations increased during berry ripening, and then suddenly decreased before

harvest for both varieties (Figs. 5.4C, 5.5C). The spectral measure of total phenolics detects a range of compounds that absorb at 280nm, including anthoycanins, tannins, flavonols, caffeic acid (hydroxy cinnamates) as well as protein and DNA (Allen, 1998). These compounds are mainly present in the skin and seeds and are synthesised well before the harvest date. In the case of anthocyanin synthesis, these compounds are formed within the first few weeks after the onset of veraison (Downey et al., 2004). Alternatively, tannin accumulation occurs between flowering and veraison, and tannin maturation occurs after veraison during berry ripening (Robinson, 2006). Flavonol synthesis is UV-dependent and can be regulated by light interception of the bunch (Price et al., 1996). With this in mind the sudden decrease in total phenolic concentration near harvest in 2005 could not be the result of the SDI treatments affecting phenolic biosynthesis. Rather there is something else that caused the decrease such as a change in one or more of the phenolic compounds that was undetectable at A_{280}. Alternatively, the possibility of berry shrivelling near harvest may have lead to the loss of membrane integrity of the skin cells, and the subsequent loss of those phenolics that would reside in the skin (Somers, 1976; Rogiers et al., 2004).

At harvest (spectral analyses):

In earlier water deficit studies, particularly on Cabernet Sauvignon, an increase in berry total anthocyanin concentration (mg/g) at harvest was attributed to an increase in the amount of total anthocyanins per berry due to water deficit exposure (Dry et al., 2000; Roby et al., 2004). The SDI treatments in this study did produce significantly lighter berries than the control (Chapter 4, Tables 4.1, 4.3). Therefore it was anticipated the SDI treatments would produce higher concentrations of total anthocyanins (mg/g) and total phenolics (au/g) due to the lighter berries having a greater skin area to volume ratio than the heavier berries (Refer to berry size discussion in section 5.4.4). However, higher concentrations of these compounds at harvest associated with less irrigation were observed only in some years for both varieties and significant differences occurred mostly between the lowest (43% Cabernet Sauvignon; 34% Shiraz) irrigation treatment and the control. After three seasons of SDI treatments, Cabernet Sauvignon treated with 43% SDI had significantly higher concentrations of total anthocyanins (22%) and total phenolics (10%) per gram of berry than the control. Conversely for Shiraz, whilst not significantly different, the total anthocyanin concentrations were approximately 15% higher in the 45% SDI-treated berries than the control, whereas total phenolics (au/g) showed no significant difference between SDI treatment and the control.

These differences may be associated with those SDI treatments which had the least water having lower levels of photosynthesis during the early weeks of veraison at a time when there may have been an opportunity to influence anthocyanin synthesis. This is supported by the physiology results in Chapter 3 where the 45% and 43% SDI-treated Cabernet Sauvignon and Shiraz vines respectively had significantly lower stomatal conductance levels compared to the controls that in turn may have reduced overall photosynthesis and assimilate availability. Many studies have investigated the effects of sunlight exposure on the concentration of anthocyanins and phenolic compounds in berries (Price et al., 1995; Dokoozlian & Kliewer, 1996; Haselgrove et al., 2000; Kennedy et al., 2002; Spayd et al., 2002). In general in hot climates the concentration of anthocyanins is not directly associated with increased sunlight intensity, but rather reaches a maximum near 50% ambient light (Ristic, 2004). At harvest, the increase in total anthocyanin (mg/g) and total phenolic (au/g)concentrations of berries from vines experiencing the lower SDI treatments may be due to the interaction between decreased photosynthesis and subsequent canopy growth reductions increasing light infiltration to the bunches during the early veraison period. As illustrated in Chapter 3, leaf area index (Figure 3.17) for both varieties was significantly reduced for the SDI treatments that received 50% or less water from day 120 after budburst. This reduction in canopy growth thereby allowed more sunlight infiltration to the developing bunches that would have coincided with the period of anthocyanin biosynthesis.

As discussed in Chapter 3, the SDI treatments increased xylem sap [ABA] levels in both the Cabernet Sauvignon and Shiraz vines exposed to water deficit. Consequently, the elevated ABA levels in the xylem sap of the SDI-treated vines than the control could be altering anthocyanin concentration in the berry skin. Antolín et al., (2003) found that berries from field grown Tempranillo vines exposed to water deficit had higher berry ABA levels than the control vines. Furthermore, there was a strong correlation between xylem sap ABA and berry ABA, particularly after veraison when anthocyanin synthesis would have commenced (Antolín et al., 2003; Antolín et al., 2006). At veraison, most of the ABA in the leaves is transported to the berry where it has been found to increase anthocyanin levels in grape berries by altering enzyme expression to form specialised anthocyanin-forming cells (anthocyanoplasts) (Hiratsuka et al., 2001). Consequently, there is a possibility that increased xylem sap ABA levels of the SDI-treated vines may have altered anthocyanin biosynthesis and increased formation of anthocyanoplasts. Furthermore, the tendency for increases in berry skin

anthocyanins of the SDI vines may also be related to the way in which isohydric or anisohydric vines synthesise ABA due to their hormonal and/or hydraulic responses to water deficit conditions (Soar et al., 2004; Soar et al., 2006b).

At harvest (HPLC analyses):

HPLC analyses were performed on berry skins to determine the effect of SDI treatments on individual anthocyanin profiles. The application of SDI to these varieties, whilst not being significant, resulted in an increase in total anthocyanin (mg/g skin) as less water was applied. These results follow similar trends found in other studies where berries of vines experiencing soil water deficits had higher berry skin anthocyanin concentrations than the control (Matthews & Anderson 1988; Brossaud et al., 1999; Ojeda et al., 2002). In 2006, while there were no significant differences in anthocyanin concentrations between the control and SDI treatments, the Cabernet Sauvignon and Shiraz had 25% and 10% higher total skin anthocyanin concentration in berries exposed to the lowest (43% and 34%) SDI than the control (Tables 5.5, 5.7). For both varieties there were lower concentrations of total anthocyanins produced in 2005 than 2006. Whilst berries exposed to direct sunlight generally produce higher anthocyanin levels, there can be a tendency for high temperatures to inhibit the synthesis of phenolic compounds (Kliewer, 1977; Bergqvist et al., 2001). Studies by Pirie (1977) on Shiraz suggest that the optimum temperature range for enzymes involved in anthocyanin biosynthesis is 17 to 26^{o}C. During the 2004/2005 season, there were higher temperatures than the average monthly maximum during early veraison (Appendix A) that could have potentially caused higher berry temperatures to develop. The possible increase in berry temperature may then have been due to direct sunlight exposure on bunches (Kliewer, 1970; Kliewer, 1977; Jackson & Lombard, 1993) or indirectly by the increased ambient temperature influencing photosynthesis and subsequent metabolic processes via a stress response (Chaves et al., 2003; Antolín et al., 2006).

For both varieties studied there were minimal differences in anthocyanin content at harvest between irrigation treatments, but larger differences between seasons. In Cabernet Sauvignon at harvest, malvidin-derived anthocyanidins were always present at the highest levels compared to the derivatives of delphinidin, peonidin, petunidin and cyanidin (Tables 5.4, 5.5). The Shiraz berries at harvest had the highest levels of malvidin, followed by peonidin, petunidin, delphinidin and cyanidin (Tables 5.6, 5.7). These differences in the order of

anthocyanidin composition are probably the result of genetic differences between these varieties (Arozarena et al., 2002; García-Beneytez et al., 2002; Romero-Cascales et al., 2005). For both varieties, total malvidin anthocyanins were present in the greatest proportions while cyanidin concentrations were the lowest, which is consistent with findings by Roggero et al., (1986). PRD field trials on Cabernet Sauvignon found that total malvidin was reduced in the PRD berries, whereas derivatives of delphinidin, petunidin and peonidin were increased (Stoll, 2000). These differences were possibly a result of increased light intensity in the bunch zones due to reduced vine vigour and canopy density as a result of the PRD response to water deficit (Stoll, 2000).

Alternatively, a more recent PRD study of potted Cabernet Sauvignon has indicated increased levels of total malvidin-3-*O*-glucoside in PRD treated berries compared to the control when bunches were exposed to a uniform level of sun-exposure (Bindon, 2004). This latter research partially supports previous findings on the effects of sun-exposure where the total levels of malvidin-3-*O*-glucosides have been enhanced under increased light intensity (Haselgrove et al., 2000; Spayd et al., 2002). However, as the results of Bindon (2004) were obtained from partially shaded, potted vines it is difficult to compare them with mature, field-grown vines grown under ambient light conditions. Under the conditions of this study, where the Cabernet Sauvignon and Shiraz vines were grown under field situations that were similar to commercial practices, the SDI-treated vines consistently (but not significantly) produced higher total malvidin levels than the control. As for the other anthocyanidin derivative, there was minimal effect of the SDI treatments on the compositional levels within a season. There did not appear to be any single anthocyanidin responsible for a shift in total anthocyanin concentrations for a particular irrigation treatment.

5.4.2 Effect of SDI on tannin concentrations

Tannin accumulation in grape skins and seeds occurs during two phases of berry development. Tannin synthesis occurs in the berry skins between flowering and veraison (Czochanska et al., 1979; de Freitas & Glories, 1999; Downey et al., 2003a; Robinson, 2006). Seed tannins however are synthesised from flowering until 1-2 weeks after veraison, whereby they begin to mature as the grapes ripen and soften (Romeyer et al., 1986; Katalinic & Males, 1997; Ristic & Iland, 2005; Robinson, 2006). Thus, at veraison the largest amounts of tannins can be

extracted per berry before they decrease post-veraison (Amrani-Joutei et al., 1994; Kennedy et al., 2000; Downey et al., 2003a). Even though skin tannins are synthesised early in berry formation, tannin concentrations are known to be lower at harvest than at the beginning of veraison (Robinson & Walker, 2006). Over the ripening period of this study, total skin tannin concentration for Cabernet Sauvignon and Shiraz followed a similar trend, being at the highest concentration at the beginning of veraison before decreasing until harvest (Figure 5.6). The reason for the decrease in tannin concentration is difficult to conclude and maybe related to the effect of the expanding berry diluting tannin concentration or changes in tannin extractability (Robinson, 2006).

At harvest, there were no significant differences in total skin tannin concentration between irrigation treatments for each variety in 2005 (Table 5.8). By 2006, as less water was applied there tended to be an increase in total skin tannin concentrations. For Cabernet Sauvignon, the 43% SDI-treated berries had approximately 15% higher concentration of skin tannin than the control, whereas 45% SDI Shiraz had similar tannin levels to the control. Work by Roby et al., (2004) noted that for Cabernet Sauvignon treated with deficit irrigation, the concentrations of skin tannin were relatively similar irrespective of berry size, and that the berries from least irrigated vines produced higher skin tannin concentration than the most irrigated vines. Consequently, the lower SDI treatments for Cabernet Sauvignon may have altered skin tannin synthesis during the early stage of berry formation that led to increased skin tannin concentration, or decreased the ability for the tannin polymers to become conjugated to other compounds, thereby increasing extractability levels (Kennedy et al., 2000; Downey et al., 2003a).

5.4.3 Effect of SDI on flavonol levels at harvest

Sunlight exposure (Price et al., 1995, Haselgrove et al., 2000) and nitrogen availability (Kliewer, 1977) can influence flavonol glucosides, such as quercitin-3-O-glucoside which is a major skin flavonol in grapes that acts as an UV protectant for the berry (Flint et al., 1985). Since deficit irrigation is known to alter grapevine vegetative growth (Williams & Matthews, 1990), thus allowing more light penetration, there is potential for sun-exposed bunches to have increased flavonol levels. Findings from this study show that the water deficit treatments tended to influence the total flavonol concentration, with some of the SDI treatments

producing significantly higher flavonol concentrations in some seasons. Furthermore, in the case of Cabernet Sauvignon (Figure 5.7), there appears to be a trend emerging in respect to the irrigation treatments, with the 52% and 43% SDI-treated vines having lower LAI and higher skin flavonol concentrations than the 100% and 70% treatments.

5.4.4 Is it berry weight or SDI influencing phenolic composition of berries?

Since the SDI-treated vines did produce berries of lower weight than the control vines it could be assumed that the increase in anthocyanin and phenolic concentration was due to the direct effect of berry size reduction (Figs. 5.7A, B). This has also been observed in other water deficit trials of grapevines where anthocyanin concentration increased in response to a berry size reduction (Williams & Matthews 1990; McCarthy, 1997a). However, the perception that berry size is the determining factor to altered anthocyanin or phenolic concentrations is debatable. Recent water deficit studies by Bindon (2004), Roby et al., (2004), Roby & Matthews (2004) and Walker et al., (2005) have questioned the assumption that berry size is the determining factor leading to increased anthocyanin and phenolic concentrations. Rather, these studies found that the effects of irrigation treatment on the final levels of anthocyanins and phenolics (seed and skin tannins) were independent of berry size. This suggests that while water deficit can have a direct effect on berry size (skin surface area/volume), a water deficit may also have an indirect effect on the synthesis of phenolic compounds due to possible skin thickness changes altering the number of phenolic producing cells.

In this study, anthocyanins and phenolics were measured as content (per berry) and concentration (per gram of fresh berry weight or skin) at harvest (calculations illustrated in section 5.2.1). These per berry measures were done on a homogenate of skin, pulp and seeds, thereby allowing anthocyanin and phenolic compounds to be measured simultaneously from the same sample, but at different wavelengths. The Cabernet Sauvignon vines, after three seasons of SDI irrigation showed no significant differences in anthocyanin (mg/berry) or phenolic (au/berry) content, but significant differences when expressed on a berry concentration (mg/g or au/g) basis (Table 5.2). Overall, the 43% SDI-treated Cabernet Sauvignon berries produced approximately 18% and 11% higher total anthocyanin concentration (mg/g) and total phenolic concentration (au/g) respectively than the 100%, 70% and 52% irrigation treatments. By contrast however, Shiraz vines subject to SDI did not necessarily produce higher concentrations of anthocyanins or phenolics (mg/g) in each season

even though berry size was reduced (Table 5.3). Furthermore while berry weight was reduced as less water was applied, the ratio of skin to berry weight was fairly uniform, (Chapter 4, Tables 4.1, 4.3). Calculations were performed using Cabernet Sauvignon berry data from the 2005 and 2006 harvests to determine mg anthocyanins per unit skin area (Appendix D, Table D.1). The Cabernet Sauvignon deficit (43%) vines tended to produce higher concentrations of total anthocyanins (mg/g) than the control, yet the concentration of anthocyanins per unit skin area did not follow the same trend. Furthermore, the concentration of anthocyanins (mg/g) tended to be higher in the deficit berries than the control berries. However, the differences in mg anthocyanins per unit skin area decreased for the deficit berries compared to the control berries. This indicates that despite a berry weight decrease there are probably other factors (i.e. irrigation treatment) that alter the final phenolic composition in the berry during the synthesis of these compounds.

In Roby et al., (2004), Cabernet Sauvignon vines were exposed to different irrigation regimes (high, control and low) that were created by applying different volumes of irrigation. When berries from each irrigation treatment were graded into a range of size categories, the content and concentration of anthocyanins and tannins in berries of the same size category were found to increase as less water was applied (Robey et al., 2004). Thus, the increase in concentration of skin anthocyanins and tannins could be solely attributed to the effect of irrigation treatment due to the berries having similar surface area: pulp volume ratios. Complimentary work by Roby & Matthews (2004) suggested the water deficit treatments could have affected skin anthocyanin and tannin levels by primarily altering the growth of the skin and inner mesocarp tissue. This caused the least irrigated berries to have a greater skin mass per berry and thus a larger number of skin cells where the anthocyanins would be localised.

5.5 Conclusions

Application of SDI treatments to commercial vineyards was able to alter phenolic composition in Cabernet Sauvignon and Shiraz berries over successive seasons. Knowing that SDI, even at a minimal level, may alter the synthesis and concentration of phenolic compounds in Cabernet Sauvignon and Shiraz berries raises a further question that is discussed in Chapter 6 as to how this may influence wine composition and quality.

- During berry ripening the development of total anthocyanins or total phenolics for Cabernet Sauvignon or Shiraz that were influenced by the SDI treatments, occurred irrespective of changes in berry weight. The concentrations of total anthocyanins and total phenolics during berry ripening and at harvest may have been to be more affected by variation in seasonal conditions or other factors, such as reduced canopy growth and increased light exposure of bunches, or possibly changes in phenolic synthesis (ABA levels altering athocyanin synthesis). At harvest the SDI treatments that applied irrigation at approximately 50% or less of the control for three seasons, had higher total anthocyanin concentrations in Cabernet Sauvignon and Shiraz berries by 22% and 15% respectively.

- Percent composition of the individual anthocyanidins only showed minimal differences between the control and SDI treatments. Total malvidin for both varieties tended to increase under SDI with total cyanidin having the lowest abundance. As less water was applied there was an increase in malvidin with less effect on delphinidin, peonidin, petunidin and cyanidin for Cabernet Sauvignon and peonidin, petunidin, delphinidin and cyanidin for Shiraz.

- In general total skin tannin concentrations tended to increase under SDI conditions being higher for the lowest irrigation levels. Furthermore the SDI-treated Cabernet Sauvignon appeared to produce a higher skin tannin concentration than the SDI-treated Shiraz that may be related to the water deficits influencing skin tannin synthesis between flowering and veraison and eventually extractability levels at harvest.

- Total skin flavonol concentration, that can be used to indicate the degree of sun-exposure of berries, tended to be higher in those irrigation treatments receiving the least irrigation, particularly for Cabernet Sauvignon.

- Given the consistency between skin weight to berry weight ratios between SDI treatments for both varieties, and the fact that the SDI treatments were applied all season, there is a strong likelihood the water deficits had an indirect effect (altering phenolic synthesis) on the final anthocyanin, tannin and phenolic levels, than berry size alone.

CHAPTER 6: Influence of SDI on Red Wine Composition

6.1 Introduction

Phenolic compounds in red wine are important indicators of the potential colour, sensory character and ageability of a red wine. A quest of the Australian grape and wine industry has been to utilise key phenolic constituents measured in the berry to predict these characteristics in red wine. Many compounds present in wine are undetectable in berries and tend to develop during the fermentation process (Nagel & Wulf, 1979; Sacchi et al., 2005). Consequently, trying to predict the potential quality attributes of a wine at the berry stage is difficult. Furthermore, the composition of phenolic compounds (anthocyanins, tannins) and subsequent conjugates in wine, while unique to each grape cultivar, can be influenced by vineyard management and climatic conditions (Matthews et al., 1990; Jackson & Lombard, 1993; Kennedy et al., 2002).

Anthocyanins and other phenolic compounds, i.e. tannins, are the major compounds that are commonly investigated in red wine. Anthocyanins are responsible for red wine colour, and diffuse into must and wine from the skins of red grapes during fermentation. However, highly coloured red grapes do not necessarily produce the most intensely coloured red wines (Jackson et al., 1978; Johnstone et al., 1996). This may be related to the extractability of anthocyanins from grape skins into the must (Gonzalez-Neves et al., 2004). Whether this is due to the fermentation conditions (Sims & Bates, 1994; Sipiora & Gutiérrez Grande, 1998) and/or other external conditions, such as water deficit or climate that in turn are influencing berry composition is not always clear. Furthermore, industry perception is that red wine "quality" is generally related to wine colour density, with lighter coloured red wines having lower flavour and less favourable mouth-feel characteristics than highly coloured red wines (Somers & Evans, 1974; Johnstone et al., 1996). In young red wines, anthocyanins can form interactions with themselves (self-association) or in a complex with phenolic compounds (procyanidins, flavonols, quercetin and caffeic acid) known as copigmentation (Boulton, 2001). This copigmentation leads to a shift in wine colour from red to purple due to the formation of complexes between the anthocyanins and copigments. Overall copigmentation can account for

30 to 50% of the colour in young red wines (Boulton, 2001) and an increase in long-term stability of wine colour (Somers, 1971).

Wine composition is the product of a dynamic process that is commonly altered by winemaking practices, but has been shown to also be influenced at the vineyard level (Freeman & Kliewer, 1983; McCarthy & Coombe, 1985; Bravdo & Hepner, 1987; Matthews et al., 1990). Since the adoption of strategic irrigation practices in Australia, it is well known that controlled water deficit will alter vegetative growth (McCarthy, 1998; Dry et al., 1999), berry development and maturation (McCarthy, 1997b; Roby et al., 2004) and photosynthesis and assimilate partitioning (Petrie et al., 2004; Loveys et al., 2005). Reduced canopy size, smaller berries and changes in berry composition, particularly increases in anthocyanin and other phenolic compounds are the direct result of vine water deficits (Matthews & Anderson, 1988; Medrano et al., 2003; Roby et al., 2004). While many irrigation trials have studied the influence of water deficits on berry composition, there is limited knowledge regarding the effects of vine water deficits on wine composition or the relationships between berry and wine composition (Bravdo et al., 1985; Hepner et al., 1985; Matthews et al., 1990; Sipiora & Gutiérrez Grande, 1998; Kennedy et al., 2002; Walker et al., 2005a). In Australia, there have been numerous studies into the effects of PRD and RDI on berry composition, but little research investigating the influence of deficit irrigation on wine quality (Stoll, 2000; Bindon, 2004). Research by Bindon (2004) suggested that improved wine composition and stability from PRD treated Shiraz could be due to an alteration in phenolic biosynthesis in the berry that leads to an increase in wine colour due to a change in the degree of copigmentation and polymerisation of the wine anthocyanins.

Given the demonstrated potential of SDI for altering vine physiology responses and berry weight and composition (Chapters 3, 4 and 5), the decision was made to investigate whether the differences in berry composition at harvest would be reflected in the wine composition after fermentation and early ageing. Furthermore, any changes in total colour of wines from SDI-treated vines may not be solely due to berry composition, but also to an increase in colour intensity due to copigmentation compounds. Consequently, the aim of this study was to test the hypothesis that differences in berry composition induced by SDI could translate into differences in wine quality of Cabernet Sauvignon and Shiraz grown in the Murray-Darling region.

6.2 Materials and Methods

6.2.1 Micro-wine ferments

The micro-ferment wines were made from grapes harvested in 2006 using a method adapted from the mini-lot fermentation methods published by the Australian Wine Research Institute, Adelaide, Australia (Holt et al., 2006). Micro-wines were made from bunches harvested between 23.5-24.7 ^{o}Brix (refer to Chapter 4 for actual ^{o}Brix range for each variety) that had been frozen at -20.0 ^{o}C. Micro-wines were compared at bottling and after 6 months ageing.

For logistical reasons it was impossible to make individual wines for each field replicate. Consequently for each irrigation treatment, bunches were collected from the field replicates and pooled together to be representative of an individual treatment as a whole across the vineyard. For each irrigation treatment, berries were plucked from all the bunches whilst frozen before random division into four wine replicates of approximately 800g per treatment and placed into individual zip lock bags to defrost overnight at room temperature. Before sealing the bags, 50 ppm (0.05 g/L) SO_2 (as potassium metabisulphite-PMS) was added to control oxidation and microbial growth (see below). The next day the bagged fruit was placed in a temperature controlled room set to 26^{o}C and left to equilibrate to temperature for approximately 4 h before being gently crushed by hand in the plastic bags. The crushed fruit was transferred to a pre-weighed ferment container and a 10.0 mL sample of the juice taken and placed in a plastic centrifuge tube in a water bath set at 35-40^{o}C for starting a yeast culture (see section following additions calculations). Immediately after crushing additions of 50 ppm (0.05 g/L) SO_2 (as PMS), 100 ppm (0.1 g/L) Diammonium Phosphate (DAP) and 1.5 g/L tartaric acid (H_2T- to adjust pH to 3.5) were made, followed by the yeast culture. Initially only 50% of the calculated amount of H_2T was added at this stage and the pH checked. The pH was re-checked and a further addition of H_2T was made if required. The ferment room was kept at a constant temperature of 26^{o}C with adequate ventilation to prevent accumulation of CO_2 gas emissions. Calculations for the volumes required for each of these additions, based on individual ferment juice volumes are given below:

SO_2 additions (8% SO_2)

Volume (µL) of 8% $SO_{2\ added}$ = 50 ppm x (juice volume mL/1000) x (1000/40,000 ppm*) x 10^3

* PMS is equivalent to 50% SO_2, therefore an 8% (w/v = 80,000 ppm) solution of PMS is equivalent to 40,000 ppm SO_2.

DAP additions (8% DAP=80,000 ppm)

Volume (µL) of 8% DAP_{added} = 100 ppm x (juice volume mL/1000) x (1000/80,000 ppm) x 10^3

Tartaric acid additions (pH=3.5)

Volume (µL) of H_2T_{added} = 1.5 g/L x (juice volume mL/1000) x 0.1 x 10^3

Yeast additions

mg of $yeast_{to\ ferment}$ = 0.15 g x (juice volume mL/1000) x 10^3

A yeast (*Saccharomyces cerevisiae*- strain ICV254D, Lavlin, France) starter culture was made. Dry yeast was sprinkled on top of 1.0 mL of distilled water that was warmed to 35-40^{o}C and allowed to incubate for 10 min. Rates of yeast addition (0.15 g/L) were determined from the manufacturer's recommendation for final yeast additions to the ferments. Once bubbling was observed, 10.0 mL of warmed grape juice was gradually added to the yeast suspension and gently mixed. This culture was then incubated in the water bath for 20 min. The culture was poured over the top of the ferment and not mixed into the crushed grapes until the next day to allow the yeast time to multiply. The yeast culture was the last addition to be made to the crushed grapes. Ferments were weighed and the gas traps attached. The gas traps contained an 8% SO_2 solution and were inserted in the lids to allow excess CO_2 to escape from the ferment container while preventing any oxygen or microbial contamination.

Ferments were stored at 26^{o}C until the end of fermentation or when the ferment reached 2.5 g/L sugar. At the start of each day the ferments were weighed, temperature, pH and ^{o}Brix recorded and the fruit plunged to ensure consistent contact between skins and juice (Figure 6.1). Once the ferment TSS had dropped by 10 ^{o}Brix, remaining sugar was measured using Clinitest® tablets. After five days maceration, the fermented must was pressed to remove the

skins and seeds and the juice transferred to a 250 mL Schott bottle to complete fermentation. These bottles were capped with a rubber bung and ferment trap containing 8% SO_2 and stored at 26^{o}C (Figure 6.1).

At the end of fermentation the wine was racked off the gross lees into a clean 250 mL Schott bottle leaving the layer of dead yeast cells behind. A further 50 ppm (0.05 g/L) of SO_2 was added. Any ullage (air gap between wine and top of bottle) was removed by adding clean glass marbles to the bottle before sealing. The wine was then stored for 14 days at 4^{o}C for cold settling. At the end of this cold settling period the wine was racked again by decanting into the final bottles with the addition of a further 50 ppm (0.05 g/L) SO_2 (Figure 6.1). Before the SO_2 was added a 10.0 mL sample of wine was taken for wine spectral measures.

Figure 6.1 Grapes were crushed and fermented in plastic containers with gas traps (above left). Ferments were plunged daily to ensure even skin contact during the maceration process (above right). Wine was pressed off into glass Schott bottles and capped with gas traps to allow the gross lees to settle before racking and cool storage (below left). After 2 weeks cool storage the wine was bottled (below right).

6.2.2 Wine analyses

Spectrophotometric and HPLC analyses were performed on micro-ferment wines made from Cabernet Sauvignon and Shiraz grapes from each sustained deficit irrigation treatment and controls. Total wine colour and total phenolic levels (Somers and Evans, 1977; Iland et al., 2004), copigmentation complexes (Levengood, 1996; Lambert, 2002) and tannin levels (Harbertson et al., 2002; Downey & Adams, 2005) were determined spectrophotometrically using the methods and equations developed by the cited authors and as described in section 6.2.

Red wine colour composition (Somers & Evans, 1977; Iland et al., 2004) and copigmentation complexes (Levengood, 1996; Lambert, 2002) were measured using a spectrophotometer (GBC UV-Visible Cintra 10e, GBC, Sydney, Australia) in either 10 or 2 mm cuvettes. The spectral bandwidth was set to 1.5 nm. Measurements performed using the 2 mm cuvettes, were multiplied by 5 to correct for pathlength difference. For the composition measures the wine was adjusted to pH 3.5, whereas for the copigmentation measures the wine was adjusted to pH 3.6 by adding either 1M NaOH or 1M HCl. HPLC wine anthocyanin profiles were determined using the methods and equipment outlined in Chapter 5 (Section 5.2.3).

HPLC wine analysis:

For wine, a 500 µL sample was placed into an eppendorf tube and centrifuged for 10 min at 13,000 x g (Eppendorf 5415D, Eppendorf, Sydney, Australia). A 200 µL sample of supernatant was aliquoted into a HPLC autosampler vial. The anthocyanin concentration was determined using a standard curve prepared from malvidin-3-*O*-glucoside standard (Extrasynthese, Ganay, France) and expressed as mg of malvidin-3-*O*-glucoside equivalents per litre of wine (Figure 6.2). Figure 5.1A (section 5.2.3) illustrates the separation and identification of the main anthocyanins measured in the wine samples

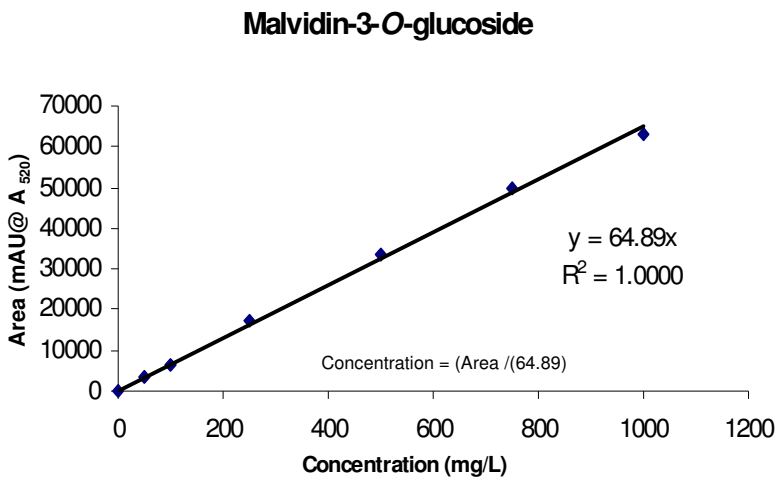


Figure 6.2 Standard curve for calculating concentration of mg of malvidin-3-*O*-glucoside equivalents per litre of wine extract.

Total tannin analysis for red wine:

The tannin extraction method for red wines is similar to the method for skin tannin extraction as described in section 5.2.4. The difference being that the wine sample is diluted 1:1 using Buffer A (model wine) (Appendix C). A 500 µL aliquot of the diluted wine sample is then added to 1.0 ml of protein solution (Appendix C). From this point on the protocol is identical to the skin tannin method in section 5.2.4.

Spectral measures for red wine colour composition:

At each sampling point, spectrophotometric analysis of wines were performed using the methods and calculations by Somers and Evans (1977) and Iland et al., (2004) as outlined below. Appendix E (Table E.1) provides definitions for each measure of red wine colour composition, as well as an indication of the value range for each analysis for Cabernet Sauvignon and Shiraz wines.

Methods for red wine colour composition

- **A_{520} and A_{420}**; Add 250 µL wine to a 2 mm glass cuvette. Read absorbance at 520 nm and 420 nm.

- $\mathbf{A^{HCl}}$; Transfer 10.0 mL of 1M HCl to glass test tubes. Add 100 µL of wine, mix and incubate at room temperature for 3h away from direct light. Read at 520 nm and 280 nm in 10 mm cuvettes. The reading is corrected for dilution by multiplying by 101.
- $\mathbf{A^{acet}}$; Transfer 2.0 mL of wine to a glass test tube. Add 20 µL of 10% acetaldehyde, mix and incubate at room temperature for 45 min. Read at 520 nm and 420 nm using 2 mm glass cuvettes.
- $\mathbf{A^{SO_2}}$; Transfer 2.0 mL wine to a glass test tube. Add 30 µL of 25% sodium metabisulphite, mix and stand for 1 min. Read at 520nm and 420nm using 2mm cuvettes.
- **10% acetaldehyde**; Add 3.2 mL of acetaldehyde (>99%) to 10 mL distilled water and make up to 25 mL in a volumetric flask.
- **25% sodium metabisulphite**; Weigh 6.25 g of sodium metabisulphite and dissolve in 25 mL of distilled water in volumetric flask.

Calculations for red wine colour composition

- Wine colour density (au) measure describes the concentration of red coloured pigments (A_{520}) and yellow/brown pigments (A_{420}) of a wine.

$= (A_{520} + A_{420})$

- Wine colour hue describes the hue (brownness) of a wine

$= (A_{420} / A_{520})$

- Total anthocyanins (mg/L) is an estimate of the concentration of anthocyanins in a wine

$= 20 \times (A^{HCl}_{520} - 5/3 \times A^{SO_2}_{520})$

- Ionised anthocyanins (mg/L) are the concentration of ionised anthocyanins in a wine.

$= 20 \times (A_{520} - A^{SO_2}_{520})$

- Degree of red pigment colouration (%) provides a percentage of the total wine pigments that are coloured.

$= (A_{520} / A^{HCl}_{520}) \times 100$

- Total red pigments (au) gives an estimate of the concentration of all the coloured anthocyanins, oligomers and polymers in a wine.

$= A^{HCl}_{520}$

- Total phenolics (au) is an indicator of the concentration of all phenolic material in a wine.

$= (A^{HCl}_{280} - 4)$

Copigmentation measures for red wine complexes:

At each sampling point spectrophotometric analyses of wines were performed using the methods and calculations outlined below (Levengood, 1996; Lambert, 2002).

Methods for red wine copigmentation

- **A^{100}** ; Transfer 10 mL acidic buffer to a glass test tube. Add 100 µL wine sample and incubate at room temperature for 3h. Read at 520 nm in a 10 mm cuvette. The reading was corrected for dilution by multiplying by 101.
- **A^{acet}** ; Transfer 2.0 mL of wine to a glass test tube. Add 20 µL of 10% acetaldehyde, mix and incubate at room temperature for 45 min. Read at 520 nm using 2 mm glass cuvettes. The reading was corrected for dilution by multiplying by 5.
- **A^{20}** ; Add 100 µL of wine sample to 1.9 mL wine condition buffer to a glass test tube, mix and read at 520 nm in a 10 mm cuvette. The reading was corrected for dilution by multiplying by 20.
- **A^{SO_2}**; Transfer 2.0 mL wine to a glass test tube. Add 30 µL of 25% sodium metabisulphite, mix and stand for 1 min. Read at 520 nm and 420 nm using 2 mm cuvettes. The reading was corrected for dilution by multiplying by 5.
- **Acidic buffer**; Add 47.14 mL of 32% HCl and 60 mL absolute ethanol to distilled water and make up to 500 mL.
- **Wine condition buffer**; Add 1.25 g potassium hydrogen tartrate (KHT) and 60 mL absolute ethanol to distilled water and make up to 500 mL.

Calculations for red wine copigmentation

- Concentration of monomeric anthocyanins (mg/L)

  $[A] = ((A^{100}-A^{SO_2})/E^{A}) - [C]$
- Concentration of anthocyanins in copigment complex (mg/L)

  $[C] = (A^{acet} - A^{20})/(E^{C} - (E^{A} \times frac))$
- Colour of anthocyanins in the polymeric pigments (au)

  $[P] = A^{SO_2}$
- Total red colour (au)

  $TC = (E^{A} \times [A] \times frac) + (E^{C} \times [C]) + [P]$

- % colour due to anthocyanins
  = (E^A x [A] x *frac*)/TC x 100
- % colour due to copigment complex
  = (E^C x [C])/TC x 100
- % colour due to polymeric pigments
  = [P]/TC x 100

where:

E^A = 0.066 au/mg; based on the molar absorptivity and molecular weight of malvidin-3-glucoside (Linskens & Jackson, 1988)

E^C = 0.198 au/mg; based on the enhancement of using pelargonidin-3-glucoside in an aqueous solution (Davies & Mazza, 1993)

frac = 0.086; estimate from the dissociation curve for malvidin using pKa values (Brouillard & Delaporte, 1977)

6.3 Results

6.3.1 Changes in red wine colour composition of SDI-treated wines

Cabernet Sauvignon:

Spectral results of wines made from Cabernet Sauvignon grapes exposed to SDI treatments are presented in Figure 6.3 and Table 6.1. Wine colour density was higher at bottling than after 6 months ageing for all irrigation treatments (Figure 6.3A). The 43% SDI-treated wines had significantly higher wine colour density levels than the other irrigation treatments at both bottling and after 6 months ageing. Wine hue was significantly higher in the 52% SDI wines than the other irrigation treatments at 6 months age (Figure 6.3B). Total anthocyanin concentrations were significantly higher in the control, 52% and 43% SDI wines compared to the 70% SDI treatment at bottling (Figure 6.3C). After 6 months, the total anthocyanin concentrations increased significantly with increasing intensity of SDI (anthocyanin concentration was significantly higher in the 43% than 52% > 70% > 100% wines) compared to the control (Figure 6.3C). Generally, the pattern of total anthocyanin concentrations had increased after 6 months ageing in all treatments. Total phenolic concentrations also increased significantly with increasing intensity of SDI after 6 months ageing (Figure 6.3D). The 43% SDI wines had significantly higher total phenolic concentrations compared to the other treatments at bottling and after 6 months.

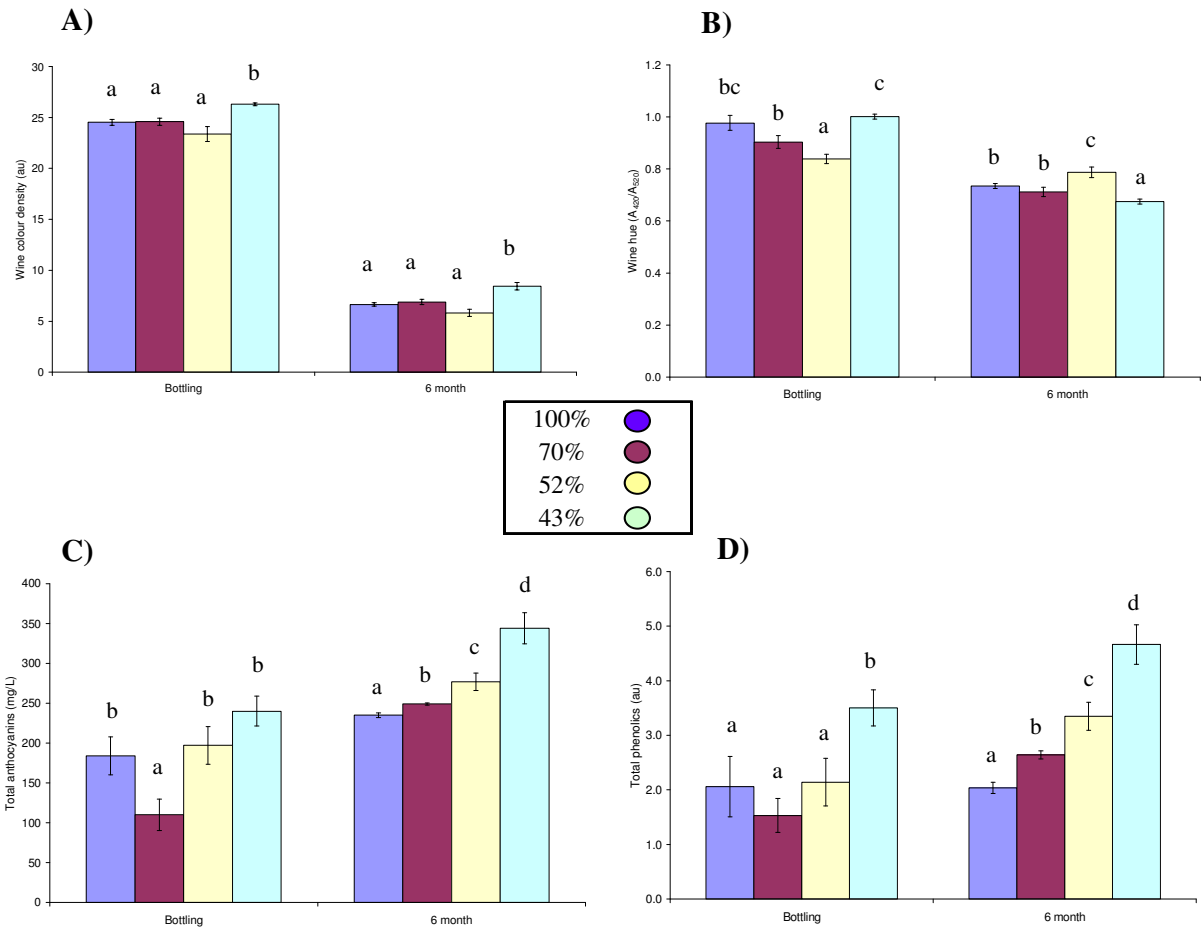


Figure 6.3 Development of wine colour from Cabernet Sauvignon micro-ferments at bottling and 6 month age for A) wine colour (au), B) wine hue (A_{420}/A_{520}), C) total anthocyanins (mg/L) and D) total phenolics (au). Grapes were collected at the 2006 harvest from Control (100%), 70%, 52% and 43% SDI treatments. Means at bottling or 6 months followed by the same letter are not significantly different at P=0.05. Data are represented as mean values ± s.e. (n=4).

For Cabernet Sauvignon, total red pigments were significantly higher in the 43% SDI wines than the control at both bottling and 6 months ageing (Table 6.1). The degree of red pigment colouration was significantly higher in the 70% SDI wines compared to the other irrigation treatments at bottling (Table 6.1). However, the degree of red pigmentation decreased across all irrigation treatments, with the 52% SDI wines having a significantly lower percentage of red pigments than the other irrigation treatments after 6 months age. Ionised pigments were significantly higher for all the SDI treatments compared to the control at bottling (Table 6.1). After 6 months ageing the concentration of ionised pigments had decreased for all irrigation treatments, with the 52% SDI wine having a significantly lower concentration of ionised pigments than the other irrigation treatments.

Table 6.1 Concentration of total red pigments (au), degree of red pigment colouration (%) and ionised pigments (mg/L) from Cabernet Sauvignon micro-ferments at bottling and 6 month age. Grapes were collected at the 2006 harvest from Control (100%), 70%, 52% and 43% SDI treatments. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4).

2006 Cabernet Sauvignon	**Irrigation Treatments**			
Spectral wine	**100%**	**70%**	**52%**	**43%**
BOTTLING				
Total red pigments (au)	19.4[b]	15.2[a]	19.6[b]	25.0[c]
Degree of red pigment colouration (%)	51.5[a]	86.2[b]	65.2[a]	52.9[a]
Ionised pigments (mg/L)	77.9[a]	132.5[b]	136.2[b]	107.5[b]
6 MONTHS				
Total red pigments (au)	15.5[a]	17.0[b]	18.8[b]	23.2[c]
Degree of red pigment colouration (%)	24.7[b]	23.6[b]	17.4[a]	21.8[b]
Ionised pigments (mg/L)	31.4[b]	25.7[b]	11.7[a]	28.9[b]

Shiraz:

Spectral results of wines made from Shiraz grapes exposed to SDI treatments are presented in Figure 6.4 and Table 6.2. Wine colour density was significantly lower at bottling for the 65% SDI wines than the other irrigation treatments (Figure 6.4A). However at 6 months, the 34% SDI wines produced significantly higher wine colour density than the other irrigation treatments (Figure 6.4A). Wine hue was significantly lower for the 100% and 45% SDI wines compared to the 70% and 34% SDI treatments at bottling and 6 months (Figure 6.4B). Total anthocyanin concentrations were significantly higher in the 45% and 34% SDI wines than the control at bottling (Figure 6.4C). After 6 months, the SDI wines had significantly higher total anthocyanin concentrations than the control (Figure 6.4C). Total phenolic concentrations showed no significant differences between irrigation treatments at bottling (Figure 6.4D). However after 6 months, total phenolic concentrations increased significantly with increasing intensity of SDI (total phenolic concentration was significantly higher in the 34% than 45% > 65% > 100% wines) compared to the control (Figure 6.4D).

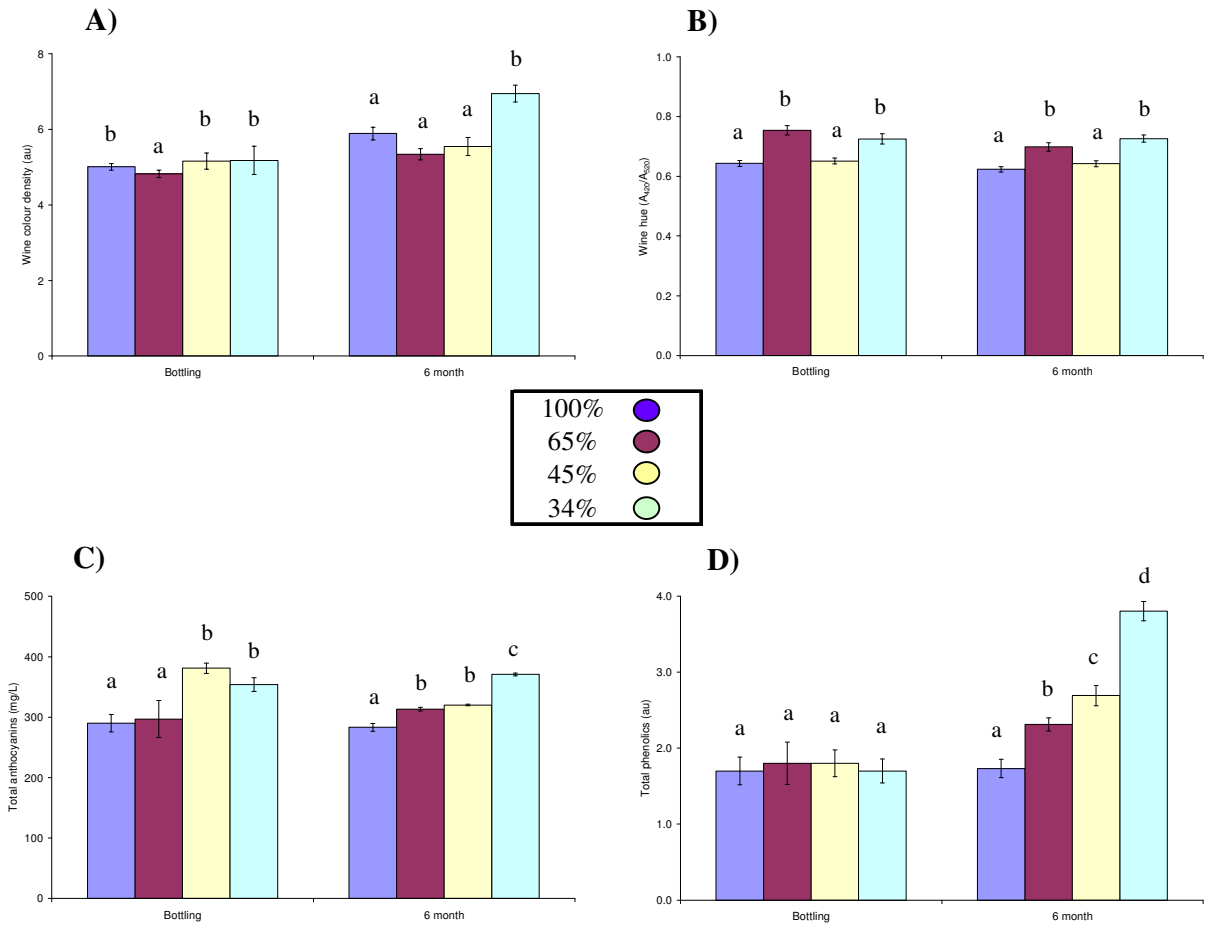


Figure 6.4 Development of wine colour from Shiraz micro-ferments at bottling and 6 month age for A) wine colour (au), B) wine hue (A_{420}/A_{520}), C) total anthocyanins (mg/L) and D) total phenolics (au). Grapes were collected at the 2006 harvest from Control (100%), 65%, 45% and 34% SDI treatments. Means at bottling or 6 months followed by the same letter are not significantly different at P=0.05. Data are represented as mean values ± s.e. (n=4).

For Shiraz, total red pigments showed no significant differences between irrigation treatments at bottling, but after 6 months ageing were significantly higher in the SDI wines than the control (Table 6.2). The degree of red pigment colouration was significantly higher in the control wines than the SDI wines after 6 months ageing (Table 6.2). Ionised pigments were significantly lower across all SDI wines than the control wine after 6 months ageing (Table 6.2). The concentration of ionised pigments was significantly lower for the SDI wines than the control after 6 months ageing.

Table 6.2 Concentration of total red pigments (au), degree of red pigment colouration (%) and ionised pigments (mg/L) from Shiraz micro-wines at bottling and 6 month age. Grapes were collected at the 2006 harvest from Control (100%), 65%, 45% and 34% SDI treatments. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4). No letters signify ns between irrigation treatments within a row.

2006 Shiraz	**Irrigation Treatments**			
Spectral wine	**100%**	**65%**	**45%**	**34%**
BOTTLING				
Total red pigments (au)	16.1	16.4	20.7	19.5
Degree of red pigment colouration (%)	20.7	17.9	15.2	16.6
Ionised pigments (mg/L)	42.3	36.8	42.8	42.7
6 MONTHS				
Total red pigments (au)	16.5[a]	18.4[b]	19.2[c]	22.8[d]
Degree of red pigment colouration (%)	22.1[b]	17.1[a]	15.3[a]	17.7[a]
Ionised pigments (mg/L)	44.6[b]	29.5[a]	20.3[a]	29.7[a]

6.3.2 HPLC anthocyanidin profiles of SDI-treated wines

Cabernet Sauvignon:

HPLC analyses of the Cabernet Sauvignon wines at bottling and after 6 months ageing are presented in Table 6.3. The parent anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, malvidin) were all significantly higher in the 43% SDI wines than the other irrigation treatments at bottling and 6 months ageing (Table 6.3). Total malvidin was significantly more abundant at bottling and 6 months age than the other parent anthocyanidins and was significantly higher in the 43% SDI wines than the control. The concentrations of dihydroxylated anthocyanidins did not vary much between bottling and 6 months age, whereas the trihydroxylated anthocyanidins were much lower after 6 months ageing than at bottling. After 6 months, the 43% SDI wines had significantly higher concentrations of di- and trihydroxylated anthocyanidins than the control wines. For all irrigation treatments, total -3-*O*-p-coumaroylglucosides were the least abundant followed by total -3-*O*-acetylglucoside and total -3-*O*-glucosides. Total anthocyanins were significantly higher in the 43% SDI wines than the other irrigation treatments at bottling and 6 months. After 6 months ageing the 43% SDI wine had 20% more total anthocyanins than the control wines.

Table 6.3 Concentration of total wine anthocyanins and parent anthocyanidin, di- and trihydroxylated anthocyanidin, and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanidins from Cabernet Sauvignon wines expressed as mg/L of wine at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 70%, 52% and 43% SDI treatments. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4).

2006 Cabernet Sauvignon HPLC wine (mg/L)	**Irrigation Treatments**			
	100%	**70%**	**52%**	**43%**
BOTTLING				
Total Delphinidin	6.9[a]	6.8[a]	6.5[a]	8.9[b]
Total Cyanidin	2.2[a]	2.3[a]	1.9[a]	2.7[b]
Total Petunidin	7.6[a]	7.6[a]	7.1[a]	9.6[b]
Total Peonidin	6.4[b]	6.0[b]	5.9[a]	7.1[c]
Total Malvidin	194.3[a]	192.2[a]	188[a]	223.8[b]
Total Dihydroxyylated (Cy + Peo)	8.6[b]	8.3[b]	7.7[a]	9.9[c]
Total Trihydroxylated (Del + Pet + Mal)	208.8[a]	206.5[a]	201.5[a]	242.3[b]
Total -3-*O* -glucoside	128.7[a]	129.1[a]	124.6[a]	149.7[b]
Total -3-*O* -acetylglucoside	77.9[a]	75.7[a]	74.4[a]	89.0[b]
Total -3-*O* -p-coumaroylgluciside	10.7[a]	10.1[a]	10.4[a]	13.5[b]
Total Anthocyanins (mg/L)	217.3[a]	214.8[a]	209.3[a]	252.2[b]
6 MONTHS				
Total Delphinidin	9.2[a]	9.7[a]	8.1[a]	12.2[b]
Total Cyanidin	5.5[ab]	5.9[bc]	4.0[a]	6.1[c]
Total Petunidin	2.5[a]	2.4[a]	2.5[a]	3.6[b]
Total Peonidin	2.2[a]	2.0[a]	2.3[a]	2.8[b]
Total Malvidin	76.5[a]	70.1[a]	79.5[a]	96.3[b]
Total Dihydroxyylated (Cy + Peo)	7.7[b]	8.0[bc]	6.3[a]	8.9[c]
Total Trihydroxylated (Del + Pet + Mal)	88.2[a]	82.1[a]	90.0[a]	112.2[b]
Total -3-*O* -glucoside	53.8[a]	50.6[a]	55.7[ab]	68.6[b]
Total -3-*O* -acetylglucoside	38.9[a]	36.7[a]	37.4[a]	47.8[b]
Total -3-*O* -p-coumaroylgluciside	3.1[a]	2.8[a]	3.2[a]	4.7[b]
Total Anthocyanins (mg/L)	95.9[a]	90.1[a]	96.3[a]	121.1[b]

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

Shiraz:

HPLC analyses of the Shiraz wines at bottling and after 6 months ageing are presented in Table 6.4. There were no significant differences in the concentration of parent anthocyanidins between irrigation treatments at bottling. Furthermore after 6 months there were only a few significant differences between irrigation treatments for total delphinidin and total petunidin. Total malvidin concentrations were significantly higher in the 34% SDI wines than the control

wines at bottling. However after 6 months there were no significant differences between malvidin levels for any irrigation treatment. Total trihydroxylated and total-3-O-glucosides concentrations were significantly higher for the 65% and 34% SDI wines than the 45% SDI wine at bottling. However after 6 months ageing these differences were no longer apparent. There were no significant differences between total anthocyanin concentrations for any irrigation treatment at bottling or 6 months age. Overall the concentration of total anthocyanins decreased between bottling and 6 months.

Table 6.4 Concentration of total wine anthocyanins and anthocyanidin derived anthocyanins, dihydroxylated and trihydroxylated anthocyanins, and -3-*O*-glucoside, acetylglucoside, and coumaroylglucoside anthocyanins from Shiraz wines expressed as mg/L of wine at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 65%, 45% and 34% SDI treatments. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4).

2006 Shiraz	**Irrigation Treatments**			
HPLC wine (mg/L)	**100%**	**65%**	**45%**	**34%**
BOTTLING				
Total Delphinidin	8.9[a]	7.3[a]	8.6[a]	8.5[a]
Total Cyanidin	0.7[a]	1.8[a]	0.7[a]	0.6[a]
Total Petunidin	3.2[a]	10.4[a]	4.3[a]	3.0[a]
Total Peonidin	7.8[a]	9.8[a]	10.7[a]	8.9[a]
Total Malvidin	168.5[ab]	179.7[bc]	153.5[a]	181.8[c]
Total Dihydroxyylated (Cy + Peo)	9.5[a]	11.3[a]	11.5[a]	8.5[a]
Total Trihydroxylated (Del + Pet + Mal)	180.0[ab]	192.6[b]	171.2[a]	193.9[b]
Total -3-*O* -glucoside	87.3[b]	89.8[b]	75.0[a]	92.8[b]
Total -3-*O* -acetylglucoside	68.0[a]	73.3[a]	64.2[a]	67.7[a]
Total -3-*O* -p-coumaryoylgluciside	34.2[a]	40.9[a]	43.6[a]	42.0[a]
Total Anthocyanins (mg/L)	189.5[a]	204.0[a]	182.8[a]	202.5[a]
6 MONTHS				
Total Delphinidin	8.8[b]	7.5[a]	9.0[b]	8.0[ab]
Total Cyanidin	2.3[a]	1.6[a]	2.1[a]	2.0[a]
Total Petunidin	1.0[a]	1.0[a]	1.4[b]	1.0[a]
Total Peonidin	4.6[a]	4.8[a]	6.0[a]	4.5[a]
Total Malvidin	85.4[a]	75.5[a]	89.1[a]	81.5[a]
Total Dihydroxyylated (Cy + Peo)	6.8[a]	6.4[a]	8.1[b]	6.5[a]
Total Trihydroxylated (Del + Pet + Mal)	95.1[a]	83.9[a]	99.4[a]	90.6[a]
Total -3-*O* -glucoside	46.9[a]	40.4[a]	49.0[a]	45.8[a]
Total -3-*O* -acetylglucoside	35.4[a]	31.8[a]	37.6[a]	34.6[a]
Total -3-*O* -p-coumaryoylgluciside	19.6[a]	18.2[a]	20.9[a]	16.7[a]
Total Anthocyanins (mg/L)	101.9[a]	90.3[a]	107.5[a]	97.1[a]

(Cy = Cyanidin; Del = Delphinidin; Pet = Petunidin; Peo = Peonidin; Mal = Malvidin)

6.3.3 Changes in tannin concentration of SDI-treated wines

For Cabernet Sauvignon, wine tannin concentration was significantly higher in the 52% SDI wine at bottling than the other irrigation treatments (Table 6.5). After 6 months ageing, the control and 52% SDI wines had significantly higher tannin concentrations than the 70% and 43% SDI wines. For Shiraz, the control wines had significantly lower tannin concentrations than the other irrigation treatments at bottling and 6 months ageing. For both varieties, the tannin concentrations were similar for each irrigation treatment between bottling and 6 months ageing.

Table 6.5 Concentration of total wine tannins from Cabernet Sauvignon and Shiraz grapes harvested in 2006 from Control (100%) and SDI treatments expressed as mg of catechin equivalent/L of wine. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4).

Total Wine Tannin (mg CE/L)	**Irrigation Treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
Bottling	242^{b}	213^{a}	269^{c}	223^{ab}
6 month age	246^{b}	217^{a}	249^{b}	215^{a}
Shiraz	**100%**	**65%**	**45%**	**34%**
Bottling	8^{a}	82^{b}	113^{b}	99^{b}
6 month age	15^{a}	85^{b}	111^{b}	98^{b}

6.3.4 Influence of SDI on wine copigmentation

Figure 6.5 illustrates the relationship between irrigation water applied (ML/ha) and various copigmentation complexes from the Cabernet Sauvignon and Shiraz micro-ferment wines at 6 months age. The relationship between volume of irrigation water applied and concentration of monomeric anthocyanins varied between the varieties (CS, R^2=0.0092; SHZ, R^2=0.4277) (Figure 6.5A). There was a weak relationship between the volume of irrigation applied and concentration of copigment anthocyanins for either variety (CS, R^2=0.0768; SHZ, R^2=0.0026) (Figure 6.5B). Alternatively, there was a strong, negative relationship between the volume of irrigation applied and the concentration of polymeric pigment (CS, R^2=0.933; SHZ, R^2=0.887) contributing to the total colour (Figure 6.5C). Likewise, total colour increased with increasing

intensity of SDI for both varieties (CS, R^2=0.887; SHZ, R^2=0.865) (Figure 6.5D). Despite having similar concentrations of monomeric and copigment anthocyanins, the Cabernet Sauvignon wines tended to produce more polymeric pigments and have higher total colour levels than the Shiraz wines.

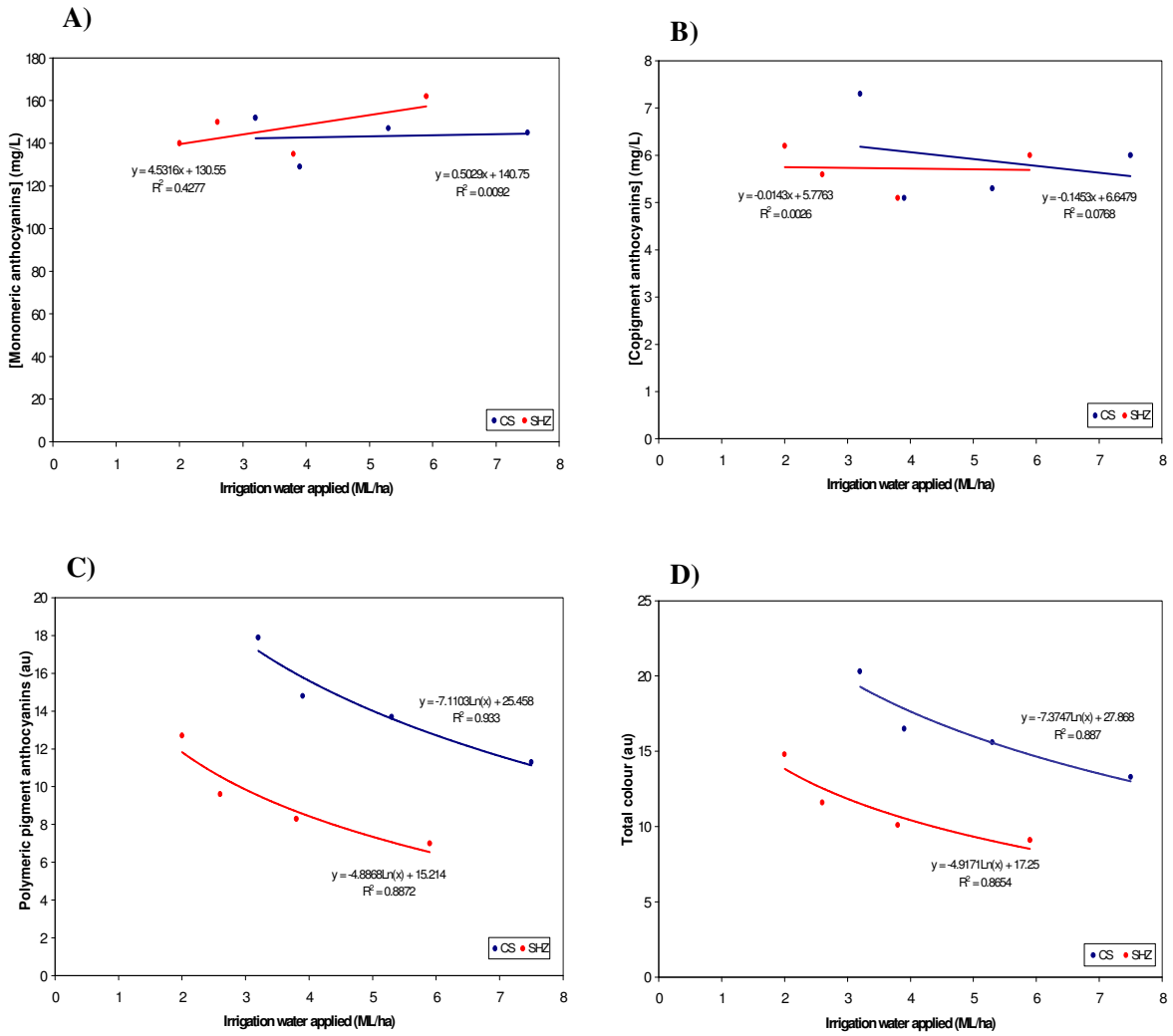


Figure 6.5 Regression analyses of monomeric anthocyanins, anthocyanins in the copigment complex, polymeric pigments and total colour against the amount of irrigation water applied for Cabernet Sauvignon (CS) and Shiraz (SHZ) wines after 6 months. A) monomeric anthocyanins, B) copigment anthocyanins, C) polymeric pigment anthocyanins and D) total colour. (n=4)

The distribution of monomeric, copigment complex and polymeric pigment anthocyanins in the wines was different for each variety between bottling and 6 months ageing (Figures 6.6,

6.7). For Cabernet Sauvignon at bottling, the greatest proportion of anthocyanins tended to be in the copigment complex, followed by polymeric pigment and monomeric anthocyanin forms for all treatments (Figure 6.6). After 6 months ageing this pattern had reversed and the greatest proportion tended to be as polymeric pigment anthocyanins, followed by the copigment complex and monomeric anthocyanins (Figure 6.6). After 6 months the proportion of polymeric pigment anthocyanins was higher in the SDI wines than the control.

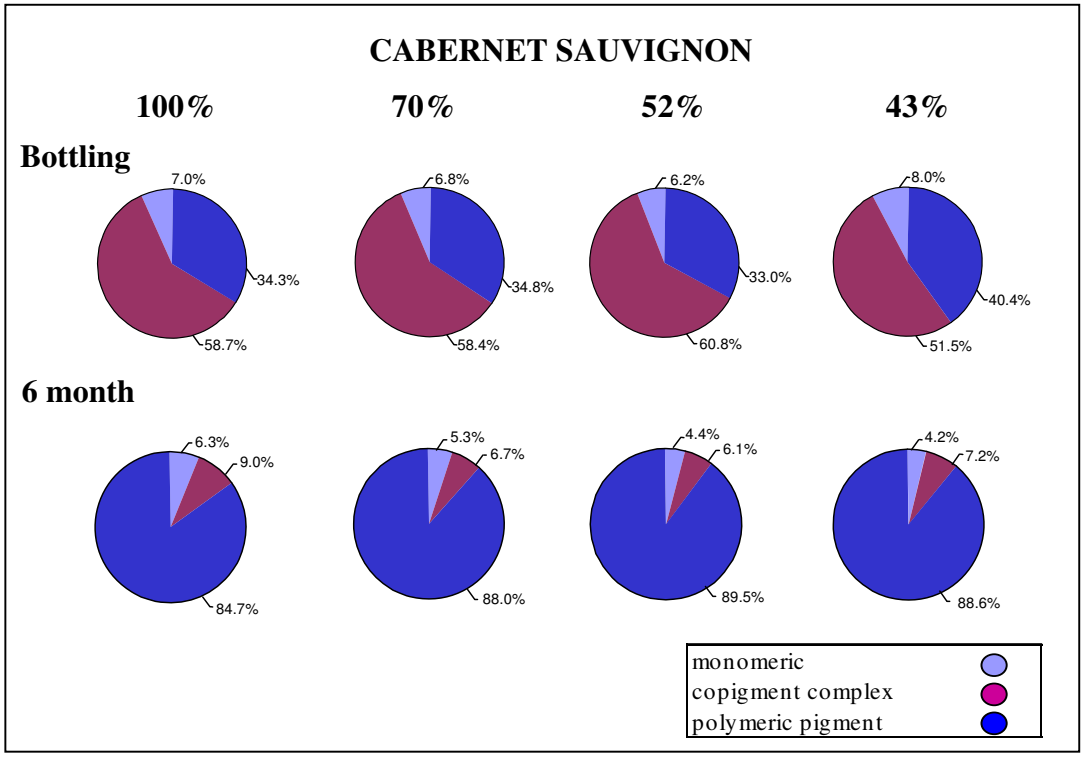


Figure 6.6 Percent colour due to monomeric, copigment complex and polymeric pigment anthocyanins of Cabernet Sauvignon wines at bottling and 6 month age. Grapes were collected at the 2006 harvest from control (100%), 70%, 52% and 43% SDI treatments.

For Shiraz at bottling, the greatest proportions of anthocyanins were in the copigment complex, followed by monomeric and polymeric pigment anthocyanins in all treatments (Figure 6.7). After 6 months ageing this pattern had reversed and the greatest proportion was polymeric pigment anthocyanins followed by the copigment complex and monomeric anthocyanins in all treatments (Figure 6.7). After 6 months ageing, the control wines tended to produce higher proportions of monomeric and copigment anthocyanins than the SDI treatments, whereas the SDI treatments had higher proportions of polymeric pigment anthocyanins than the control.

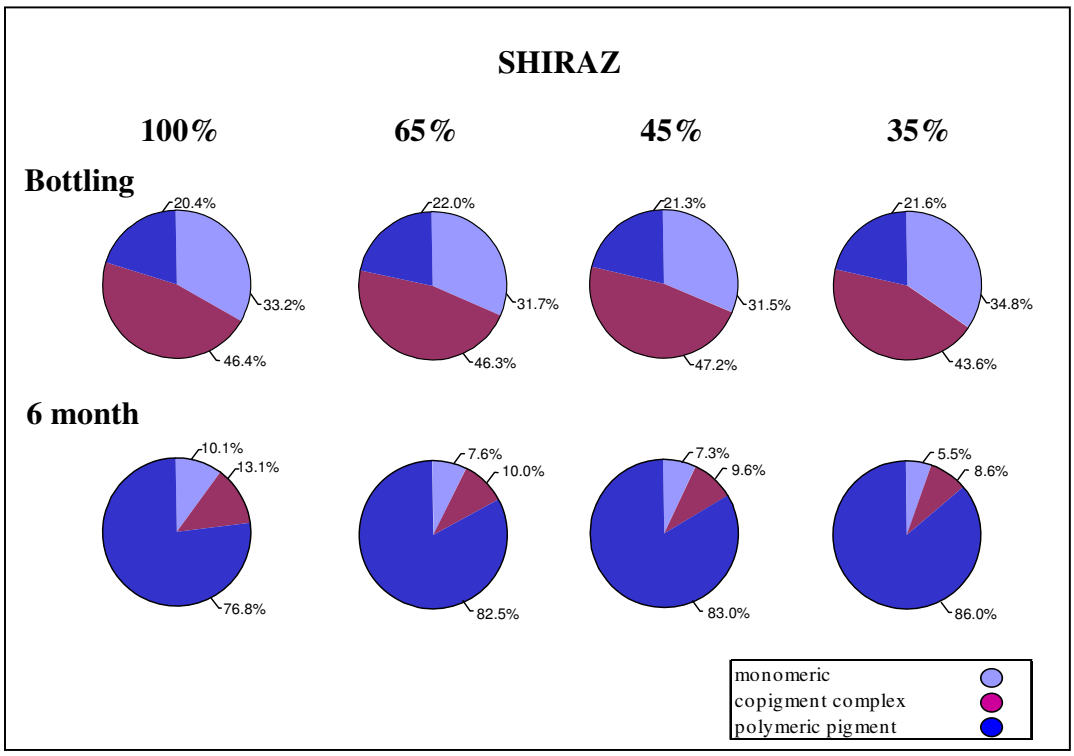


Figure 6.7 Percent colour due to monomeric, copigment complex and polymeric pigment anthocyanins of Shiraz wines at bottling and 6 month age**.** Grapes were collected at the 2006 harvest from control (100%), 65%, 45% and 34% SDI treatments.

6.4 Discussion

To explore the influence of SDI on wine composition, a series of micro-ferments were conducted on grapes from the 2006 vintage according to the methods developed by Holt et al., (2006). Certain fermentation conditions, such as skin contact time, fermentation temperature, ferment pH, yeast strain and ratio of skins to must have been found to augment or diminish the differences in wine colour (anthocyanins) and phenolic composition (Singleton, 1972; Scudamore-Smith et al., 1990; Sims & Bates, 1994; Sipiora & Gutiérrez Grande, 1998). To ensure any differences in wine composition were the result of the irrigation treatments all micro-ferments were maintained at similar ferment temperature and pH level, fermented using the same yeast strain, macerated on skins for five days and bottled on the same day.

6.4.1 Wine colour

For both varieties there were significant changes in the wine colour levels with increasing intensity of SDI. Wine colour density was significantly increased in the 43% and 34% SDI wines for Cabernet Sauvignon and Shiraz respectively after 6 months ageing. Likewise the total anthocyanin and total phenolic concentrations of all wines after 6 months ageing were significantly increased with reduced water application applied for both varieties by comparison with bottling. These changes in wine composition are similar to results observed in a study by Matthews et al., (1990) on Cabernet Franc exposed to different irrigation treatments (continual irrigation, early deficit and late deficit). In that study wines made from grapes exposed to deficit irrigation had the highest wine colour density and greatest concentration of total anthocyanins and total phenolics compared to the continually irrigated vines (Matthews et al., 1990). The authors also suggested that the timing of the water deficit effected the wine composition. The early deficit irrigation treatments (water withheld before veraison) produced wines with higher concentrations of total anthocyanins and phenolics than late deficit where water was withheld after veraison. In the current trial since the SDI treatments were applied throughout the irrigation season, it is possible that soil:water deficits could have directly influenced phenolic synthesis during the pre-veraison stage in the berry and composition of the resulting wine. As mentioned in Chapter 5, anthocyanins develop in the grape skins in the first few weeks after veraison. Since the SDI treatments were still being applied during this period there is once again potential for the water deficits to alter anthocyanin biosynthesis and concentration in the berry skin cells.

For both varieties, wine total red pigments were significantly increased in wines with increasing intensity of SDI treatments after 6 months ageing (Tables 6.1, 6.2). For the Cabernet Sauvignon and Shiraz wines exposed to approximately 50% less water (43% and 45% SDI treatments respectively), the total red pigments increased by about 15% compared to the control wine. PRD trials on Cabernet Sauvignon found significantly higher colour density, total phenolics and total red pigments in PRD wines after 12 months ageing (Bindon, 2004). The author suggested that this increase in red pigments of the PRD wines was thought to be due to an increase in copigmented or polymeric forms of the anthocyanins rather than a change in anthocyanin concentration alone. For this current study, copigmentation measures (further discussed under section 6.4.3) showed that after 6 months ageing the SDI wines were

producing significantly higher proportions of polymeric pigment anthocyanins than the control wines.

6.4.2 Wine phenolic composition

Phenolic compounds in red wine, particularly anthocyanins and tannins, are important components contributing to colour and mouth-feel characteristics. In young red wine, such as the wine from these micro-ferments, the phenolic concentration and composition is possibly more related to the berry composition, as opposed to older wines that have undergone a longer ageing process that would also indirectly alter the final wine composition (Ribéreau-Gayon & Glories, 1987). Since anthocyanins are primarily responsible for red wine colour, it is thought that changes in particular aspects of anthocyanin composition in the wine would be influencing final wine colour or even copigmentation properties (Ribéreau-Gayon & Glories, 1987; Boulton, 2001).

For both Cabernet Sauvignon and Shiraz, significant differences observed in the wine anthocyanin concentrations for each irrigation treatment than the berry total anthocyanin concentrations between SDI treatments and the control (Chapter 5, Tables 5.2, 5.3). The Cabernet Sauvignon wines from the 43% SDI treatment had a 20% increase in total anthocyanins compared to the control at 6 months ageing. The Shiraz wines from the 45% SDI treatment had higher total anthocyanin concentrations than the control wines after 6 months ageing, although in this case the difference was not significant. Although the anthocyanin profiles for each variety would be influenced by its genetic background (García-Beneytez et al., 2002; Brouillard et al., 2003; Romero-Cascales et al., 2005), in the current trial there were obvious changes occurring during the early stages of young red wine development that may be influenced by the SDI treatments. From the HPLC analyses, the concentrations of total malvidin for both varietieswhile not being significant, decreased by approximately 50% between bottling and 6 months ageing in all treatments.

Anthocyanins have a relatively simple structure within the grape skin vacuoles (Chapter 1, Figure 1.3), that when extracted can undergo various reactive changes within themselves or by forming associations with other compounds by a process known as copigmentation (Boulton, 2001). Berries that are exposed to high temperatures tend to produce higher proportions of the

anthocyanidins malvidin-, petunidin-, and peonidin-3-*O*-coumaroyl that have a lower extractability during wine fermentation than the non-acylated glucoside and acetylglucosides anthocyanins (Roggero et al., 1984). Since the SDI treatments did affect canopy density (Chapter 3) there is a possibility that greater light interception by developing bunches could have resulted in changes to the developing anthocyanin profiles during the early stages of veraison (Chaves et al., 2003; Antolín et al., 2006). As shown in Chapter 3, the 43% and 45% SDI treatments resulted in increased ABA levels in the xylem sap. This may also have affected anthocyanin concentrations in the skin by forming more anthocyanin-forming cells (Hiratsuka et al., 2001; Antolín et al., 2003; Antolín et al., 2006). An increase in anthocyanoplasts could subsequently allow more anthocyanins to be synthesised and released into the wine ferment during the maceration period, thereby increasing total wine colour.

Total wine tannin concentrations for both varieties did appear to be influenced by the water deficit treatments at bottling and after 6 months ageing (Table 6.5). Cabernet Sauvignon wines from the 100% (control) and 52% SDI treatments had significantly higher tannin concentrations than the 70% and 43% SDI treatments after 6 months ageing. In Shiraz, wines from all SDI treatments had significantly higher tannin concentrations than control wines. Studies by Brossaud et al., (2001) found that wine made from small berries contained a higher proportion of seed and skins in the ferment and produced higher tannin concentrations than ferments made from larger berries that would have contained greater pulp and juice volumes to dilute the tannin concentrations. As berries from the Shiraz SDI treatments were significantly smaller than berries from the control, there may have been a greater proportional weight of seeds (not measured) within the SDI treatments and thus a greater level of extractable tannin in the must than the larger control berries. Another explanation for changes in tannin concentrations between the irrigation treatments could be that the skin and seed tannin polymers of the control became chemically conjugated to other compounds, which in turn decreased the extractability level of the wine tannin (Kennedy et al., 2000; Downey et al., 2003a; Hazak et al., 2005; Robinson, 2006).

6.4.3 Wine copigmentation

In young red wines, a phenomenon known as copigmentation can result in enhanced colour due to the formation of new complexes between anthocyanins and colourless co-factors

(Levengood, 1996; Boulton, 2001; Levengood & Boulton, 2004). During ageing of red wines the copigmentation of anthocyanins becomes less important to the colour and stability of the pigmented compounds (Harbertson & Spayd, 2006). Hence for the purpose of trying to understand the effect of SDI on wine composition, the application of the copigmentation measures on these wines at bottling and 6 months age was feasible. To measure red wine copigmentation the sample needs to be adjusted to pH 3.6 to eliminate colour differences due to pH (Levengood & Boulton, 2004).

The increase in total colour after 6 months by comparison with bottling values for both varieties with reduced water application does appear to be influenced more by the polymeric pigment anthocyanin, than the monomeric or copigment anthocyanins. For both Cabernet Sauvignon and Shiraz, when irrigation was reduced by approximately a half (52% or 43%, Cabernet Sauvignon; 45%, Shiraz) there was an increase of almost 30% in polymeric pigments of the wine after 6 months ageing compared to the control wines. The increase in polymeric pigments at this irrigation level was also reciprocated in the total colour measures. Cabernet Sauvignon and Shiraz wines both increase in total colour of approximately 25% compared to control wines. The ability for anthocyanins to form more stable interactions with themselves or more complex associations with polymeric compounds is known to increase the long-term stability of wine colour due to their resistance to pH adjustments or bleaching effects of sulphur dioxide (Somers, 1971; Boulton, 2001).

The higher concentration of tannin in the SDI-treated berry skins and wine (Chapter 5, Table 5.8; Table 6.5) may have provided additional binding opportunities for the anthocyanins to form more stable polymeric pigment compounds (Ribéreau-Gayon & Glories, 1987). It has been observed that the most stable copigmentation complex is between the main anthocyanin, malvidin-3-*O*-glucoside, and the flavonols of quercitin and quercitin-3-*O*-glucoside (Baranac et al., 1997; Lambert, 2002). In the current study the greatest proportion of anthocyanin detected in the berries across all the irrigation treatments was as malvidin, particularly for the SDI treatments (Chapter 5, Tables 5.4, 5.5, 5.6 & 5.7). The higher proportion of total malvidin and higher concentration of tannin in the berries may explain the increased total colour for SDI wines. Flavonols, mainly the quercitin glycosides, are known to be particularly effective as cofactors in copigmentation of anthocyanins in flowers and fruit (Asen et al., 1972; Boulton, 2001). As the SDI treatments did decrease canopy growth, there is a possibility of

increased light exposure to the SDI-treated bunches that may have resulted in increased levels of quercitin-3-*O*-glucoside in the berry skins (Price et al., 1995; Haselgrove et al., 2000; Bindon, 2004). For the 2006 berries, while flavonol levels may not have consistently increased with increasing intensity of SDI (Chapter 5, Table 5.9), there is potential for SDI to indirectly affect quercitin glycosides in the berry skin due to increased light exposure.

Examination of total colour and the percentage of colour due to monomeric, copigment and polymeric pigment anthocyanins not only showed how total colour varied between the varieties and irrigation treatments, but also the distribution of anthocyanin compounds within the colour complex. At bottling all the irrigation treatments for the Cabernet Sauvignon wines had less than 10% monomeric anthocyanin, whereas the Shiraz wines had at least 30% monomeric anthocyanins (Figs. 6.6, 6.7). For Cabernet Sauvignon wines, there was a large shift of copigment complex anthocyanins to the polymeric pigment form between bottling and 6 months ageing (Figure 6.6). By contrast, the large proportion of polymeric pigment anthocyanins of the Shiraz wines after 6 months ageing appear to be due to both the monomeric and the copigment complex anthocyanins forming new complexes (Figure 6.7). This knowledge will be important to understand the subsequent effect of water deficits on wine colour intensity and stability and provide further direction for linkage of irrigation research with grape and wine composition.

6.4.4 Influence of berry size on wine composition

Clearly in this study there have been changes occurring in the phenolic composition of wines across the irrigation treatments that were not observed in the berries at harvest (Table 6.6). While both varieties showed a decrease in berry weight with reduced water application, the skin weight to berry weight ratios were similar between irrigation treatments (Chapter 4., Tables 4.1, 4.3). Furthermore, while there was a significant increase in berry total anthocyanin and berry total phenolic concentrations for Cabernet Sauvignon exposed to 43% SDI compared to the control, there were no significant differences observed between irrigation treatments for the Shiraz (Table 6.6). If these berry measures were to be used as the main indicators of subsequent wine composition there is potential to overlook the likely effect of the SDI treatments on wine colour and phenolic development during fermentation and ageing. Total wine anthocyanin and phenolic concentrations for Cabernet Sauvignon and Shiraz wines

all showed a significant increase with reduced water application (Table 6.6). This finding is similar to other Australian research (Freeman, 1983) that found increased colour in wine from deficit-irrigated Shiraz, even when there was no change in fruit anthocyanins. Recent berry size work by Walker et al. (2005a) on Shiraz reported no significant differences between wine colour density, wine colour hue, wine total anthocyanins or wine total phenolics between the small and large berries, thereby ruling out any effect from differences in berry size/weight (Walker et al., 2005a). While my study did not compare the effect of SDI across specific berry size categories, it did show that wine quality could not be completely pre-determined at the berry level.

Table 6.6 Means of berry weight, total anthocyanins and total phenolics from grapes collected at the 2006 harvest. Total wine anthocyanin and phenolic concentrations are from micro-ferment wines after 6 months ageing. Grapes were collected from Cabernet Sauvignon and Shiraz exposed to a control (100%) and SDI treatments. Means followed by the same letter are not significantly different at P=0.05 (n=8*, n=4).

2006 Vintage	**Irrigation Treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
Berry wt. (g)*	1.09^{c}	1.04^{bc}	0.96^{ab}	0.95^{a}
Berry anthocyanins (mg/g)*	0.95^{a}	0.94^{a}	0.98^{a}	1.16^{b}
Berry phenolics (au/g)*	1.41^{a}	1.42^{a}	1.46^{a}	1.55^{b}
Wine anthocyanins (mg/L)	235	249	277	343
Wine phenolics (au/L)	2.03	2.64	3.35	4.66
Shiraz	**100%**	**65%**	**45%**	**34%**
Berry wt. (g)*	1.08^{c}	0.99^{bc}	0.95^{b}	0.82^{a}
Berry anthocyanins (mg/g)*	0.99^{a}	1.08^{a}	1.14^{a}	1.11^{a}
Berry phenolics (au/g)*	1.41^{a}	1.24^{a}	1.43^{a}	1.42^{a}
Wine anthocyanins (mg/L)	283	313	320	370
Wine phenolics (au)	1.73	2.31	2.66	3.80

6.5 Conclusions

This study explored the influence of SDI on wine colour and phenolic composition and found that changes in wine composition are complex and may not necessarily correspond to differences in phenolic composition of the berry at harvest. Overall this study found that:

- Wine colour measures for both varieties were influenced by the SDI treatments after 6 months ageing. Total anthocyanin and total phenolic concentrations for both varieties showed significant increases with increasing intensity of SDI. Total red pigments for Cabernet Sauvignon (52% and 43% SDI) and Shiraz (45% SDI) wines were approximately 15% higher compared to the control.

- HPLC analyses provided an insight into the effect of SDI on wine composition during early ageing. In both varieties there tended to be a decrease in the concentration of individual and different classes of anthocyanidins at bottling and after 6 months ageing. After 6 months ageing, total anthocyanins (mg/g of skin) were significantly increased in the 43% SDI wines for Cabernet Sauvignon than the control. For Shiraz there were no significant differences observed between the total anthocyanin concentrations across the irrigation treatments.

- Wine tannin concentrations were similar at bottling and 6 months for both varieties. The Shiraz tannin levels at bottling and 6 months were significantly lower in the control than the SDI treatments that may be related to a dilution effect or extractability level of the tannin polymers.

- Copigmentation measures provided further insight into colour development and how deficit irrigation can affect young red wine colour. For both varieties, the changes in total colour concentrations at bottling and 6 months were probably due more to the increase in polymeric anthocyanins than to the monomeric and copigment anthocyanins. These changes were further influenced by the SDI treatments, as Cabernet Sauvignon (52% and 43% SDI) and Shiraz (45% SDI) had tended to be higher percent composition of polymeric pigments than the control.

- A change in wine composition was the result of the SDI treatments and not changes in berry weight. The concentration differences observed between Cabernet Sauvignon and Shiraz for total berry anthocyanins (mg/g) and total berry phenolics (mg/g) at harvest were not reflected in the wine samples.

CHAPTER 7: General Discussion and Conclusions

In this thesis, several investigations have been presented that contribute to the understanding of sustained deficit irrigation (SDI) on red winegrape varieties. Overall this study aimed to understand the physiological behaviour of selected wine grape cultivars to SDI and how this deficit irrigation strategy might influence yield and composition of the grapes and wine. The trials were conducted during 2003-2006 on the cultivars Cabernet Sauvignon and Shiraz grafted to 140 Ruggeri (*V. berlandieri* x *V. rupestris*) rootstock and grown in the Murray-Darling region of Australia. The vines were drip irrigated providing 100% of estimated ET_c (control) and three graded sustained water deficits (Cabernet Sauvignon 70%, 52% and 43% of the control; Shiraz 65%, 45% and 34% of the control). Furthermore, this research studied how compositional changes in the phenolic compounds of berries from Cabernet Sauvignon and Shiraz exposed to SDI may impact on subsequent wine composition and quality. A discussion of the results for each objective, as described in Chapter 1 is presented below.

7.1 Physiology and Growth

Objective: *Explore the physiological responses of Vitis vinifera varieties to water deficits and any subsequent impacts on abscisic acid synthesis.*

In the current study, there has been an attempt to identify physiological responses of field-grown grapevines that respond to a sustained water deficit of approximately 50% ET_c to understand whether there are differences between cultivars that might make them better suited to an induced water stress. Like the results from previous water deficit studies (Matthews et al., 1987; Goodwin & Jerie, 1992; McCarthy, 1997a) there was a reduction in stomatal conductance and leaf water potential in those grapevines receiving SDI compared to the controls. Various water deficit studies on grapevine physiology have shown there are differences in water relations and photosynthetic responses between *Vitis vinifera* cultivars (Schultz, 1996; Schultz, 2003; Soar et al., 2006b). Accordingly, grapevines have been broadly classified into two ecological categories based on their stomatal response to water deficit as being either isohydric (pessimistic-drought avoiding) or anisohydric (optimistic-drought tolerant). Research by Schultz (2003) and Soar et al., (2006b) on Grenache and Shiraz has shown that these varieties respond differently to potentially stressful environmental conditions

(water stress and changes in atmospheric conditions) and can be classified as isohydric and anisohydric respectively. Cabernet Sauvignon and Shiraz are the main red winegrape varieties grown in the Murray-Darling region, and anectodally, they are observed to respond differently to hot, dry conditions when managed under similar irrigation regimes.

It was evident during this study that Cabernet Sauvignon displayed more isohydric-like behaviour than the anioshydric-like response of Shiraz to water deficit. These varieties showed differences in diurnal patterns of stomatal conductance, particularly in the afternoon, with Shiraz tending to have similar values throughout the day, whereas Cabernet Sauvignon tended to have lower stomatal conductance during the afternoon than the morning. Although no statistical comparison can be made, Shiraz tended to have higher leaf water potentials (less negative) as well as higher stomatal conductance than Cabernet Sauvignon that suggests a difference in hydraulic conductivity between these varieties. Such differences in leaf water potential and stomatal conductance may explain the anecdotal observations that Cabernet Sauvignon appears to have conservative growth compared to the vigorous growth response of Shiraz when both varieties are exposed to similar irrigation practices and climatic conditions. While no direct measures of photosynthesis were conducted, the reduction in stomatal conductance and most likely gas exchange may have resulted in reduced carbohydrate assimilation and shoot growth of the Cabernet Sauvignon than Shiraz. This was also supported in the canopy growth results, whereby pruning weights and LAI were more reduced relative to the control for Cabernet Sauvignon exposed to approximately 50% SDI than Shiraz. These differences in canopy development could be a reflection of the isohydric-like and anisohydric-like responses of these varieties to water deficit that may ultimately influence carbohydrate dynamics and the long-term sustainability under SDI management.

Differences in stomatal control between grapevine varieties have been linked to hydraulic ‘signals’, chemical signals or both (Tardieu & Simonneau, 1998; Schultz, 2003; Soar et al., 2006b). Isohydric plants may regulate stomatal closure using a combination of hydraulic and hormonal signals, whereas an anisohydric plant tends to control stomatal aperture by hormonal signals (Tardieu et al., 1996). In this study, Cabernet Sauvignon and Shiraz exposed to an SDI of approximately 50% had higher xylem sap [ABA] throughout the day than the controls. This result is supportive of other ABA studies where deficit irrigated vines produced higher levels of xylem sap [ABA] than fully-irrigated or non-stressed controls (Soar et al., 2006b; Stoll et

al., 2000b). However, the results from this study do not take into consideration whether the source of the xylem sap [ABA] is root or canopy derived. Consequently, since ABA was only measured in xylem sap, and not the leaves, it is not possible to make any conclusions as to the degree to which leaf ABA may have influenced stomatal control. The ABA and stomatal physiology work by Soar et al., (2006b) suggested that for Grenache (isohydric) the most likely factor controlling stomatal aperture was enhanced regulation of ABA in the cell apoplast in combination with hydraulic signals, rather than xylem sap [ABA] sourced from the roots. Overall, the differences in xylem sap [ABA] between Cabernet Sauvignon and Shiraz may be due to the balance between hydraulic and hormonal signals. Furthermore, since this work did not investigate osmotic adjustment then it may be that an anisohydric response was not reliant on hormonal control at all and that hydraulic and osmotic factors dominated.

While it is evident that deficit irrigation techniques, notably PRD, can manipulate root-derived ABA levels and subsequent xylem sap [ABA] (Loveys et al., 1998; Stoll et al., 2000b), there is suggestion that rootstock genotypes may vary in sensitivity to water deficit (Soar et al., 2006a). Both varieties used in this study were grown on the same rootstock - 140 Ruggeri (*V. berlandieri* x *V. rupestris*). This rootstock has previously been rated as being highly drought tolerant based on its ability to maintain higher transpiration rates than less tolerant rootstocks (i.e. 5BB Kober, 420A) under potted conditions with Cabernet Sauvignon (Carbonneau, 1985). However, in another trial on scion/rootstock combinations, field-grown Shiraz grown on 140 Ruggeri was more sensitive to water stress than own-rooted Shiraz due to lower stomatal conductance and transpiration rates (Soar et al., 2006a). With this in mind, the scion/rootstock interaction becomes important in understanding the potential to which an anisohydric or isohydric vine may perform under water deficit conditions. Since anisohydric vines potentially rely on hormonal signals from the roots to regulate stomatal aperture, then varieties like Shiraz may not perform as well if grown on a rootstock that is unable to supply adequate ABA levels to the scion canopy. By contrast, an isohydric vine, being drought-avoiding or conservative, has a dual control mechanism to water loss from hormonal and hydraulic responses, hence providing a buffering mechanism if the rootstock is sensitive to water deficit and is unable to produce sufficient ABA levels for stomatal regulation. This suggests that, with use of SDI of at least 50% ET_c in existing vineyards, there would need to be some consideration given to the scion/rootstock combination and whether the rootstock would be capable of supporting an anisohydric or isohydric scion. As speculated by Soar et al.

(2006a), less vigorous rootstocks that have developed relatively compact root systems may be more appropriate for SDI as the reduced volume of water applied may be able to wet up a larger proportion of the root profile at each irrigation event

This work has shed some light on the differential response of Cabernet Sauvignon and Shiraz to SDI. However, further work needs to be done to determine how SDI influences carbohydrate dynamics and the long-term viability of scion/rootstock growth, particularly at SDI levels less than 50% ET_c. With current water restrictions for many winegrape growers across the Murray-Darling, i.e. NSW and SA growers are potentially faced with 50-60% reductions in water allocations, there is real applicability to use this knowledge to tailor irrigation management strategies to try and optimise or maintain viable growth and grape ripening. Furthermore this knowledge could also be used to take into account differences between cultivars in their responses to water deficit and to optimise their effectiveness in enhancing water use efficiency or preserving vineyard viability during such periods of reduced water allocations.

7.2 Yield and Yield Components

Objective: *Determine the effect of SDI on yield components and berry composition relevant to the Australian wine industry.*

Various water deficit studies have noted that yield and berry weight/size at harvest are dependent on when the irrigation is applied in relation to canopy growth and berry development (Hardie & Considine, 1976; McCarthy, 1997b; Kennedy et al., 2000). The strategic irrigation techniques of RDI and PRD are currently used within the Australian grape and wine industry to manipulate canopy growth, yield, berry size and ultimately berry composition and wine quality. Figure 1.4 in Chapter 1 illustrates how SDI differs from PRD or RDI, in that this form of deficit irrigation strategy has the potential to influence berry formation, development and ripening in conjunction with canopy growth. After three seasons, the SDI treatments had caused yield reductions in both varieties, primarily due to a reduction in berry weight that generally occurred from the beginning of veraison through to harvest. Overall both varieties still produced comparable yields per hectare to the district average (20-25 t/ha) even after being exposed to a sustained water deficit of 50% or less ET_c all irrigation season.

Like the results from previous PRD studies (Dry et al., 1996; Stoll et al., 2000), bunch numbers for each variety were unaffected even after continued application of the SDI treatments. Thereby suggesting the SDI treatments were not influencing bunch primordial initiation during these particular seasons (Krstic et al., 2005). Berry weight for both varieties was sensitive to the SDI-treatments and was reduced for all SDI-treated vines as less water was applied. For this study, the SDI treatments were applied throughout the irrigation season, in contrast to how RDI and PRD are managed. This suggests that an SDI of 50% or less ET_c may affect berry growth by altering cell division and expansion during the post-flowering period, rather than just berry expansion after veraison.

Berry weight/size has widely been accepted, in the past, as a major factor influencing final grape and wine quality. The general assumption has been that smaller berries (lower mass) have a greater skin area to pulp volume ratio that results in improved composition (phenolic compounds) relative to larger berries (Hardie & Considine, 1976; Williams & Matthews, 1990; Kennedy et al., 2002). From this study, even though berry weight was reduced as less water was applied; the ratio of skin weight to berry weight was fairly uniform across the irrigation treatments for both Cabernet Sauvignon and Shiraz. Consequently, the suggestion that changes in surface area to volume ratio can not solely explain increased phneolic concentrations in smaller berries. With this in mind, and since SDI was applied throughout the entire irrigation season, there is a likelihood the water deficits altered cellular growth of the skin tissue. This may have resulted in the smaller berries developing more cell layers per unit of skin that in turn would contain more anthocyanin-forming cells (Hardie & Considine, 1976; Roby & Matthews, 2004; Adams, 2006).

Other water deficit studies have also found that improvements in phenolic composition are due more to the effect of the irrigation treatment than any effect on berry size (Roby et al., 2004; Roby & Matthews, 2004; Walker et al., 2005a; Matthews & Kriedemann, 2006). Furthermore, since anthocyanin and tannin synthesis can occur in different parts of the berry (Adams, 2006; Robinson, 2006), the SDI treatments could have affected phenolic synthesis and accumulation in a number of ways due to the deficit irrigation coinciding with specific times of berry development and ripening. Since no berry grading experiments were conducted during this study, there needs to be further research into the primary (cellular) effect of SDI, or water deficit per se, on berry skin morphology and phenolic synthesis and/or maturation.

7.2 Berry Composition

Objectives: *Determine the effect of SDI on yield components and berry composition relevant to the Australian wine industry.*

Examine the effect of SDI on grape phenolic concentration and composition during berry ripening and harvest

Assessment of grape quality is complex as it is determined by a combination of the berry attributes such as sugar, acid, juice pH and other secondary metabolites (e.g. anthocyanins, tannins) that ultimately influence the colour, sensory characteristics and ageability of the wine. These compounds are produced at different stages of berry development and ripening that eventually influence their final concentration in the grape juice and wine (Figure 1.4, Chapter 1). For this study there were no significant differences between total soluble solids, juice pH and titratable acidity for both varieties exposed to SDI and the control. The general trend for both varieties was that as less water was applied the juice pH increased and the titratable acidity levels decreased (Tables 4.1 & 4.3, Chapter 4). The decrease in titratable acidity as less water was applied may have been due to higher bunch exposure due to a decrease in leaf area index for the SDI than the control treatment that in turn increased bunch temperatures and malate respiration (Iland & Gago, 2002). Furthermore, grape juice from the SDI berries may also be more prone to microbial oxidation and spoilage reactions during fermentation, due to the resulting higher pH values, than a fully irrigated vine (Iland & Gago, 2002).

Phenolic compounds of particular interest include the anthocyanins, tannins and flavonols. In this study total anthocyanins and total phenolics were measured using ultraviolet and visible spectrophotometric methods that have been widely adopted in the Australian grape and wine industry (Iland et al., 2004). Measures of total anthocyanins and total phenolics from whole berries can be expressed either as content (mg/berry) or concentration (mg/g) (Iland et al., 2004). In light of the previous discussion regarding berry size, it was initially anticipated that the SDI treatments would produce higher concentrations of total anthocyanin and total phenolics due to the reduction in berry weight. This was not the case, as higher concentrations of these compounds at harvest associated with less water were observed only in some years for both varieties. Only the lowest SDI treatments for Cabernet Sauvignon (43% SDI) and Shiraz (34% SDI) consistently produced higher total anthocyanin and total phenolic concentrations. Anthocyanin synthesis, however, is known to occur in the berry skin during the first few

weeks of veraison and is completed well before harvest (Haselgrove et al., 2000; Spayd et al., 2002; Downey et al. 2004; Robinson, 2006). With this in mind, the timing of irrigations for the SDI treatments in relation to phenolic synthesis in the skin, pulp and seeds of the berry may have influenced the final skin concentrations.

High performance liquid chromatography (HPLC) analyses of the individual anthocyanidin profiles indicated that as less water was applied, there was an increase in skin total anthocyanins concentration (mg/g skin). For both varieties, total malvidin tended to increase under SDI with total cyanidin having the lowest levels. As less water was applied there was an increase in total malvidin concentration for both varieties, with less effect on delphinidin, peonidin, petunidin and cyanidin for Cabernet Sauvignon and peonidin, petunidin, delphinidin and cyanidin for Shiraz. These differences in the order of anthocyanidin composition are probably the result of genetic differences between these varieties (Arozarena, et al., 2002; García-Beneytez, et al., 2002, Romero-Cascales et al., 2005). No single anthocyanin derivative was responsible for a shift in total anthocyanin concentrations for a particular SDI treatment.

Other studies have shown deficit irrigation to alter vegetative growth and light penetration through the canopy to the bunch, thereby increasing flavonol content (Price et al., 1995; Bindon, 2004). Results from this study showed that SDI could decrease leaf area index particularly for Cabernet Sauvignon vines exposed to the 52% and 43% SDI treatments, with subsequent increases in total skin flavonol concentrations. In general total skin tannin and flavonol concentrations tended to increase under SDI conditions, being highest in those irrigation treatments receiving the least volume of water. These results, while not being conclusive as to how SDI can ultimately affect flavonol and tannin concentration in the vineyard, have provided some preliminary insight into how the phenolic composition of commercial vines may be affected by sustained water deficits.

7.3 Wine Composition

Objective: *Investigate the composition and sensory attributes of wines made from SDI-treated red winegrapes.*

Knowing that SDI has the capacity to alter the concentration of phenolic compounds in Cabernet Sauvignon and Shiraz berries raises the question as to how this may influence wine

composition and quality. Previous work has found increased colour in wine from deficit-irrigated vines, even when there has been no change in fruit anthocyanins (Freeman, 1983). Likewise, Walker et al., (2005a) reported that improved wine quality from smaller rather than larger berries was due to the treatment (pruning or water deficit) not the reduction in berry size. Increases in wine colour of deficit-irrigated vines may be due to copigmentation interactions with other phenolic compounds (Boulton, 2001). Research by Bindon (2004) on Cabernet Sauvignon found wine colour was enhanced under PRD compared to the control. Furthermore, it was suggested that increases in wine colour were due to changes in the molecular properties of the anthocyanins to polymeric forms, rather than any change in anthocyanin concentration alone (Bindon, 2004).

For this study, the development of wine colour in the first six months of ageing was improved under SDI. As the intensity of the SDI treatment increased, the total wine anthocyanins and total wine phenolics also increased, with those vines receiving less than 50% water producing approximately 20-30% higher wine anthocyanin and phenolic concentration. Other studies, which applied pre- and post veraison water deficits, have also shown increases in total wine anthocyanins and total wine phenols compared to fully irrigated vines (Matthews et al., 1990; Bindon, 2004). The increases in wine colour with SDI treatment may be the result of changes in the synthesis of the phenolic compounds within the berry during development. Alternatively, the increases in red wine colour could possibly be due to a change in chemical properties of the anthocyanins to polymeric forms during the winemaking or ageing process (Levengood, 1996; Levengood & Boulton, 2004).

Copigmentation measures performed in this study provided insight into the development of red wine colour during the early stages of ageing. The increase in wine colour from vines treated with approximately 50% SDI appears to be due to an increase in polymeric compounds. The enhancement of wine colour due to copigmentation is the result of new complexes developing between anthocyanins and other cofactors or polymeric pigments (tannins) (Levengood, 1996; Boulton, 2001; Levengood & Boulton, 2004). Both varieties had increased levels of malvidin-3-O-glucoside in SDI-treated berries, and this is considered the most stable anthocyanin to form copigmentation complexes (Baranac et al., 1997; Lambert, 2002). However, it is unclear from these results whether the measured tannin concentration in the berries had any subsequent effect on the formation of the polymeric pigmented

anthocyanins. Consequently, an area of future work could be to explore how deficit irrigation influences anthocyanin and tannin concentration in conjunction with copigmentation properties of young red wines.

As previously discussed (Chapters 4 and 5), the importance of berry size as a determining factor influencing grape and therefore wine composition is unresolved. It was also shown that a change in wine composition was the result of the SDI treatments and not changes in berry weight. Table 6.6 (Chapter 6) supports this finding as the differences observed between Cabernet Sauvignon and Shiraz for total berry anthocyanins (mg/g) and total berry phenolics (au/g) at harvest were not reflected in the wine samples. If berry size/weight is used as the main indicator of subsequent wine composition there is potential to underestimate the potential wine colour and phenolic development of SDI-treated wines during fermentation and ageing.

In addition to the micro-wine ferments conducted in this thesis, a separate wine sensory project was performed to determine the effectiveness of the irrigation treatments in terms of their impact on the composition and quality of the resultant wines. Using grapes from the 2006 harvest, small-scale wines (20L ferments) were produced for both varieties and each irrigation treatment (Appendix F) (Chalmers, 2006 in press). After 6 months ageing, the wines were tasted by panels of winemakers from wine companies in the Murray-Darling and were ranked from most (rank number 1) to least preferred (rank number 12) based on sensory characteristics (colour, aroma and palate). No descriptive wine analyses were performed.

Table 7.1 Wine ranking scores for small-scale wines produced from a control (100%) and SDI treated Cabernet Sauvignon (70%, 52%, 43%) and Shiraz (65%, 45%, 34%) grapes harvested in 2006. Lower scores represent a more preferred wine. Means followed by the same letter within a row are not significantly different at P=0.05, (n=4).

Wine Ranking	**Irrigation treatments**			
Cabernet Sauvignon	**100%**	**70%**	**52%**	**43%**
	7.87[b]	7.65[b]	5.19[a]	4.81[a]
Shiraz	**100%**	**65%**	**45%**	**34%**
	5.96[a]	7.21[a]	6.53[a]	5.99[a]

Preliminary results indicate that for Cabernet Sauvignon the wines produced from grapes exposed to 52% and 43% SDI had significantly more preferred sensory characteristics than the control and 70% SDI wines (Table 7.1). Conversely, for Shiraz there were no significant sensory differences between the control and the SDI wines (Table 7.1).

Results from the SDI study have been encouraging and indicate that even though there may be differences in berry composition as a result of the water deficits, the wine differences appear to be intensified under SDI. Additonally, it seems that the differences between berry and wine composition after exposure to SDI are complex. Further research needs to be conducted in the area of deficit irrigation and young red wine composition, particularly in relation to how water deficit can alter phenolic levels in the berry that could then potentially affect wine colour (copigmentation) and wine ageing.

7.5 Management and Economic Implications of SDI

For this study, the irrigation treatments were created by various dripper flow rates that under the same irrigation interval and timing applied different volumes of water all season to the rootzone (Table 2.1, Chapter 2). This is in contrast to the strategic deficit irrigation techniques of PRD and RDI that work on irrigating to a refill point that re-wets part of the root zone (Kriedemann & Goodwin, 2003). When applying SDI, the water is restricted to the upper soil layers thereby resulting in lack of deep drainage to flush away any salts or other compounds (boron, calcium carbonate) that may build up in the top soil and potentially affect healthy vine function. Additionally, the application of SDI to the upper soil layers may also initiate “hydraulic redistribution” whereby water in the lower soil layers moves to the drier roots in the upper soil layers due to higher water potentials created when upper soil layers dry out. Other studies have also shown horizontal transfers or lateral movement of water from irrigated to non-irrigated roots along a hydraulic gradient as soil layers dry out (Stoll et al., 2000; Smart et al., 2005; Burgess & Bleby, 2006). An observation with SDI is that over time the soil water content stabilised at different ranges for each irrigation treatment (Figure 2.4). This suggests that SDI, like RDI, could be implemented into an existing irrigation system, without changing irrigation infrastructure, simply by lowering the re-fill point per irrigation so as the rootzone is not completely re-wetted.

Overall, if reduced water allocations are going to compel more Australian winegrape growers to use some form of deficit irrigation there needs to be a better understanding of vineyard water movement between soil layers and within root systems. Such research also needs to be incorporated with real time measures of soil moisture and soil potential, as well as plant based responses (isohydric vs anisohydric, scion/rootstock interactions), so that more detailed information on plant water demand and/or availability can be matched with soil water availability.

WUE, i.e. the tonnes of fruit produced per megalitre of water applied, increased as less water was applied for both Cabernet Sauvignon and Shiraz (Tables 4.2 & 4.4, Chapter 4). Even though there were significant reductions in yield and berry weights for some of the SDI treatments compared to the control, each variety still produced commercially acceptable yields when compared to the district average. Furthermore, for both varieties, significant yield reductions from the SDI treatments when compared to the control were not apparent until the second season. While this may suggest improvements in WUE for a particular irrigation season, it still does not clarify whether there would be better financial returns for adopting SDI practices.

The adoption of RDI or PRD has provided an opportunity to improve red winegrape quality that wine companies have also been willing to pay bonuses for in contractual agreements, thus compensating for any yield reductions. At the same time there was a shortage of quality red winegrapes in the warm climate regions that provided an incentive for many growers to adopt some form of strategic deficit irrigation technique. However, it should also be noted that the production of high tonnages from full irrigation has also been occurring across many Australian vineyards and has often meant zero return for the grower. The reason being that wineries are prepared to pay bonuses for better quality fruit (eg higher grape colour) that tends to occur in grapes grown on vineyards exposed to some form of deficit irrigation.

Currently across the Murray-Darling region winegrape growers are confronted with the prospects of low winegrape prices due to an oversupply of quality red winegrapes (particularly from the cool climate regions) and changes in market prices for bottled wine. From an economic perspective, this decline in red winegrape prices (Appendix G, Figure G.1) is often a disincentive for growers to conserve water as payment of winegrapes is not necessarily based

on potential quality, but rather on quantity. Consequently, as the cost of water is relatively inexpensive (i.e. A$60-120/ML in the Murray-Darling) there is a temptation to produce larger yields in an attempt to improve economic returns. Figure 7.1 shows the net return per hectare that incorporates operating costs (Appendix H, Table H.1) for Sunraysia at each SDI treatment imposed on the Cabernet Sauvignon and Shiraz. For the net return, a three year average price was calculated using the weighted average prices in Appendix G (Figure G.1) from 2003-2006 (Cabernet Sauvignon - $425/t, Shiraz-$516/t).

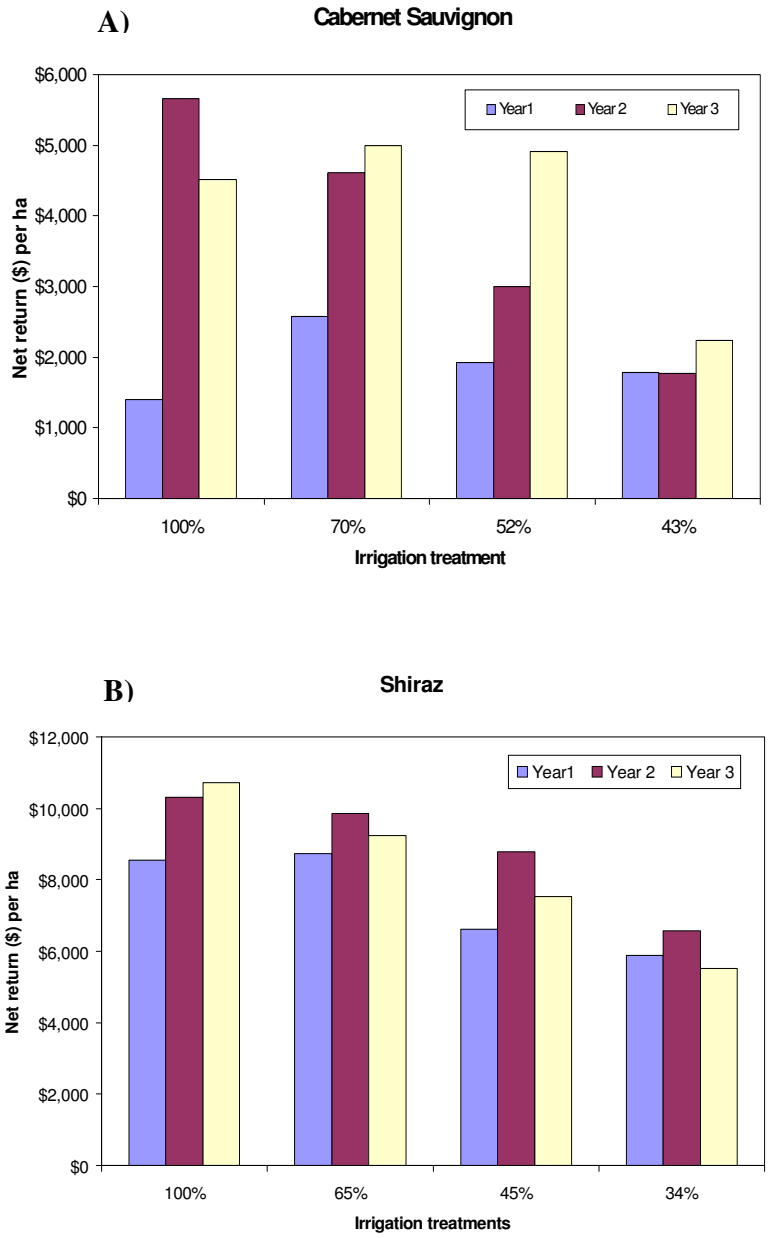


Figure 7.1 Net return per hectare for the control and SDI treatments for A) Cabernet Sauvignon and B) Shiraz during 2003-2006.

An important factor to consider in the net return per hectare for Cabernet Sauvignon (Figure 7.1A) is that for Year 1 there was a lower volume of water applied (Chapter 4, Table 4.2) across all irrigation treatments compared to Years 2 and 3. This reduction in water across all irrigation treatments could explain the reason for reduced returns in the first year than the following years. In Year 2, a progressive reduction in net returns resulted as the level of SDI intensified. However, in Year 3 the Cabernet Sauvignon vines exposed to 70% and 52% SDI showed slightly higher net return than the control. The effect of SDI on the Shiraz net returns (Figure 7.1B) appears to be more consistent and progressively reduces as less water is applied. Overall, after 3 seasons of receiving SDI, the Cabernet Sauvignon vines exposed to 70% and 52% SDI were more economically viable than the control. Furthermore, the 52% Cabernet Sauvignon vines continued to produce better grape composition that was reflected in better quality wine both from a compositional and sensory perspective (Table 7.1). As for Shiraz, these net returns show that SDI is not cost-effective under the current economic conditions (water prices). Furthermore, there were no sensory differences between the control and SDI Shiraz wines in 2006.

The net return takes into account the annual reduced costs (non-capital) of electricity and water (Appendix G) and variances in yield for each SDI. Based on data from Appendix H, the cost of water and electricity makes up less than 10% of overall input costs per hectare. Therefore an SDI of approximately 50% ET_c is only going to save about 4% of production costs. Consequently, net returns are not going to be largely affected by using SDI if there is no difference in grape price/tonne. For example, if there was a 30% reduction in yield resulting from SDI than the control there would need to be a 50% increase in grape price/tonne for a grower to maintain comparable net returns. If water becomes a more highly valued resource and priced accordingly, which is a likely outcome resulting from current water shortages, then a larger increase in net return will result from SDI. Consequently for SDI to be adopted, water needs to be valued as a more “limited” resource and priced accordingly. Additionally, wineries would need to offer price incentives to produce lower yields for winegrape growers in the Murray-Darling to adopt SDI as a strategic deficit irrigation technique.

7.6 Future Recommendations

- In the short-term, if the Australian grape and wine industry was faced with water restrictions of 50% or less of available water allocations then the adoption of SDI may be a more appropriate solution to maintaining winegrape production. It is important to note that current water allocations for Sunraysia range between 9.0-14.0 ML/ha depending on whether the vineyard is located in Victoria or NSW. However with current water transfers many growers sell a portion of this original water allocation before the irrigation season commences, thereby reducing the net amount of water available for the vineyard. For example, many growers across Sunraysia may have between 6.0-7.0 ML/ha at the beginning of the irrigation season that is further reduced to 3.0-3.5 ML/ha under water restrictions. Under this situation, implementation of SDI into existing vineyards could be used as an "emergency" response as it would require no change to existing irrigation systems, as opposed to PRD that requires extra capital outlay for additional irrigation infrastructure. For this study drippers of various flow rates were used to create the desired water volumes for similar irrigation intervals. In commercial vineyards, to manage SDI under existing irrigation systems a possible method would be to apply shorter irrigations that would not refill the entire soil profile yet still allow the vineyard to receive irrigation all season. The application of soil moisture monitoring tools or equipment would be required to schedule irrigations and monitor soil water patterns in order to avoid detrimental water stress during critical berry development (flowering, berry set) and ripening stages (early veraison), as well as visual observations or some plant-based method to assess vine water stress.

- In the long-term, there needs to be further research into the sustainability of SDI and the impacts on the soil environment (salt build-up, microbial activity) and grapevine viability. In light of the fact that Cabernet Sauvignon and Shiraz can be potentially categorised as isohydric-like (pessimistic) or anisohydric-like (optimistic), such responses to water deficit could ultimately influence the carbohydrate dynamics of the grapevine and subsequent viability. Given that this study was only conducted over three growing seasons it is difficult to conclude how the SDI may have affected carbohydrate reserves and assimilate partitioning to woody and fruiting structures.

- A key finding in this study was that berry weight was not a key driver of berry and wine composition and quality. It is more likely that the effect of the deficit irrigation on primary pathways such as phenolic synthesis at different stages of berry development and ripening are causing changes in berry composition. With this in mind, future research could continue to explore how deficit irrigation can be used to manipulate phenolic composition at different stages of berry development. Given that SDI is applied throughout the entire irrigation season there is potential for this deficit irrigation strategy to also alter those phenolic compounds that are synthesised pre-veraison as well as post-veraison in a similar manner as to PRD and RDI. Future research could explore how the timing of deficit irrigation may affect the production of specialised skin cells that influence phenolic concentration, or even skin thickness in conjunction with specific berry sizes.

- With the realisation that some form of deficit irrigation management will probably be necessary to grow winegrapes in the future, there needs to be greater knowledge of the effects that deficit irrigation will have on wine quality. As previously discussed, while SDI did increase the concentration of berry phenolic compounds, how this translates to wine is more complex. From the SDI findings there were differences in wine composition that were not apparent in the berry. Whether this is due to phenolic changes occurring at the berry level that are undetectable until transformed into wine, or due to copigmentation reactions occurring during young red wine ageing needs to be further explored. Since this current study only focussed on red winegrapes it would also be beneficial to research the implications of SDI on white winegrapes and how this deficit irrigation technique may influence sensory characteristics of premium white wine varieties.

- Through understanding the translation of grape composition into wine, these findings should be able to provide additional knowledge to the Australian wine and grape industry as to how sustained deficit irrigation can be used to manipulate grape composition for the production of sustainable wine styles. The emerging question this work raises is whether SDI is regarded more as a short-term, emergency response to water reductions or a viable option for long-term, vineyard sustainability?

REFERENCES

Adams, D.O. (2006) Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture* **57**, 249-256.

Alexander, D.McE. (1965) The effect of high temperature regimes or short periods of water stress on development of small fruiting sultana vines. *Australian Journal of Agricultural Research* **16**, 817-823.

Allen, M. (1998) Phenolics demystified. In 'Proceedings of the ASVO Oenology seminar held 9 October, 1997, Adelaide, S.A.' pp. 4-11. Australian Society of Viticulture and Oenology, Adelaide, Australia.

Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. (1998) Crop evapotranspiration – Guidelines for computing crop water requirements. 'FAO Irrigation and Drainage Paper no. 56.' Food and Agriculture Organization of the United Nations, Rome, Italy.

Amrani-Joutei, K., Glories, Y. and Mercier, M. (1994) Localization of tannins in grape berry skins. *Vitis* **33**, 133-138.

Antolín, M., Baigorri, H., DeLuis, I., Aguirrezábal, F., Geny, L., Broquedis, M. and Sánchez-Díaz, M. (2003) ABA during reprodcutive development in non-irrigated grapevines (*Vitis vinifera* L. cv. Tempranillo). *Australian Journal of Grape and Wine Research* **9**, 169-176.

Antolín, M., Ayari, M. and Sánchez-Díaz, M. (2006) Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. *Australian Journal of Grape and Wine Research* **12**, 13-20.

Archer, E. and Strauss, H.C. (1989) Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. *South African Journal of Enology and Viticulture* **10**, 74-76.

Arozarena, I., Ayestarán, B., Cantalejo, M.J., Vera, M.N.M., Abril, I. and Casp, A. (2002) Anthocyanin composition of Tempranillo, Garnache and Cabernet Sauvignon grapes from high- and low-quality vineyards over two years. *European Food Research and Technology* **214**, 303-309.

Asen, S., Stewart, R.N. and Norris, K.H. (1972) Co-pigmentation of anthocyanins in plant tissues and its effect on color. *Phytochemistry* **11**, 1139-1144.

Atwell, B.J., Kreidemann, P.E. and Turnball, C.G. (1999) 'Plants in action – adaption in nature, performance in cultivation.' Macmillan Education Pty Ltd, South Yarra, Australia.

Australian Bureau of Statistics (2006) 'Australian wine and grape industry.' Cat. No. 1329. ABS, Canberra, Australia.

Bakker, J. and Timberlake, C.F. (1985) The distribution and content of anthocyanins in young port wines as determined by high performance liquid chromatography. *Journal of the Science of Food and Agriculture* **36**, 1325-1333.

Baranac, J.M., Petranovič, N.A. and Dimitrič-Markovič, J.M. (1997) Spectroscopic study of anthocyanin copigmentation reactions. 2. Malvin and the nonglycosidized flavone quercitin. *Journal of Agricultural Food Chemistry* **45**, 1694-1697.

Becker, N. and Zimmermann, Z. (1984) Influence de divers apports d'eau sur des vignes en pots, sur la maturation des sarmants, le developpment des baies et la qualité du vin. *Bulletin de L'O.I.V.* **57**, 548-596.

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

Bindon K. (2004) 'Influence of partial rootzone drying on aspects of grape and wine quality.' PhD Thesis, University of Adelaide, Adelaide, Australia.

Boss, P.K., Davies, C. and Robinson, S.P. (1996) Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiology* **4**, 1059-1066.

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

Bravdo, B. and Hepner, Y. (1987) Water management and effect on fruit quality in grapevines. In 'Proceedings of the Sixth Australian Wine Industry Technical Conference held 14-17 July 1986, Adelaide, S.A.' pp. 150-158. Winetitles, Adelaide, Australia.

Bravdo, B., Hepner, Y., Loinger, C., Cohen, S. and Tabacman, H. (1985) Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. *American Journal of Enology and Viticulture* **36**, 132-139.

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

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

Brouillard, R., Chassaing, S. and Fougerousse, A. (2003) Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? *Phytochemistry* **64**, 1179-1186.

Brouillard, R. and Delaporte, B. (1977) Chemistry of anthocyanin pigments. 2.[1] Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of the American Chemical Society* **99**, 8461-8468.

Burgess, S.S.O., Adams, M.A., Turner, N.C. and Ong, C.K. (1998) The redistribution of soil water by tree root systems. *Oecologia* **115**, 306-311.

Burgess, S.S.O. and Bleby T.M. (2006) Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany* **57**, 3283-3291

Burns, J., Mullen, W., Landrault, N., Teissedre, P-L., Lean, M.E.J. and Crozier, A. (2002) Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon hybrid grapes. *Journal of Agricultural and Food Chemistry* **50**, 4096-4102.

Buttrose, M.S., Hale, C.R. and Kliewer, W.M. (1971) Effect of temperature on the composition of 'Cabernet Sauvignon' berries. *American Journal of Enology and Viticulture* **22**, 71-75.

Caldwell, M.M., Dawson, T.E. and Richards, J.H. (1998) Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* **113**, 151-161.

Candolfi-Vasconcelos, C.M., Candolfi, M.P and Kobet, W. (1994) Retranslocation of carbon reserves from the woody storage tissues into the fruit as a reponse to defoliation stress during the ripening preiod in *Vitis vinifera* L. *Planta* **192**, 567-573.

Carbonneau, A. (1985) The early selection of grapevine rootstocks for resistance to drought conditions. *American Journal of Enology and Viticulture* **36**, 195-198.

Chaves, M. M., Maroco, J. P. and Pereira, J. S. (2003) Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology* **30**, 239-264.

Cheynier, V., Prieur, C., Guyot, S., Rigaud, J., Moutounet, M. (1997) The structures of tannins in grapes and wines and their interactions with proteins. In 'Wine: Nutritional and Therapeutic Benefit's.' (Ed. Watkins, T.R.) pp. 81-93. American Chemical Society: Washington D.C.

Cheynier, V. and Rigaud, J. (1986) HPLC separation and characterisation of flavonols in the skins of *Vitis vinifera* var. Cinsault. *American Journal of Enology and Viticulture* **37**, 248-252.

Cifre J., Bota, J., Escalona, J. M., Medrano, H. and Flexas, J. (2005) Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): an open gate to improve water-use efficiency? *Agriculture, Ecosystems and Environment* **106**, 159-170.

Comstock, J. (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *Journal of Experimental Botany* **53**, 195-200.

Coombe, B.G. (1992) Research on development and ripening of the grape berry. *American Journal of Enology and Viticulture* **43**, 101-110.

Coombe, B.G. and Iland, P.G. (2004) Grape berry development and winegrape quality. In 'Viticulture Volume 1-Resources. 2nd Edition.' (Eds. Dry, P.R. and Coombe, B.G.) pp. 210-246. Winetitles, Adelaide, Australia.

Coombe, B.G. and McCarthy, M.G. (2000) Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research* **6**, 131-135.

Correia, M.J., Pereira, J.S., Chaves, M.M., Rodrigues, M.L. and Pacheco, C.A. (1995) ABA xylem concentrations determine maximum daily leaf conductance of field-grown *Vitis vinifera* L. plants. *Plant, Cell and Environment* **18**, 511-521.

Crothers, S. (2001) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Agriculture Victoria, Mildura, Vic., Australia.

Crothers, S. (2002) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Agriculture Victoria, Mildura, Vic., Australia.

Crothers, S. (2003) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Department of Primary Industries, Mildura, Vic., Australia.

Crothers, S. (2004) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Department of Primary Industries, Mildura, Vic., Australia.

Crothers, S. (2005) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Department of Primary Industries, Mildura, Vic., Australia.

Crothers, S. (2006) (Comp.) 'Australian regional wine grape crush survey: Victoria/NSW Murray Valley.' Department of Primary Industries, Mildura, Vic., Australia.

Davies, W.J. and Gowing, D.J.G. (1999) Plant responses to small perturbations in soil water status. In 'Physiological Plant Ecology.' (Eds. Press, M.C., Scholes, J.D. and Barker, M.G.) pp. 27-89. Blackwell Science, Oxford, UK.

Davies, A.J. and Mazza, G. (1993) Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. *Journal of Agricultural and Food Chemistry* **41**, 716-720.

Davies, W.J., Wilkinson, S. and Loveys, B. (2002) Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytologist* **153**, 449-460.

Davies, W.J. and Zhang, J. (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 55-76.

do Ó-Marques, J., Reguinga, R., Laureano, O., Ricardo-da-Silva, J. (2005) Changes in grape seed, skin and pulp condensed tannins during berry ripening: effect of fruit pruning. *Ciência e Técnica Vitivinícola* **20**, 35-52.

Dokoozlian, N.K. (1996) Influence of light on grape berry growth and composition varies during fruit development. *Journal of American Society for Horticultural Science* **12**, 869-874.

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

Downey, M. and Adams, D. (2005) Practical use of a total tannin assay for red winegrapes. *Australian Viticulture* **9** (3), 68-71.

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

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

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

Dry, P.R. (2004) Grapevine varieties. In 'Viticulture Volume 1 – Resources. 2nd Edition.' (Eds. Dry, P.R. and Coombe, B.G.) pp. 132-149. Winetitles, Adelaide, Australia.

Dry P.R. and Loveys B.R. (1999) Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis* **38**, 151-146.

Dry, P., Loveys, B.R., Botting, D.D. and Düring, H. (1996) Effects of partial root-zone drying on grapevine vigour, yield, composition of fruit and use of water. In 'Proceedings of the Ninth Australian Wine Industry Technical Conference held 16-19 July, 1996, Adelaide, SA.' pp. 128-131. Winetitles, Adelaide, Australia.

Dry, P. R., Loveys, B. R., Iland, P. G., Botting, D. G., McCarthy, M. G. and Stoll, M. (1999) Vine manipulation to meet fruit specifications. In 'Proceedings of the Tenth Australian Wine Industry Technical Conference held 2-5 August, 1998, Sydney, NSW.' pp. 208-214. Winetitles, Adelaide, Australia.

Dry, P., Loveys, B., Stoll, M. and McCarthy, M. (2002) Commercial implementation of partial rootzone drying. *Australian and New Zealand Wine Industry Journal* **17**, 46-48.

Dry, P.R., Loveys, B.R., Stoll, M., Steward, D. and McCarthy, M.G. (2000) Partial rootzone drying – an update. *Australian Grapegrower and Winemaker,* Annual Technical Issue **438a**, 35-39.

Escalona, J.M., Flexas J. and Medrano, H. (1999) Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Australian Journal of Plant Physiology* **26**, 421-433.

Esteban, M.A., Villanueva, M.J. and Lissarrague, J.R. (2001) Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (*Vitis vinifera* L.) grape berries during ripening. *Journal of the Science of Food and Agriculture* **81**, 409-420.

Flint, S.D., Jordon, P.W. and Caldwell, M.M. (1985) Plant protective response to enhanced UV-B radiation under field conditions: leaf optical properties and photosynthsis. *Photochemistry and Photobiology* **41**, 95-99.

Freeman, B.M. (1983) Effects of irrigation and pruning of Shiraz grapevines on subsequent red wine pigments. *American Journal of Enology and Viticulture* **1**, 23-26.

Freeman, B. M. and Kliewer, W. M. (1983) Effect of irrigation, crop level and potassium fertilization on Carignane vine. II. Grape and wine quality. *American Journal of Enology and Viticulture* **34**, 197-207.

Freeman, B.M., Lee, T.H. and Turkington, C.R. (1980) Interaction of irrigation and pruning level on grape and wine quality of Shiraz vines. *American Journal of Enology and Viticulture* **31**, 124-135.

García-Beneytez, E., Cabello, F. and Revilla, E. (2003) Analysis of grape and wine anthocyanins by HPLC-MS. *Journal of Agricultural and Food Chemistry* **51**, 5622-5629.

García-Beneytez, E., Revilla,E. and Cabello, F. (2002) Anthocyanin pattern of several red grape cultivars and wines made from them. *European Food Research and Technologyl* **215**, 32-37.

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

Gawel, R., Oberholster, A. and Francis, I.L. (2000) A 'Mouth-feel Wheel': Terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research* **6**, 203-207.

Gibberd, M.R., Walker, R.R., Blackmore, D.H. and Condon, A.G. (2001) Transpiration efficiency and carbon-isotope discrimination of grapevines grown under well-watered conditions in either glasshouse or vineyard. *Australian Journal of Grape and Wine Research* **7**, 110-117.

Ginestar, C., Eastham, J., Gray, S. and Iland, P. (1998) Use of sap-flow sensors to schedule vineyard irrigation. II. Effects of post-veraison water deficits on composition of Shiraz grapes. *American Journal of Enology and Viticulture* **49**, 421-428.

Gishen M., Iland, P.G., Dambergs, R.G., Esler, M.B., Francis, I.L., Kambouris, A., Johnstone, R.S. and Høj P.B. (2002) Objective measures of grape and wine quality. In 'Proceedings of the Eleventh Australian Wine Industry Technical Conference held 7-11 October, 2001, Adelaide, SA.' pp. 188-194. Winetitles, Adelaide, Australia.

Gladstones, J. (1999) 'Viticulture and Environment.' Winetitles, Adelaide, Australia.

Gonzalez-San Jose, M.L., Barron, L.J.R., Junquera, B. and Robredo, L.M. (1991) Application of principle component analysis to ripening indices for wine grapes. *Journal of Food Composition and Analysis* **4**, 245-255.

González-Neves, G., Charamelo, D., Balado, J., Barreiro, J., Bochicchio, R., Gatto, G., Gil, G., Tessore, A., Carbonneau, A. and Moutounet, M. (2004) Phenolic potential of Tannat, Cabernet-Sauvignon and Merlot grapes and their potential correspondence with wine composition. *Analytica Chimica Acta* **513**, 191-196.

Goodwin, I. (1995) 'Irrigation of vineyards: a winegrape grower's guide to irrigation scheduling and regulated deficit irrigation.' Agriculture Victoria, Tatura, Australia.

Goodwin, I. and Jerie, P. (1992) Regulated deficit irrigation: from concept to practice. *Australian and New Zealand Wine Industry Journal* **7,** 258-261.

Goodwin, I. and Macrae, I. (1990) Regulated deficit irrigation of Cabernet Sauvignon grapevines. *Australian and New Zealand Wine Industry Journal* **5**, 131-133.

Grimes, D.W. and Williams, L.E. (1990) Irrigation effects on plant water relations and productivity of Thompson Seedless grapevines. *Crop Science* **30**, 255-260.

Hale, C.R. and Buttrose, M.S. (1974) Effect of temperature on ontogeny of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Journal of American Society for Horticultural Science* **99**, 390-394.

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

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

Harbertson, J.F. and Spayd, S. (2006) Measuring phenolics in the winery. *American Journal of Enology and Viticulture* **3,** 280-288.

Hardie, W.J. and Considine, J.A. (1976) Response of grapes to water-deficit stress in particular stages of development. *American Journal of Enology and Viticulture* **27**, 55-61.

Harris, J.M., Kriedemann, P.E. and Possingham, J.V. (1968) Anatomical aspects of grape berry development. *Vitis* **7**, 106-119.

Hartung, W. and Radin, J.W. (1989) Abscisic acid in the mesophyll apoplast and in the root xylem sap of water-stressed plants: the significance of pH gradients. *Current Topics in Plant Biochemistry and Physiology* **8**, 110-124.

Hartung W., Sauter A. and Hose E. (2001) Abscisic acid in the xylem: where does it come from, where does it go? *Journal of Experimental Botany* **53**, 27-32.

Hazak, J.C., Harbertson, J.F., Adams, D.O., Ho Lin, C. and Ro, B. (2005) The phenolic components of grape berries in relation to wine composition. *Acta Horticulturae* **689**, 189-196.

Haselgrove, L. (1997) 'The effect of manipulated bunch exposure on the accumulation of quercitin glycosides and anthocyanins in *Vitis Vinifera* L. cv Shiraz.' Masters Thesis, University of Adelaide, Adelaide, Australia.

Haselgrove, L., Botting, D., van Heeswijck, R., Høj, P.B., Dry, P.R., Ford, C. and Iland, P.G. (2000) Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research* **6**, 141-149.

Hennessey, T.L. and Field, C.B. (1991) Circadian rhythm in photosynthesis. *Plant Physiology* **96**, 831-836.

Hepner, Y., Bravdo, B., Loinger, C., Cohen, S. and Tabacman, H. (1985) Effect of drip irrigation schedules on growth, yield, must composition and wine quality of Cabernet Sauvignon. *American Journal of Enology and Viticulture* **1**, 77-85.

Hiratsuka, S., Onodera, H., Kawai, Y., Kubo, T., Itoh, H. and Wada, R. (2001) ABA and sugar effects on anthocyanin formation in grape berry cultured in vitro. *Scientia Horticulturae* **90**, 121-130.

Holt, H., Iland, P. and Ristic, R. (2006) A method for mini-lot fermentation for use in research and commercial viticultural and winemaking trials. *Australian and New Zealand Grapegrower and Winemaker,* Annual Technical Issue **509a**, 74-77.

Iland, P. (1998) The tannin project - the role of the vineyard. In 'ASVO seminar on Phenolics and Extraction.' (Eds. Allen, M., Wall, G. and Bullied, N.) pp. 40-41. Australian Society of Viticulture and Oenology, Australia.

Iland, P. (2004) Development and status of Australian viticulture. In 'Viticulture: Volume 1 Resources. 2nd Edition.' (Eds. Dry, P.R. and Coombe, B.G.) pp. 1-16. Winetitles, Adelaide, Australia.

Iland, P., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) 'Chemical analysis of grapes and wine: techniques and concepts.' Patrick Iland Wine Promotions Pty Ltd, Adelaide, Australia.

Iland, P. and Gago, P. (2002) 'Australian wine: styles and tastes.' Patrick Iland Wine Promotions Pty Ltd. Adelaide, Australia. pp. 22-51.

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

Jackson, M.G., Timberlake, C.F., Bridle, P. and Vallis, L. (1978) Red wine quality: correlations between colour, aroma, and flavour, and pigment and other parameters of young Beaujolais. *Journal of Science, Food and Agriculture* **29**, 715-727.

Johnstone, R. S., Clingeleffer, P. R. and Lee, T. H. (1996) The composition of Shiraz grape berries- implications for wine. In 'Proceedings of the Ninth Australian Wine Industry Technical Conference held 16-19 July, 1996, Adelaide, SA.' pp. 105-108. Winetitles, Adelaide, Australia.

Jones, H.G. (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* **49**, 387-398.

Jones, H.G. (1980) Interaction and integration of adaptive responses to water stress - the implications of an unpredictable environment. In 'Adaption of plants to water and high temperature stress.' (Eds. Turner, N.C. and Kramer, P.J.) pp. 353-365. Wiley, New York.

Jones, H.G. and Sutherland, R.A. (1991) Stomatal control of xylem embolism. *Plant, Cell & Environment* **14**, 607-612.

Jones, H.G. and Tardieu, F. (1998) Modelling water relations of horticultural crops: a review. *Scientia Horticulturae* **74**, 21-46.

Kennedy, J.A., Matthews, M.A. and Waterhouse, A.L. (2000) Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* **55**, 77-85.

Kennedy, J.A., Matthews, M.A. and Waterhouse, A.L. (2002) Effect of maturity and vine water status on grape skin and wine flavonoids. *American Journal of Enology and Viticulture* **53**, 268-274.

Kerridge G. and Antcliff, A. (1996) 'Wine grape varieties of Australia.' CSIRO Publishing, Melbourne, Australia.

Kliewer, W.M. (1970) Effect of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. *Journal of American Society of Horticultural Science* **95**, 693-697.

Kliewer, W.M. (1977) Influence of temperature, solar radiation and nitrogen coloration and composition of Emperor grapes. *American Journal of Enology and Viticulture* **28**, 96-103.

Kliewer, W.M., Freeman, B.M. and Hosssom, C. (1983) Effect of irrigation, crop level and potassium fertilization on Carignane vines I. Degree of water stress and effect on growth and yield. *American Journal of Enology and Viticulture* **34**, 186-195.

Kliewer, W.M. and Torres, R.E. (1972) Effect of controlled day and night temperatures on grape coloration. *American Journal of Enology and Viticulture* **23**, 71-77.

Kramer, P.J. (1988) Changing concepts regarding plant water relations. *Plant, Cell and Environment* **11**, 565-568.

Kriedemann, P.E. and Goodwin, I. (2003) 'Regulated deficit irrigation and partial rootzone drying: an overview of principles and applications.' Land & Water Australia, Canberra, ACT, Australia.

Kriedemann, P.E. and Smart, R.E. (1971) Effects of irradiance, temperature, and leaf water potential on photosynthesis of vine leaves. *Photosynthetica* **5**, 6-15.

Krstic, M., Moulds, G., Panagiotopoulos, B. and West, S. (2003) 'Growing Quality Grapes to Winery Specification'. Winetitles, Adelaide, Australia.

Krstic, M., Clingeleffer, P., Dunn, G., Martin, S. and Petrie, P. (2005) Grapevine growth and reproduction: an overview. In 'Transforming Flowers to Fruit.' ASVO Proceedings held 29 July, 2005, Mildura, Vic., Australia. pp. 7-10.

Lambers, H., Chapin III, F.S. and Pons, T.L. (1998) 'Plant physiological ecology.' Springer-Verlag, New York, USA.

Lambert, S.G. (2002) 'Copigmentation and its impact on the stabilisation of red wines.' PhD Thesis. University of Adelaide, Adelaide, Australia.

Levengood, J. (1996) 'Copigmentation in Cabernet Sauvignon.' Masters Thesis. University of California, Davis, USA.

Levengood, J. and Boulton, R. (2004) The variation in the color due to copigmentation in young Cabernet Sauvignon wines. In 'Red wine color - Exploring the mysteries.' (Eds. Waterhouse, A.L. and Kennedy, J.A.). pp. 35-72. American Chemical Society Washington, DC.

Linskens, H.F. and Jackson, J.F. (1988) 'Wine Analysis.' Springer-Verlag.

Liu ,W.T., Pool, R., Wenkert, W. and Kriedemann, P.E. (1978) Changes in photosynthesis, stomatal resistance and abscisic acid of *Vitis labruscana* through drought and irrigation cycles. *American Journal of Enology and Viticulture* **29**, 239-246.

Loveys, B.R. and Düring, H. (1984) Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars. II Abscisic acid changes under semi-arid conditions. *The New Phytologist* **97**, 37-47.

Loveys, B.R. and Kriedemann, P.E. (1973) Rapid changes in abscisic acid-like inhibitors following alterations in vine leaf water potential. *Physiologia-Plantarum* **28**, 476-479.

Loveys, B. R., Soar, C. J. and Stoll, M. (2005) Root to shoot signalling and vine response to the environment. In 'Proceedings of the Twelth Australian Wine Industry Technical Conference held 24-29 July, 2004, Adelaide, SA.' pp. 45-50, Australian Wine Industry Technical Conference, Adelaide, Australia.

Loveys, B., Stoll, M. and Dry, P. (2001) Partial rootzone drying - how does it work? *Australian Grapegrower and Winemaker,* Annual Technical Issue **449a**, 25-28.

Loveys, B., Stoll, M., Dry, P.R. and McCarthy, M.G. (1998) Partial rootzone drying stimulates stress responses in grapevine to improve water use efficiency while maintaining crop yield and quality. *The Australian Grapegrower and Winemaker* **414a**, 108-111.

Maschmedt, D.J. (2004) Soils and Australian viticulture. In 'Viticulture: Volume 1 Resources 2nd Edition.' (Eds. Dry, P.R. and Coombe, B.G.) pp. 56-89. Winetitles, Adelaide, Australia.

Matthews, M.A. and Anderson, M.M. (1988) Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. *American Journal of Enology and Viticulture* **39**, 313-320.

Matthews, M.A. and Anderson, M.M. (1989) Reproductive development in grape (*Vitis vinifera* L.): reponses to seasonal water deficits. *American Journal of Enology and Viticulture* **40**, 52-60.

Matthews M.A., Anderson M.M. and Schultz H.R. (1987a) Phenologic and growth responses to early and late season water deficits in Cabernet franc. *Vitis* **26**, 147-160.

Matthews, M.A., Cheng, G. and Weinbaum, S.A. (1987b) Changes in water potential and dermal extensibility during grape berry development. *Journal of American Society for Horticultural Science* **112**, 314-319.

Matthews, M.A., Ishii, R., Anderson, M.M. and O'Mahony, M. (1990) Dependence of wine sensory attributes on vine water status. *Journal of Science, Food and Agriculture* **51**, 321-335.

Matthews, M. and Kriedemann, P.E. (2006) Water deficit, yield, and berry size as factors for composition and sensory attributes of red wine. In 'Finishing the Job.' ASVO Proceedings held 21 July, 2006, Mildura, Vic., Australia. pp. 46-54.

Mazza, G. (1995) Anthocyanins in grapes and grape products. *Critical Reviews in Food Science and Nutrition* **35**, 341-371.

McCarthy, M.G. (1997a) 'Effect of timing of water deficit on fruit development and composition of *Vitis vinifera* cv. Shiraz.' PhD Thesis. University of Adelaide, Adelaide, Australia.

McCarthy, M.G. (1997b) The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research* **3**, 102-108.

McCarthy, M.G. (1998) Irrigation management to improve winegrape quality – nearly 10 years on. *The Australian Grapegrower & Winemaker* Annual Technical Issue **414a**, 65-71.

McCarthy, M.G. (2000) Developmental variation in sensitivity of *Vitis vinifera* L. (Shiraz) berries to soil water deficit. *Australian Journal of Grape and Wine Research* **6**, 136-140.

McCarthy, M.G. and Coombe, B.G. (1985) Water status and winegrape quality. *Acta Horticulturae* **171**, 447-456.

Medrano, H., Escalona, J.M., Bota, J., Gulías, J. and Flexas, J. (2002) Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany* **89**, 895-905.

Medrano, H., Escalona, J. M., Cifre, J., Bota, J. and Flexas, J. (2003) A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biology* **30**, 607-619.

Meyer, W.S. (1999) Divining for water. In 'Proceedings of the Tenth Australian Wine Industry Technical Conference held 2-5 August, 1998, Sydney, NSW.' pp. 73-77. Winetitles, Adelaide, Australia.

Mullins, M.G., Bouquet, A. and Williams, L.E. (1992) 'Biology of the grapevine.' Cambridge University Press, Cambridge, UK.

Munns, R. and King, R.W. (1988) Abscisic acid is not the only stomatal inhibitor in the transpiration stream. *Plant Physiology* **88**, 703-708.

Myburg, P.A. (2003) Responses of *Vitis vinifera* L. cv. Sultanina to water deficits during various pre- and post-harvest phases under semi-arid conditions. *South African Journal of Enology and Viticulture* **24**, 25-33.

Nadal, M. and Arola, L. (1995) Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis* **34**, 151-154.

Nagel, C.W. and Wulf, L.W. (1979) Changes in anthocyanins, flavonoids and hyydroxycinnamic acid esters during fermentation and ageing of Merlot and Cabernet Sauvignon. *American Journal of Enology and Viticulture* **30,** 111-116.

Nicholas, P., Magarey, P. and Wachtel, M. (1994) 'Diseases and Pests – Grape production series Number 1.' Winetitles, Adelaide, Australia.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. and Deloire, A. (2002) Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *American Journal of Enology and Viticulture* **53**, 261-267.

Patterson, A. and Hill M. (2003) (Comp.) 'Grapecheque vineyard benchmarking results – Victoria 2001-2002.'Department of Primary Industries, Vic., Australia.

Peterlunger, E., Buccella, A. and Iacono, F. (2000) Physiological changes in grapevine during adjustment to water stress: ABA, leaf gas exchange and root hydraulic conductivity. *Acta Horticulturae* **526**, 201-206.

Petrie P.R., Cooley N.M. and Clingeleffer P.R. (2004) The effect of post-verasion water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following season. *Australian Journal of Grape and Wine Research* **10**, 203-215.

Pirie, M. (1977) 'Phenolics accumulation in red wine grapes (*Vitis vinifera* L.).' PhD thesis. The University of Sydney, Sydney, Australia.

Pirie, A. and Mullins, M.G. (1976) Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. *Plant Physiology* **58**, 468-472.

Pirie, A. and Mullins, M.G. (1977) Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. *American Journal of Enology and Viticulture* **28**, 204-209.

Pirie, A. and Mullins, M.G. (1980) Concentration of phenolics in the skin of grape berries during fruit development and ripening. *American Journal of Enology and Viticulture* **31**, 34-36.

Poni, S., Lakso, A.N., Turner, J.R. and Melious, R.E. (1993) The effects of pre- and post-veraison water stress on growth and physiology of potted Pinot Noir grapevines at varying crop levels. *Vitis* **32**, 207-214.

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

Price, S.F., Watson, B.T. and Valladao, M. (1996) Vineyard and winery effects on wine phenolics - flavonols in Oregon Pinot noir. In 'Proceedings of the Ninth Australian Wine Industry Technical Conference, held 16-19 July, 1995, Adelaide, SA.' pp. 93-97. Winetitles, Adelaide.

Prieur, C., Rigaud, J., Cheynier, V. and Moutounet, M. (1994) Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **36**, 781-784.

Rankine, B.C., Kepner, R.E. and Webb Dinsmoor, A. (1958) Comparison of anthocyan vinifera pigments of vinifera grapes. *American Journal of Enology and Viticulture* **9**, 105-110.

Ribéreau-Gayon P. (1982) The anthocyanins of grapes and wines. In 'Anthocyanins as Food Colours.' (Ed. Markakis, P.). Academic Press, New York.

Ribéreau-Gayon, P. and Glories, Y. (1987) Phenolics in grape and wine. In 'Proceedings of the Sixth Australian Wine Industry Technical Conference held 14-17 July, 1986, Adelaide, SA.' pp. 247-256. Winetitles, Adelaide.

Ristic, R. (2004) 'A study of seed development and phenolic compounds in seeds, skins and wines of *Vitis vinifera* L. cv. Shiraz.' PhD Thesis. The University of Adelaide, Adelaide, Australia.

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

Robinson, S. (2006) Colour and tannin during berry development and ripening. In 'Finishing the Job.' ASVO Proceedings held 21 July, 2006, Mildura, Vic., Australia. pp. 11-14.

Robinson, S.P. and Davies C. (2000) Molecular biology of grape berry ripening. *Australian Journal of Grape and Wine Research* **6**, 175-188.

Robinson, S. and Walker, M. (2006) When do grapes make tannins? *Australian and New Zealand Grapegrower and Winemaker*, Annual Technical Issue **509a**, 97-105.

Roby, G. and Matthews, M.A. (2004) Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Australian Journal of Grape and Wine Research* **10**, 74-82.

Roby G., Harbertson, J.F., Adams, D.A. and Matthews, M.A. (2004) Berry size and vine water deficits as factor in winegrape composition: anthocyanins and tannins. *Australian Journal of Grape and Wine Research* **10**, 100-107.

Roggero, J.P., Coen. S. and Ragonnet, B. (1986) High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah: an approach to anthocyanin metabolism. *American Journal of Enology and Viticulture* **37**, 77-83.

Roggero J.P., Ragonnet B. and Coen S. (1984) Analyse fine des anthocyanes des vins et des pellicules de raisin par la technique HPLC. *Vignes et vins* **327**, 38-42.

Rogiers, S.Y., Hatfield, J.M. and Keller, M. (2004) Irrigation, nitrogen, and rootstock effects on volume loss of berries from potted Shiraz vines. *Vitis* **43**, 1-6.

Romero-Cascales, I., Ortega-Regules, A., López-Roca, J.M., Fernŕndez-Fernŕndez, J.I. and Gómez-Plaza, E. (2005) Differences in anthocyanin extractability from grapes to wines according to variety. *American Journal of Enology and Viticulture* **56**, 212-219.

Rühl, E.H. and Alleweldt, G. (1990) Effect of water stress on carbohydrate accumulation in root and stem of four different grapevine varieties. *Wein Wissenschaft Viticulture and Enological Science* **45**, 156-159.

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

Schölander, P.F., Hammel, H.T., Bradstreet, D. and Hemmington, E.A. (1965) Sap pressure in vascular plants. *Science* **148**, 339-346.

Schultz, H.R. (1996) Water relations and photosynthetic responses of two grapevine cultivars of different geographic origin during water stress. *Acta Horticulturae* **427**, 251-266.

Schultz, H.R. (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell and Environment* **26**, 1393-1405.

Schultz, H.R. and Matthews M.A. (1988) Resistance to water transport in shoots of *Vitis vinifera* L. *Plant Physiology* **88**, 718-724.

Schultz, H.R. and Matthews M.A. (1993) Growth, osmotic adjustment, and cell-wall mechanics of expanding grape leaves during water deficits. *Crop Science* **33**, 287-294.

Scudamore-Smith, P.D., Hooper, R.L. and McLaren, E.D. (1990) Color and phenolic changes of Cabernet Sauvignon wine made by simultaneous yeast/bacteria fermentation and extended pomace contact. *American Journal of Enology and Viticulture* **41**, 57-67.

Shirley, B.W. (1996) Flavonoid biosynthesis: 'new' functions for an 'old' pathway. *Trends in Plant Science* **1**, 377-382.

Sims, C.A. and Bates, R.P. (1994) Effects of skin fermentation time on the phenols, anthocyanins, ellagic acid sediment, and sensory characteristics of red *Vitis rotundifolia* wine. *American Journal of Enology and Viticulture* **45**, 56-62.

Singleton, V.L. (1972) Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. *American Journal of Enology and Viticulture* **23**, 106-113.

Sipiora, M.J. and Gutiérrez Granda, M.J. (1998) Effects of pre-veraison irrigation cut off and skin contact time on the composition, color, and phenolic content of young Cabernet Sauvignon wines in Spain. *American Journal of Enology and Viticulture* **49**, 152-162.

Smart, D.R., Carlisle, E., Goebel, M. and Núñez, B.A. (2005) Transverse hydraulic redistribution by a grapevine. *Plant, Cell and Environment* **28**, 157-166.

Smart, R.E. (1974) Aspects of water relations of the grapevine (*Vitis vinifera*). *American Journal of Enology and Viticulture* **25**, 84-91.

Smart, R.E. and Coombe, B.G. (1983) Water relations of grapevines. In 'Water deficits and Plant Growth Vol. 7.' pp. 137-196. Academic Press Inc.

Soar, C.J., Dry, P.R. and Loveys, B.R. (2006a) Scion photosynthesis and leaf gas exchange in *Vitis vinifera* L. cv. Shiraz: Mediation of rootstock effects via xylem sap ABA. *Australian Journal of Grape and Wine Research* **12**, 82-96.

Soar, C.J., Spiers, J., Maffei, S.M. and Loveys, B.R. (2004) Gradients in stomatal conductance, xylem sap ABA and bulk leaf ABA along canes of *Vitis vinifera* cv. Shiraz: molecular and physiological studies investigating their sources. *Functional Plant Biology* **31**, 659-669.

Soar, C.J., Spiers, J., Maffei, S.M., Penrose, A.B., McCarthy, M.G. and Loveys, B.R. (2006b) Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. *Australian Journal of Grape and Wine Research* **12**, 2-12.

Somers, T.C. (1968) Pigment profiles of grapes and of wines. *Vitis* **7**, 303-320.

Somers, T.C. (1971) The polymeric nature of wine pigments. *Phytochemistry* **10**, 2175-2186.

Somers, T.C. (1976) Pigment development during ripening of the grape. *Vitis* **14**, 269-277.

Somers, T.C. (1978) Interpretations of colour composition in young red wines. *Vitis* **17**, 161-167.

Somers, T.C. and Evans, M.E. (1974) Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines. *Journal of the Science of Food and Agriculture* **25**, 1369-1379.

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

Somers, T.C. and Pocock, K.F. (1986) Phenolic harvest criteria for red wine vinification. *Australian Grapegrower and Winemaker* **268**, 24-30.

Somers, T.C. and Vérette, E. (1988) Phenolic composition of natural wine types. In 'Wine Analysis.' (Eds. Linskens, H.F. and Jackson, J.F.) pp. 219-257. Springer-Verlag, Berlin.

Souquet, J., Cheynier, V., Brossaud, F. and Moutounet, M. (1996a) Polymeric proanthocyanidins from grape skins. *Phytochemistry* **43**, 509-512.

Spayd, S.E., Tarara, J.M., Mee, D.L. and Ferguson, J.C. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture* **53,** 171-182.

Stafford, H.A. (1990) 'Flavonoid metabolism.' p. 298. Boca Raton, Fla: CRC Press.

Stafford, H.A. (1991) Flavonoid evolution: an enzymic approach. *Plant Physiology* **96**, 680-685.

Stoll, M. (2000) 'Effects of partial rootzone drying on grapevine physiology and fruit quality.' PhD Thesis. University of Adelaide, Adelaide, Australia.

Stoll M., Dry, P., Loveys, B., Stewart, D. and McCarthy, M. (2000a) Partial rootzone drying - effects on root distribution and commercial application of a new irrigation technique. *Australian and New Zealand Wine Industry Journal* **15** (2), 74-77.

Stoll, M., Loveys, B. and Dry, P. (2000b) Hormonal changes induced by partial rootzone drying of irrigated grapevine. *Journal of Experimental Botany* **51**, 1627-1634.

Tardieu, F. and Davies, W.J. (1993) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. *Plant, Cell & Environment* **16**, 341-349.

Tardieu, F., Lafarge, T. and Simonneau, T. (1996) Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant, Cell and Environment* **19**, 75-84.

Tardieu, F. and Simonneau, T. (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* **49**, 419-432.

Tardieu, F., Zhang, J. and Gowing, D.J.G. (1993) Stomatal control by both (ABA) in the xylem sap and leaf water status: a test model for droughted or ABA-fed field-grown maize. *Plant Cell and Environment* **16**, 413-420.

Thorngate, J.H. (1997) The physiology of human sensory response to wine: A review. *American Journal of Enology and Viticulture* **48,** 271-279.

Thorngate, J.H.I. and Singleton, V.L. (1994) Localization of procyanidins in grape seeds. *American Journal of Enology and Viticulture* **45**, 259-262.

Tyree, M.T. and Sperry, J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 19-38.

van Zyl, J.L. (1984) Response of Colombar grapevines to irrigation as regards quality aspects and growth. *South African Journal of Enology and Viticulture* **5**, 19-28.

van Zyl, J.L. and Weber, H.W. (1977) Irrigation of Chenin Blanc in the Stellenbosch area within the framework of climate-soil-water-plant continuum. In 'International OIV Symposium, 14-21 February 1977, Cape Town, South Africa.' pp. 331-350.

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R., Kerridge, G.H., Rühl, E.H. and Nicholas, P.R. (2005a) Shiraz berry size in relation to seed numbers and implications for juice and wine composition. *Australian Journal of Grape and Wine Research* **11**, 2-8.

Walker, R.R., Gibberd, M.R. and Stevens, R.M. (2005b) Improving vineyard water use efficiency. In 'Proceedings of the Twelfth Australian Wine Industry Technical Conference, held 24-29 July, 2004, Melbourne, Vic.' pp. 152-158. Winetitles, Adelaide, Australia.

Wilkinson, S. and Davies, W. J. (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment* **25**, 195-210.

Williams, L.E. and Araujo, F.J. (2002) Correlations among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera. Journal of American Society for Horticultural Science* **127,** 448-454.

Williams, L.E. and Matthews, M.A. (1990) Grapevine. In 'Irrigation of Agricultural Crops.' pp. 1019-1055. American Society of Agronomy Inc.

Williams, L.E. and Smith, R.J. (1991) The effect of rootstock on the partitioning of dry weight, nitrogen and potassium, and root distribution of Cabernet Sauvignon grapevines. *American Journal of Enology and Viticulture* **42,** 118-122.

Winkel, T. and Rambel, S. (1993) Influence of water stress on grapevines growing in the field: from leaf to whole-plant response. *Australian Journal of Plant Physiology* **20**, 143-57.

Wulf, L.W. and Nagel, C.W. (1978) High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. *American Journal of Enology and Viticulture* **29**, 42-49.

Zhang, J. and Davies, W.J. (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell and Environment* **12**, 73-81.

Zhang, J., Schurr, U. and Davies, W.J. (1987) Control of stomatal behaviour by abscisic acid which apparently originates in roots. *Journal of Experimental Botany* **38**, 1174-1181.

APPENDICES

APPENDIX A

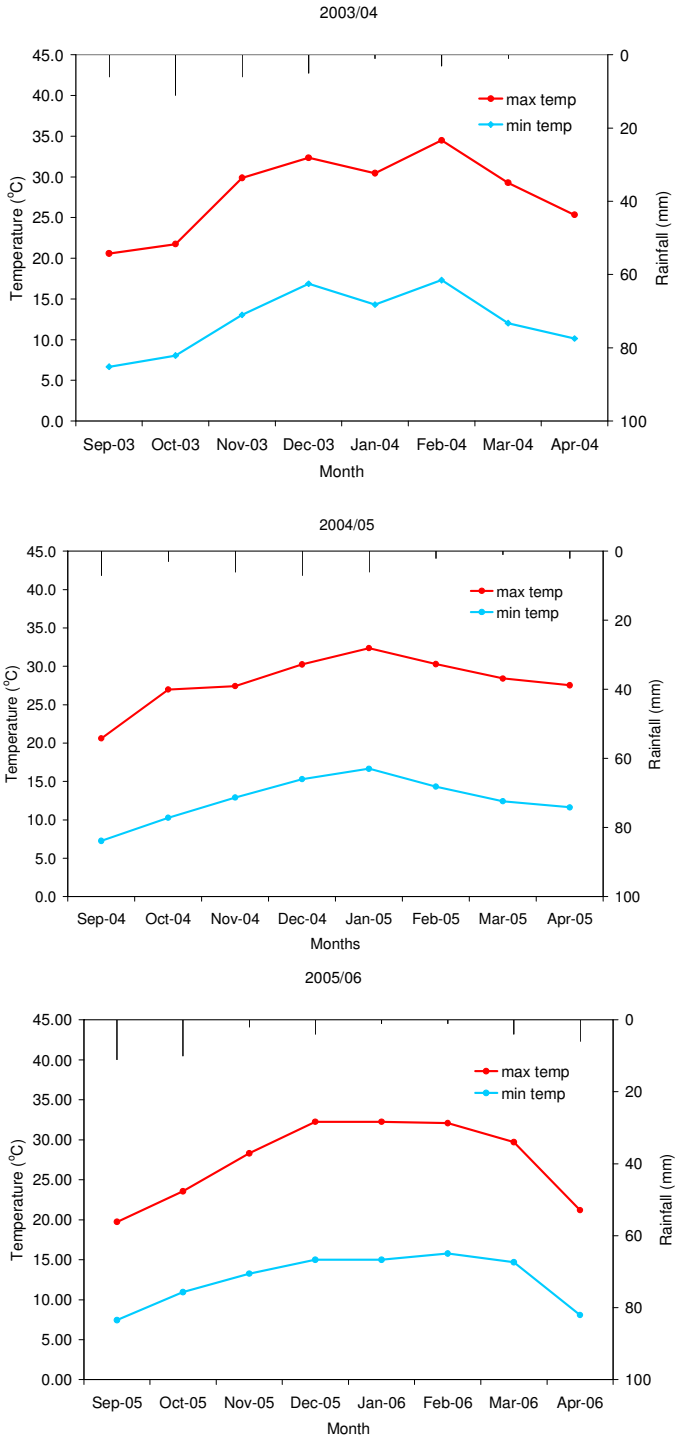


Figure A.1 Average monthly maximum and minimum temperatures (°C) and total monthly rainfall (mm) figures for Sunraysia from September to April for 2003/2004, 2004/2005 and 2005/2006. Data was collated from daily readings collected by the Bureau of Meteorology, Mildura, Vic, Australia.

APPENDIX B

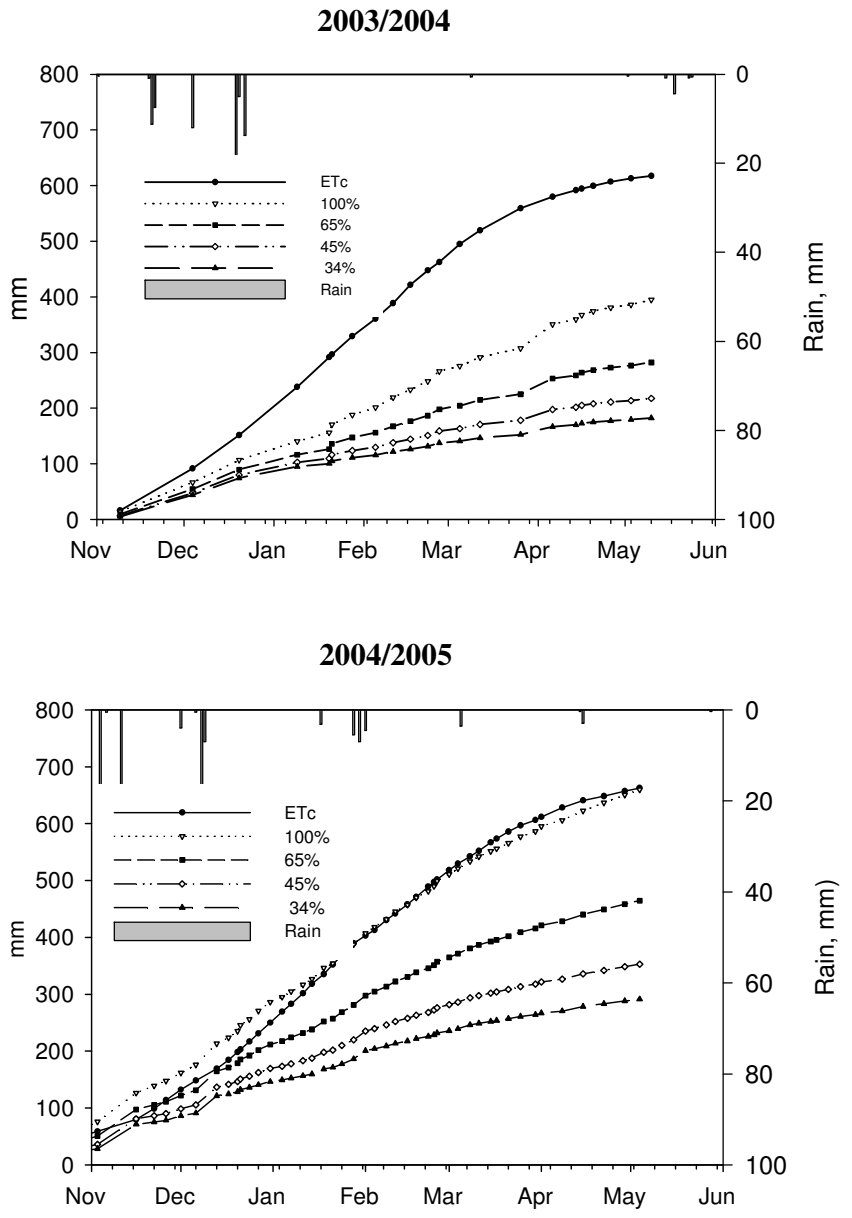


Figure B.1 Comparison of the cumulative water applied (mm of irrigation as ETc and rainfall) for each irrigation treatment at the Shiraz site in 2003/04 and 2004/05.

APPENDIX C

Table C.1 Reagents used for tannin extraction method

Name in procedure	To make 1.0 litre	Storage
Buffer A (Model wine)	Dissolve 5.0 g potassium bitartrate in 800 mL distilled water. Add 120 mL of 96% ethanol and stir for 5 min. Adjust pH to 3.3 with HCl and make volume up to 1.0 L with distilled water.	Room temperature
Buffer B (Washing buffer)	Dissolve 9.86 g sodium chloride in 500 mL distilled water. Add 12 mL glacial acetic acid and adjust to pH 4.9 with sodium hydroxide. Make volume up to 1.0 L with distilled water.	Room temperature
Buffer C (Resuspension buffer)	Dissolve 50g of SDS in 800 mL of distilled water. Add 50 mL of triethanolamine and monitor pH. When pH stabilises adjust pH to 9.4 with HCl and make volume to 1.0 L with distilled water.	Room temperature
Ferric chloride reagent	Dissolve 2.7g ferric chloride in 800 mL distilled water. Add 800 µL of concentrated HCl (12.1 N; 33-37% HCL) and make volume up to 1.0L with distilled water.	Room temperature
Protein solution (40mL)	Mix 1.0 mL of Bovine Serum Albumin (stored frozen at –80.0^{o}C) with 39 mL of Buffer B to make up 1mg/mL solution	Store at 4^{o}C

APPENDIX D

Table D.1

Cabernet Sauvignon	**Full irrigation (control)**	**Deficit irrigation (43%)**
*Av. berry wt. (g)	1.01	0.86
#Av. berry radius (mm)	5	4.75
Berry surface area (mm^2)	314	284
Berry volume (mm^3)	523	449
Berry surface area:berry volume ratio (unit skin area - mm^2)	0.60	0.63
▲Av. total anthocyanins (mg/berry)	1.07	1.04
▲Av. total anthocyanins (mg/g)	1.07	1.22
mg anthocyanins (mg/berry) per unit skin area (mm^2)	1.78	1.65
•Skin wt. to berry wt ratio	0.30	0.29

* Data is the average berry weight for 2005 and 2006

These are hypothetical figures. Since the berry weight for the deficit irrigation was 15% lighter than the control berries, the assumption is that volume would also be reduced by 15% for deficit berries. This does not apply to surface area.

▲Data is the average total anthocyanins (mg/berry or mg/g) for 2005 and 2006.

• Data is the average skin wt.: berry wt. ratio for 2005 and 2006.

APPENDIX E

Table E.1 Definitions of wine colour composition and the indicative minimum to maximum and average (in brackets) ranges for Cabernet Sauvignon and Shiraz (modified from Somers, (1978); Somers & Vérette, (1988); Iland et al., 2004).

Wine colour composition definitions		**Indicative ranges**	
		Cabernet Sauvignon	*Shiraz*
Wine colour density	▪ indication of the colour intensity of the wine.	2.7 – 16.5 (7.6)	2.5 – 15.7 (7.6)
Wine hue	▪ describes the brownness of a wine.	0.45 – 1.0 (0.70)	0.48 – 0.99 (0.72)
Total anthocyanins	▪ an estimate of the concentration of anthocyanins in a wine.	125 – 733 (357)	107 – 620 (366)
Total phenolics	▪ an indicator of the concentration of all phenolic compounds in a wine.	26 –96 (50)	23 –67 (50)
Total red pigments	▪ an estimate of the concentration of all red pigments in a wine, these may be monomeric anthocyanins or pigmented oligomers or polymers.		
Degree of red pigment colouration	▪ the percentage of pigments in the wine that are coloured.	10 – 36 (21)	11 – 36 (20)
Ionised pigments	▪ the concentration of ionised anthocyanins present in a wine.	12 – 171 (52)	12 – 150 (49)

APPENDIX F

RED WINE – Basic Small-Scale Technique

Small-scale wine ferments were made at the Experimental Winery at CSIRO Plant Industry, Merbein, Victoria. After crushing, the grapes were divided between four, 20 L buckets that later became the fermentation vessels. The buckets were filled with approximately 17 L of crushed fruit, ensuring that enough space was left at the top of the bucket to prevent excessive pressure and overflow of contents due to carbon dioxide being formed during fermentation. A sample of juice was taken from each bucket in order to determine total soluble solids (ºBrix), pH, and titratable acidity (TA) of the must (performed by winemaker at CSIRO Plant Industry, Merbein Victoria).

To the fermentation bucket 1.0 mL/L of 10% SO_2 solution (to control microbial growth) and 5.0 mL/L of diammonium orthophosphate (a yeast nutrient) was added. Since the grapes was stored in cool rooms prior to crushing, the crushed grapes were allowed to reach room temperature (25°C) before addition of 0.2g dry *Saccharomyces cerevisiae* yeast, strain ICV254D (Lalvin, France) re-hydrated in 10 mL of 35°C water. Tartaric acid was also added at this time. The amount of tartaric acid added was calculated according to titratable acidity (TA) of the crushed fruit (see Table F.1 for additions). Each bucket was equipped with a gas trap, containing 2% SO_2 (as potassium metabisulphite - PMS) solution. The fermentation vessels were then stored at 25ºC until the end of fermentation (defined as the point when the sugar content equalled 0.25% or 2.5g/L). After three-day maceration the fermenting must was pressed to remove skins and seeds, and the juice transferred to a glass bottle and returned to 25ºC to complete fermentation. At the end of fermentation the wine was racked off the gross lees into a glass bottle (with a gas trap inserted into the lid containing 2% SO_2 - as PMS) and 1.0 mL/L of 10% SO_2 (as PMS) solution added to kill the yeast and cease fermentation (first racking). After the first racking the wine was adjusted for tartaric acid levels and transferred to storage at 15ºC, where it remained until second racking.

Second racking involved transferring the wine into another glass bottle minus the sediment and other wine solids. Any adjustments to tartaric acid and SO_2 levels were made at this time (Table F.1). The wine was then transferred to cold storage at 1°C, where it remained until

bottling. At bottling the wines were filtered into glass bottles and once again if necessary the tartaric acid and SO_2 levels are adjusted.

Table F.1 Tartaric acid additions for small-scale wines produced from control and SDI-treated grapes from Cabernet Sauvignon and Shiraz in 2006 at CSIRO - Plant industries, Merbein.

2006 small-scale wine additions	Irrigation Treatment	Wine replicate	Tartaric acid (g/L) added at crushing	Tartaric acid (g/L) added at first rack	Tartaric acid (g/L) added at second rack	Tartaric acid (g/L) added at bottling
Cabernet Sauvignon	100%	1	1.5	1.4	0.2	-
	100%	2	1.6	-	1.5	-
	100%	3	1.6	1.0	0.3	-
	70%	1	1.5	1.3	0.3	-
	70%	2	1.5	-	1.3	-
	70%	3	1.2	1.8	-	-
	52%	1	1.8	1.3	-	-
	52%	2	1.9	1.4	-	-
	52%	3	1.7	-	1.5	-
	45%	1	1.7	1.5	-	-
	45%	2	1.8	1.1	1.3	-
	45%	3	1.8	1.3	0.5	-
Shiraz	100%	1	4.1	1.7	0.5	-
	100%	2	4.6	1.5	0.1	-
	100%	3	5.0	2.9	0.3	-
	65%	1	4.9	2.0	-	-
	65%	2	5.1	2.0	-	-
	65%	3	5.2	2.1	0.1	-
	45%	1	4.4	0.6	-	-
	45%	2	4.9	-	-	-
	45%	3	4.8	0.4	-	-
	34%	1	4.3	2.0	0.2	-
	34%	2	5.1	1.9	0.3	-
	34%	3	5.6	1.9	0.5	-

APPENDIX G

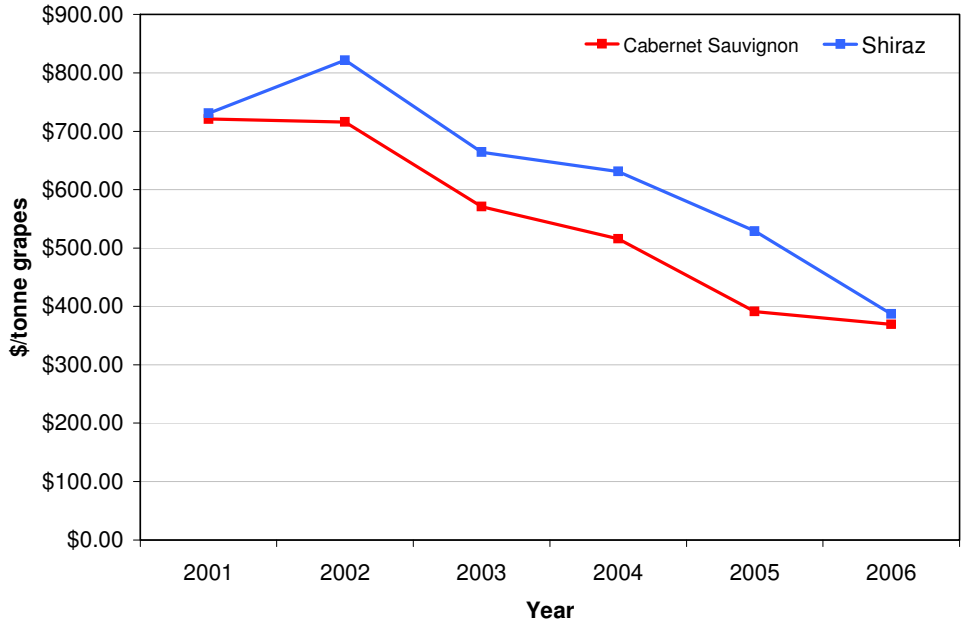


Figure G.1 Weighted average prices from 2001-2006 for Cabernet Sauvignon and Shiraz grown in the Murray-Valley region (prices were obtained from the winegrape utilisation surveys published each year by the Department of Primary Industries in support with regional wineries) (comp. Crothers 2001, 2002, 2003, 2004, 2005, 2006).

APPENDIX H

Table H.1 Total production costs for Cabernet Sauvignon and Shiraz at each irrigation treatment (comp. Patterson & Hill, 2003).

NOTE:
This figure is included on page 180 of the print copy of the thesis held in the University of Adelaide Library.