Cooperative Research Centre for Viticulture

Australian Government
Grape and Wine Research and Development Corporation

OPTIMISING WINEGRAPE QUALITY WITH PARTIAL ROOTZONE DRYING



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CRV 99/24**

Principal Investigator: **Associate Professor Peter Dry - University of Adelaide**

Research Organisation: **Cooperative Research Centre for Viticulture**

Date: **June 2004**

Project Title: Optimising winegrape quality with partial rootzone drying

CRCV Project Number: 2.1.2

Author Details: Assoc. Prof. Peter Dry and Karen Bindon (formerly PhD candidate.
School of Agriculture and Wine, University of Adelaide
Waite Campus, Glen Osmond SA 5064

Date report completed: June 2004

Publisher: Cooperative Research Centre for Viticulture

Cover picture: Bagged bunches at Nuriootpa trial site

ACKNOWLEDGEMENTS

The authors would like to acknowledge:

- the project team and industry contributors as listed in Appendix 3; and
- the members of CRCV Industry Reference Group.

The Cooperative Research Centre for Viticulture is a joint-venture between Australia's viticulture industry and leading research and education organisations. It promotes cooperative scientific research to accelerate quality viticultural management from vine to palate. Australian grapegrowers and winemakers are key stakeholders in the CRCV, contributing levies matched by the Commonwealth Government and invested by the Grape and Wine Research and Development Corporation in the Centre.

Copyright:
© Copyright in the content of this report is owned by the Cooperative Research Centre for Viticulture.

Disclaimer:
The information contained in this report is a guide only. It is not intended to be comprehensive, nor does it constitute advice. The Cooperative Research Centre for Viticulture accepts no responsibility for the consequences of the use of this information. You should seek expert advice in order to determine whether application of any of the information provided in this guide would be useful in your circumstances.

Contents

1. Abstract 4
2. Executive Summary 5
3. Background 7
4. Project aims and performance targets 8
5. Method 9
   - 5.1 Experimental sites and irrigation strategies 9
   - 5.2 Bunch exposure 9
   - 5.3 Temperature 9
   - 5.4 Shoot growth and stomatal conductance 10
   - 5.5 Berry sampling and maturity analysis 10
   - 5.6 Measures of berry composition 10
   - 5.7 Mini-winemaking procedure 11
6. Results/Discussion 12
   - 6.1 How does PRD affect secondary metabolism in grape berries? 12
   - 6.2 The contribution of berry size to the concentration of secondary metabolites 14
   - 6.3 Bunch exposure 17
     - 6.3.1 Anthocyanins 18
     - 6.3.2 Precursors to aroma compounds 19
   - 6.4 Yield versus quality 23
     - 6.4.1 Soil type, location and irrigation cycling 23
     - 6.4.2 Node number per vine 24
7. Outcomes/Conclusions 26
8. Recommendations 27
9. Appendix 1: Communication 29
10. Appendix 2: Intellectual Property 30
11. Appendix 3: References 31
12. Appendix 4: Staff 33

List of Figures

Figure 1. Summary of some of the responses of Cabernet Sauvignon grape berries to PRD observed in the current study 13
Figure 2. Linear regression of anthocyanin versus berry weight for Cabernet Sauvignon A. 2002 mg/g B. 2003 mg/g; C. 2002 mg/berry; D. 2003 mg/berry Control; PRD; n=42; ns = not significant. 15
Figure 3. Linear regression of total phenolics versus berry weight for Cabernet Sauvignon A. 2002 A_{280}units/g B. 2003 A_{280}units /g; C. 2002 A_{280}units /berry; D. 2003 A_{280}units /berry; Control; PRD; n = 42. 15
Figure 4. Linear regression analysis of the relationship between seed tannin (proanthocyanidin) and berry weight for Cabernet Sauvignon in 2003 A. concentration per gram B. content per berry; Control; PRD; n = 42, ns = not significant 16
Figure 5. The effect of PRD on the relative contribution of the five component anthocyanidin types in A. grapes (ANOVA, n = 42) and B. wines (ANOVA, n = 12). 19
Figure 6. Effect of PRD on the concentration of lutein over berry development for the 2002 vintage (ANOVA; n=42; *=P<0.05; **=P<0.01) 22
Figure 7. Effect of PRD on the concentration of β-carotene over berry development for the 2002 vintage (ANOVA; n=42; *=P<0.05) 22

List of Tables

Table 1: Wine pigmentation in response to PRD and standard irrigation treatments for the 2002 vintage (ANOVA, n=12) 17
Table 2: Total C_{13}-norisoprenoids in PRD-treated Cabernet Sauvignon grown at Langhorne Creek (ANOVA; n=42) 20

1. Abstract

The effect of PRD on the composition of secondary metabolites important to grape and wine quality has been investigated. The results of the current study show that at harvest, total anthocyanin and phenolic concentration in *Vitis vinifera* L. cvs. Shiraz and Cabernet Sauvignon fruit was either unaltered or increased with PRD over two seasons. Where an increase in anthocyanin concentration was found with PRD, this was found to be independent of berry size. Rather, increases in anthocyanin concentration are most likely due to an increase in anthocyanin production in the berry skins. In wine made from one vintage of Cabernet Sauvignon, total colour was enhanced 10% by the PRD treatment. This improvement in wine colour was due to a change in the structure of coloured wine pigments (co-pigmentation) rather than due to increased anthocyanin concentration in the wines. Preliminary results of this study on Shiraz indicated that the concentration of the precursors to the aroma compounds ß-damascenone and TDN (1, 1, 6 trimethyl dihydronaphthalene) can potentially be influenced by changes in vine vigour, induced by a water-deficit. This finding was validated by a study on Cabernet Sauvignon, where the effect of PRD on fruit C_{13}-norisoprenoids was investigated. The concentrations of the C_{13}-norisoprenoids ß-damascenone, ß-ionone and TDN were shown to be increased up to 30% with PRD in Cabernet Sauvignon fruit over two vintages. The changes in these compounds in the fruit were associated with increased levels of the carotenoids lutein and ß-carotene, which are potential precursors to the C_{13}-norisoprenoids.

2. Executive summary

The project has explored the application of the novel irrigation technique, partial rootzone drying (PRD) in two of South Australia's important growing regions: the Barossa Valley and Langhorne Creek. This technique applies water to one half of the vine's root system at a time, alternating the irrigation pattern every 7 to 10 days. Half of the vine's root system is effectively in drying soil at any one time, producing plant signals, which cause the vine to down-scale its water use. In an ideal situation, water supplied to the other half of the root system prevents the vine from experiencing water stress. Because the vine's system adjusts to a lowered level of water use through reduced stomatal conductance, this minimises the chance of yield loss due to water stress, and effectively cuts the irrigation water required by up to 50%. Under the conditions of the current study, it was found that the PRD treatment caused a decrease in leaf water potential, in addition to lowering leaf stomatal conductance, which was indicative of a mild hydraulic stress in the treatment vines relative to the controls. Moreover, in some experiments PRD caused a restriction in berry size, resulting in a small yield loss. As the application of PRD in the current study showed a different response from the expected non-hydraulically mediated reduction in stomatal conductance, it must be noted that some of the observed changes in grape berry metabolism with PRD may in part be a result of hydraulic water stress. Because the effectiveness of PRD as an irrigation strategy is largely dependent upon soil type, this will become an increasingly important consideration in the choice of irrigation strategy by grapegrowers in the future (Kriedemann and Goodwin, 2003).

The benefit of the PRD technique is that plant signals, which trigger a reduction in the vine's water use, also have an important role in the growth and development of the fruit. The outcomes of the project have shown that some compounds which are important to wine quality can be enhanced through the application of the PRD technique. Amongst these are the anthocyanins and phenolics, which determine the final colour in red wines of grape varieties such as Shiraz and Cabernet Sauvignon. Unexpectedly, this was found to be independent of berry size, which has traditionally been thought to hold an inverse relationship with the concentration of anthocyanin. The results of the current study indicate that the application of a mild water stress to developing fruit may result in increased concentration of anthocyanins in berry skins, rather than merely causing an alteration of the

ratio of skin to fruit volume. However, a 10% improvement in wine colour was shown in wines in response to PRD treatment, even where the initial analysis of fruit composition had not shown a corresponding increase in anthocyanin concentration. This was caused by enhanced binding of wine pigments to form larger, more stable coloured compounds, a chemical phenomenon knows as co-pigmentation. This is most likely caused by an increase in non-anthocyanin phenolic compounds in wines, in response to the PRD treatment. This phenomenon additionally improved the quality of wines produced from PRD-treated fruit by an increase in the long-term stability of wine colour. This research has advanced our understanding of the means by which grape and wine aroma may be manipulated. PRD produces a biochemical response in fruit, which leads to increased production of some important volatile aroma compounds called the norisoprenoids. These are present in extremely low concentrations in wines but cause a strong sensory response, so that only small changes in their production in fruit could cause a large change in what the consumer perceives as aroma and flavour in the wine. Previously, it has been thought that the amount of sunlight reaching developing berries was a primary factor governing the quantity of these aroma compounds produced in the fruit at harvest. However, the results of the current study have shown that the application of a water deficit to developing fruit may have a far greater effect on the final concentration of these compounds than bunch exposure alone. Of course it is not entirely possible to separate these effects because water deficits generally lead to less shoot vigour and more open canopies.

Taking deficit irrigation strategies like PRD a step further, it may be possible for growers to produce higher quality fruit with greater potential for both colour and aroma in the resulting wines. Although this would be a significant achievement in itself, the PRD irrigation technique has the additional advantage of minimising water use. Following continued research into the application of this technique in the different growing regions of Australia, PRD holds the potential to become a powerful tool to the Australian wine industry.

3. Background

In previous studies, PRD has been shown to improve water use efficiency by halving the amount of water applied to vines by irrigation (Dry *et al.* 2000), without causing the reduction in yield and berry size that is generally associated with other deficit irrigation strategies. Added to this, PRD was proposed to potentially improve grape composition, through increased anthocyanin and phenolic content per berry. The proposed study aimed to further explore aspects of grape and wine composition that may be affected by PRD. It was proposed that while maintaining berry size and yield, the PRD technique would positively alter the content of secondary metabolites per berry. The potential exists that other secondary metabolic pathways important to grape and wine quality may also be affected by the PRD treatment, namely the isoprenoid pathway, which is important in the determination of flavour (Antolin *et al.* 2003).

The underlying biochemical mechanism responsible for possible changes in secondary metabolism in response to PRD is unknown. In the study by Dry *et al.* (2000), PRD-treated Cabernet Sauvignon vines were found to have significantly higher berry anthocyanins and phenolics than control treatments when the bunches were better exposed by a factor of 2.7. No significant differences in these parameters were observed when bunch exposure between PRD and control vines differed by a factor of 1.4. This led to the hypothesis that improved bunch exposure due to reduced shoot growth in PRD-treated vines may influence these products in grapes. Wine produced from PRD-treated vines was also rated more highly for aroma characteristics than that made from control vines for a given season (Dry *et al*. 2000). As both phenolic and aroma compounds are potentially influenced by bunch exposure, its contribution to the final concentration of these metabolites in PRD-treated grapes and wines is relevant to the current study.

4. Project Aims and Performance Targets

- Determine the mechanisms responsible for PRD effects on fruit composition and wine quality
- Determine the effect of the interaction between PRD and pruning level on berry size and consequent fruit composition and wine quality.
- Determine the effect of a combination of early water deficit and PRD on berry size and consequent fruit composition and wine quality
- Determine the effects of PRD on wine quality and the consequences for long term use of the PRD technique.

OUTPUTS/PERFORMANCE TARGETS

Output	Performance Targets	Date
1. Information on the mechanism by which PRD may increase concentration of secondary metabolites in grape berries	Measurement of quantitative changes in phenolic compounds and other secondary metabolites with emphasis on qualitative changes in anthocyanin species and β-damascenone.	Completed 2002
2. Information on interaction between PRD and pruning level or bunch thinning on berry size and fruit composition in different varieties	Data available from existing experiments at Nuriootpa, Waite and a new experiment at Langhorne Creek.	"
3. Information on effect of interaction of cytokinins (CK) and abscisic acid (ABA) on fruit composition to provide a better understanding of the physiology of PRD.	Data from experiment to be conducted at Waite where CK and ABA will be exogenously applied to berries	June 2003
4. Recommendations on use of PRD to manipulate grape end-use specification	Collection and interpretation of data from all experiments	Dec 2003
5. PhD thesis completed		March 2004

5. Methods

5.1 Experimental sites and irrigation strategies

The experimental vineyard sites were at the PIRSA/SARDI research station at Nuriootpa, located in the Barossa Valley, SA and at a commercial vineyard, Orlando-Wyndham, Langhorne Creek, SA. The former experiment was on Shiraz, which was subjected to three different pruning levels (30, 60 and 120 nodes/vine), superimposed over PRD or standard irrigation strategies. The latter experiment was on Cabernet Sauvignon, with all vines pruned to 60 nodes. In both experiments, treatments were set up in a randomised block design, with 2 vines per experimental plot. In the Shiraz experiment at Nuriootpa, each treatment was replicated 5 times, and in Cabernet Sauvignon, 3 treatment replicates were used, with 7 two-vine sub-plots. Vines were irrigated with drip emitters, either on both sides of the root system (control), or one side at a time (PRD) for 7-10 day intervals. Drip emitters were be positioned 45 cm either side of the vine trunk.

5.2 Bunch exposure

Photosynthetically-active radiation (PAR) intercepted by developing bunches was measured throughout the growing season with a ceptometer. The ceptometer was inserted into the bunch zone of experimental vines in an east-to-west direction, and measurements taken vertically (0°) and at 45° and 90° north and south of the original measurement. To determine the effects of a combination of PRD irrigation and total shade on bunches, polyethylene boxes (designed by Mark Downey, CSIRO) were placed over developing bunches at berry set. Polyethylene boxes were prepared by painting the interior of the box black to prevent light penetration, leaving the outer surface white to maximise light reflection.

5.3 Temperature

The temperature of bunches was determined using thermocouples positioned within shaded bunches, enclosed in polyethylene boxes; and within sun-exposed bunches. Thermocouples were also positioned within the canopy. Temperature was recorded at half-hour intervals from veraison to harvest.

5.4 Shoot growth and stomatal conductance

Prior to the application of the PRD treatment, 6 shoots per plot were randomly selected from the experimental vines and tagged below the apical 10 internodes. At weekly intervals until the cessation of shoot growth, shoot lengths were determined by measuring from the node above the tag to the shoot tip. In randomly selected, sun-exposed leaves, stomatal conductance was determined diurnally and at different stages during a PRD cycle.

5.5 Berry sampling and maturity analysis

Berries were collected at weekly intervals from one month pre-veraison, until harvest at approximately 24 °Brix. Two fifty-berry samples were collected from each vine plot for a given sampling date. One fifty-berry sample was weighed and immediately processed to determine berry maturity, and a second one stored at -20ºC for later analysis. For berry maturity analysis, juice was squeezed from a fresh 50-berry sample, and centrifuged at 3500 rpm for 10 minutes. Juice samples were analysed for total soluble solids (°Brix) using a refractometer, pH using a pH meter and titratable acidity (TA) was determined on samples diluted 1:5 juice:distilled water using an automated titrator.

5.6 Measures of berry composition

Frozen 50-berry samples were divided into sets of 25 berries and homogenised at high speed using an Ultra-Turrax homogeniser. For analysis of anthocyanin and phenolic concentration, one gram of the slurry was extracted in 50% (v/v) EtOH (pH 2) for 1 h and then centrifuged at 3500 rpm for 5 min. An aliquot of the ethanol extract was diluted 1:11 in 1 M HCl, and left to stand for 3 h. The absorbance of aliquots of the diluted HCl extract solution will be determined at 520 nm and 280 nm for anthocyanins and phenolics respectively (Iland *et al*. 2000). For HPLC analysis of anthocyanins, an aliquot of the original EtOH extract was injected onto a Merck LiChrosopher 100 RP-18 (5μm) 4x250mm HPLC column with photodiode array detection at 520 nm. The HPLC system used was a Hewlett Packard HP 1100.

C_{13}-norisoprenoid aroma volatiles were determined using a combination of solid-phase-micro-extraction (SPME) and GCMS. Two g of grape homogenate samples were sealed in glass vials and heated to 100°C for 1 h in the presence of acid. A polydimethylsiloxane SPME fibre was inserted directly through the cap of the headspace vial, and exposed to the

headspace of the homogenate sample for a further 1 h. The SPME fibre was then removed and inserted into a GC injection port at 220°C, and analysed by GCMS. Carotenoids were extracted in hexane:actone:water from a further 1 g of the berry homogenate and separated by HPLC using a Merck LiChrosopher 100 RP-18 (5μm) 4x250mm HPLC column, with photodiode array detection at 450 nm.

5.7 Mini-winemaking procedure

Wine was made in one vintage from fruit harvested from the Langhorne Creek Cabernet Sauvignon experiment. Approximately 30 kg of fruit was allocated to each winemaking replicate. Fruit were de-stemmed and crushed into 18 L fermenters and 4% SO_2 solution was added during crushing at 12 mL per 10 L of must. Following this, 8% DAP was added at 1 mL per L of must and pH was adjusted to 3.6 by addition of tartaric acid. The musts were then inoculated with yeast (EC 1118 *Saccharomyces cerevisiae*) at 0.25 g/L. Wines were fermented at 15 ºC on skins for 10 days, and plunged every 6 hours. When the levels of total soluble solids were below a threshold the wines were pressed and transferred to glass 12 L fermenters equipped with fermentation locks. Fermentation was continued at 20 ºC for a further 11 days until reducing sugars were measured at below 0.25%. Wines were then racked from the lees to fresh 12 L glass fermenters and subjected to malolactic fermentation. Wines were then racked, pH was re-adjusted to 3.6 with tartaric acid. Then SO_2 was added to give a total SO_2 value of 60 ppm. Wines were bottled in 750 mL glass bottles and sealed with screw caps. Wine colour was analysed using spectrophotometric methods at bottling and after one year in storage. Total C_{13}-norisoprenoid aroma precursors were assessed on bottled wine using the method described above.

6. Results and Discussion

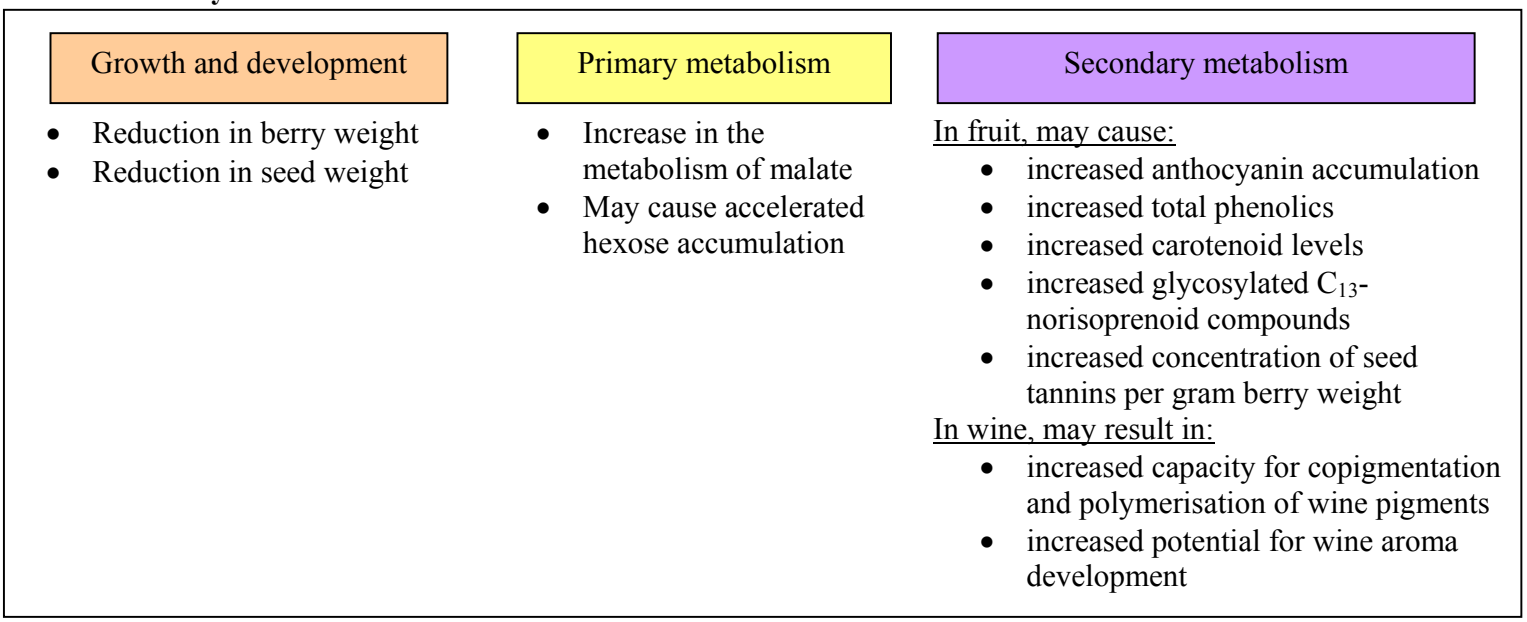
The aim of the current study was to explore the potential effects of the PRD irrigation technique on grape **secondary metabolic** pathways and their contribution to **grape and wine quality**. However, other factors have the potential to strongly influence both the concentration and proportion of the different groups of secondary metabolic compounds in the grape berry. Thus, the effect of PRD on other factors such as **primary carbon metabolism, berry size and bunch exposure** was also explored to ascertain their potential influence on secondary metabolism.

6.1 How does PRD affect secondary metabolism in grape berries?

A summary of some of the potential responses of grape berry metabolism to PRD irrigation is shown in Figure 1, drawn from the results of the PRD experiment on Cabernet Sauvignon grapevines. In that experiment, a primary response to the PRD treatment was a reduction in berry weight and seed weight from early in fruit development relative to the control treatment. This response was accompanied by increased accumulation of hexose sugars in the PRD treatment in one season of the study (2002) and an increased respiration of malic acid was observed over two seasons. Furthermore, PRD showed the potential to increase the accumulation of anthocyanins and total phenolics in grape berries, measured as milligram per gram berry weight. The increase in the concentration of total phenolics due to PRD was found to be due in part to increased contribution of seed-derived proanthocyanidins and flavan-3-ol monomers. In PRD wines, there was shown to be an improvement in colour that was independent of anthocyanin concentration. Rather, this was shown to be due to increased capacity of anthocyanins in PRD wines to form co-pigmented or polymeric associations with other compounds present in the wine. This may be due to a relatively higher contribution of non-anthocyanin phenolic compounds in wines produced from PRD-treated fruit, compared with that made from control treatments. Additionally, PRD increased metabolism of isoprenoid secondary metabolites, as estimated from the concentration of the C_{13}-norisoprenoid group of glycosylated flavour/aroma compounds, and their carotenoid precursors.

Thus it was shown that PRD has the potential to influence the concentration of two groups of secondary metabolites: the phenols, including anthocyanins, tannins and other phenolics; and the isoprenoids, including carotenoids and C_{13}-norisoprenoids. As both these groups have been shown to contribute significantly to wine quality in terms of colour, astringency, mouthfeel and aroma/flavour, these results indicate that the PRD irrigation strategy holds the potential to improve wine quality. However, for this to be best optimised, the underlying biochemical basis for these changes in fruit composition in response to PRD must be better understood.

Figure 1 Summary of some of the responses of Cabernet Sauvignon grape berries to PRD observed in the current study



6.2 The contribution of berry size to the concentration of secondary metabolites

In the current study, the production of smaller berries by Cabernet Sauvignon or Shiraz vines was shown to be a response to carbon limitation. This was either due to an increase in the size of the reproductive sink for photosynthetically-derived carbon as a response to higher node number per vine, for a smaller canopy size over which carbon could be fixed. Or, when berry size was reduced as a direct response to the PRD treatment, this may have been due to a carbon limitation resulting from a reduction in photosynthesis associated with decreased stomatal conductance due to PRD. But, it could additionally have been caused by a direct hydraulic effect of water deficit induced by the PRD treatment, which altered the expression of genes controlling cell growth and development in both berries and seeds.

In both the Cabernet Sauvignon and Shiraz PRD experiments, the opportunity was created for analysis of the contribution of berry size to the concentration of secondary metabolites. Theoretically, smaller berries should have a larger surface area to volume ratio, which means that skin-derived secondary metabolites would be extracted in a smaller volume of must during fermentation, potentially increasing their concentration in the resulting wines. However, this hypothesis does not account for the possibility of skin density or thickness being increased in large berries, and ignores the potential contribution of phenolics derived

from the seeds and isoprenoids derived from the flesh to the total measure of secondary metabolites.

The current study has shown that for a range of berry weights, produced either by varying node number per vine or as a response to water stress occurring in the PRD treatment, there is only a weak effect of berry size on the concentration of anthocyanins derived from the skin (Figure 2). Thus, smaller berries did not have an increased concentration of anthocyanins. For the measure of total phenolics, however, a different response was obtained. In Cabernet Sauvignon, decreased berry size had the potential to increase the concentration of phenolics in grapes and wines (Figure 3).

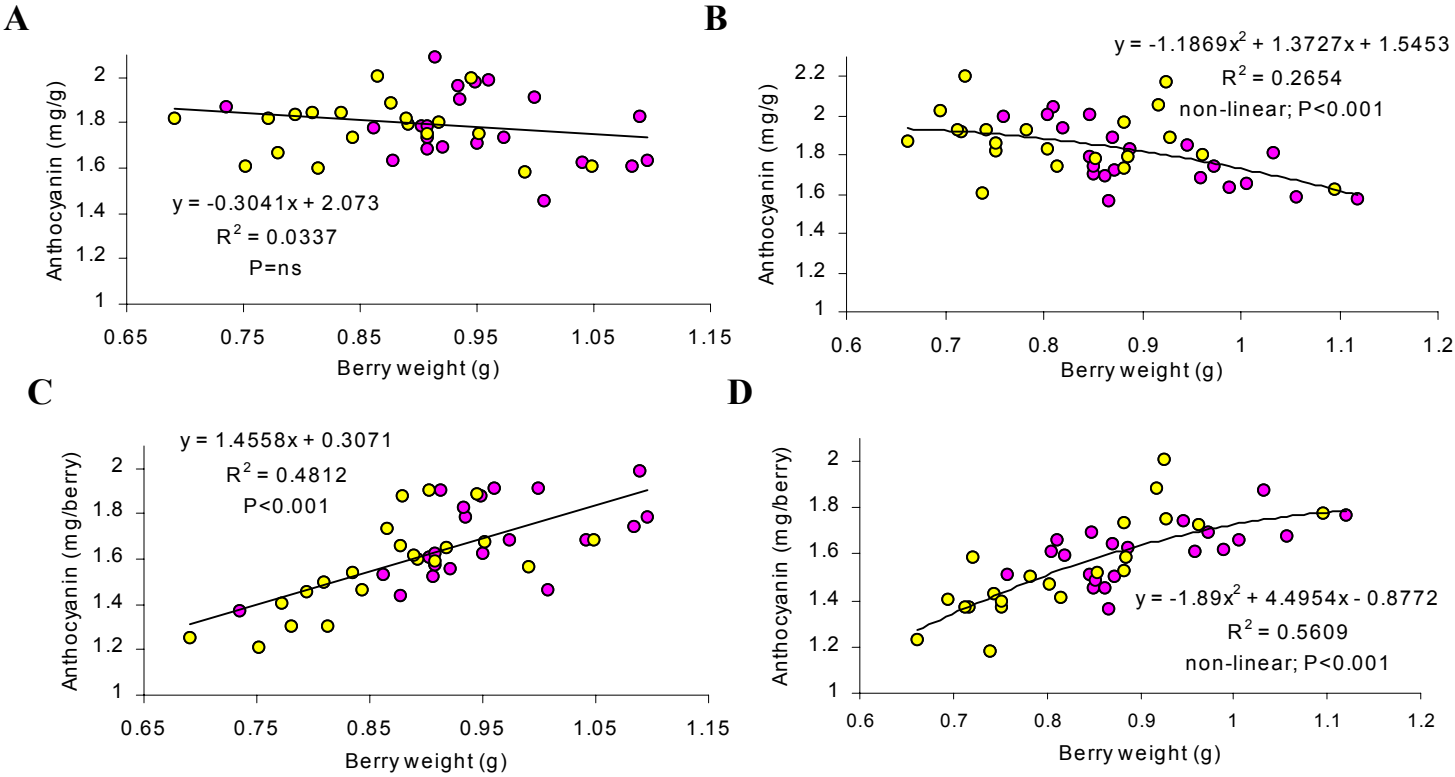


Figure 2 Linear regression of anthocyanin versus berry weight for Cabernet Sauvignon **A.** 2002 mg/g **B.** 2003 mg/g; **C**. 2002 mg/berry; **D.** 2003 mg/berry ●Control; ○ PRD; n=42; ns = not significant.

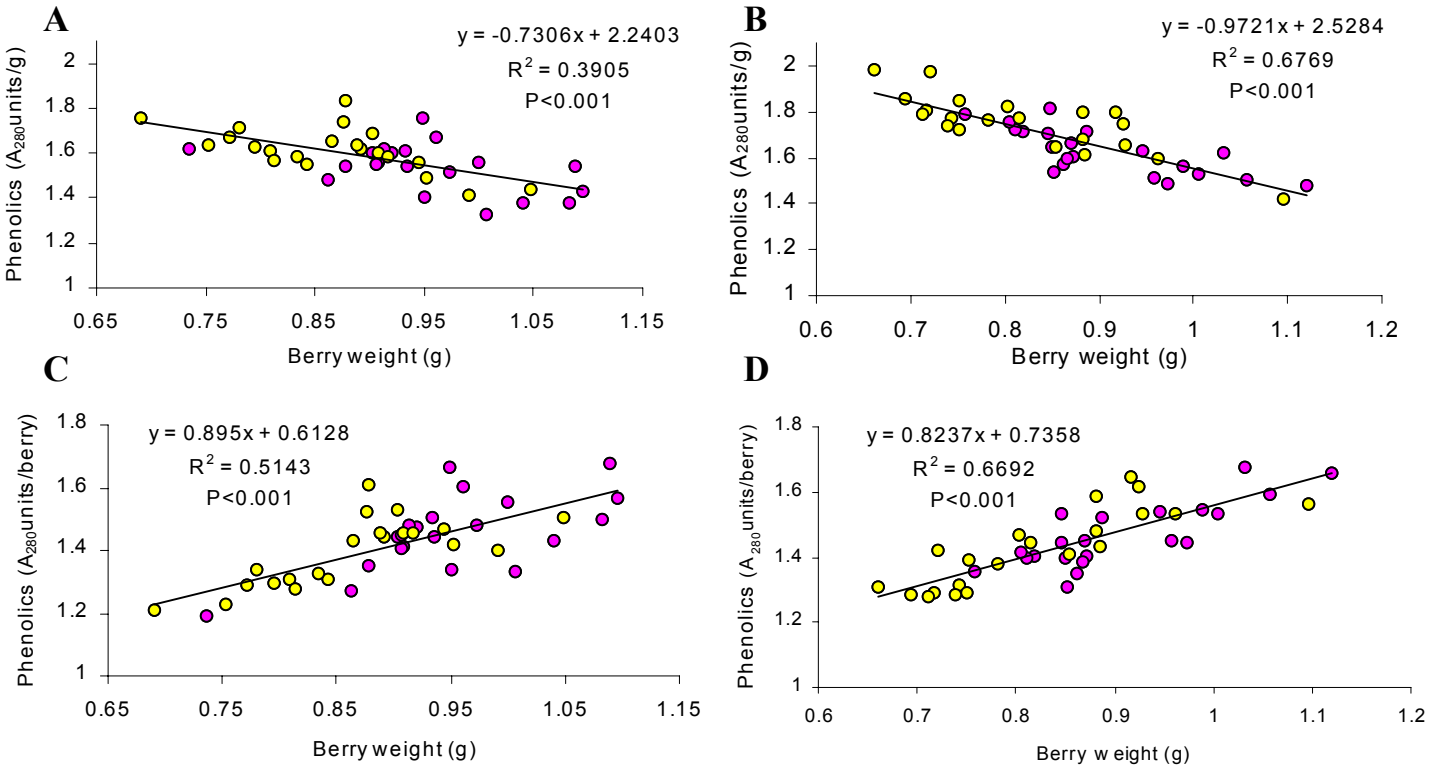


Figure 3 Linear regression of total phenolics versus berry weight for Cabernet Sauvignon **A**. 2002 A_{280}units/g **B**. 2003 A_{280}units /g; **C.** 2002 A_{280}units /berry; **D.** 2003 A_{280}units /berry; ●Control; ○ PRD; n = 42.

An analysis of the contribution of seed-derived phenolics to the measure of total phenolics showed that seed tannin concentration was increased as berry weight decreased in Cabernet Sauvignon (Figure 4). It is therefore possible that in PRD-treated fruit from the Cabernet Sauvignon experiment, there is an increased contribution of seed-derived phenolics, due to a reduction in berry size

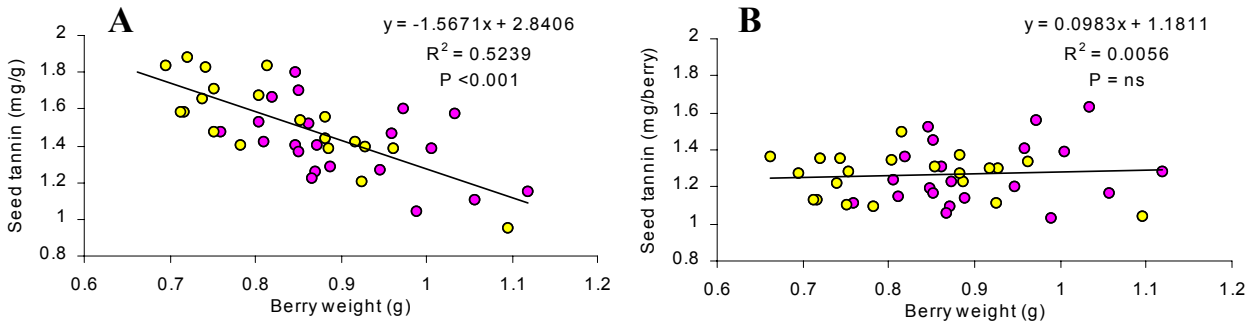


Figure 4: Linear regression analysis of the relationship between seed tannin (proanthocyanidin) and berry weight for Cabernet Sauvignon in 2003 **A**. concentration per gram **B**. content per berry; ● Control; ● PRD; n = 42, ns = not significant

These findings have important implications for our current understanding of the influence of fruit composition on wine quality. If wine colour in red varieties is used as a potential indicator of wine quality, then the winemaking experiment from the current study has shown that using PRD irrigation can improve colour by 10% (Table 1). Traditionally, this would be thought to be due to increased concentration of anthocyanins in the wine. However, our understanding of the effects of co-pigmentation and polymerisation of wine pigments has grown in recent years (Boulton, 2001) and the current results reiterate that anthocyanin concentration alone is not the primary factor influencing wine colour. Decreased berry size can potentially increase the concentration of non-anthocyanin phenolics, which in turn have the potential to act as intermolecular co-pigments in wines. This relationship may partly account for results such as those of Freeman (1983), who demonstrated a strong negative correlation between wine colour and berry weight in wines produced from irrigated versus non-irrigated fruit. In that study, however, no significant relationship was detected for berry weight and anthocyanin colour, indicating the contribution of an additional 'unknown' component in the determination of final wine colour. Analysis of phenolic compounds in wine by HPLC indicated that, although total tannin (pigmented polymer) was increased in PRD-treated wines, there was also a

significant increase in non-anthocyanin wine phenolic compounds in response to PRD. As grape seed tannins have been shown to be weak co-pigments in red wines, it is likely that the unknown co-pigment increased in PRD-treated wines falls within the latter category of phenolic compounds.

Table 1: Wine pigmentation in response to PRD and standard irrigation treatments for the 2002 vintage (ANOVA, n=12)

Red wine colour measures	Control	PRD	%	P
Wine Colour Density	7.1	8.1	15	<0.001
Wine Colour Hue	0.69	0.66	-3	ns
Degree of red pigment colouration (%)	21.9	24.3	11	<0.01
Total red pigments (A_{520} HCl)	19.2	20.1	5	<0.05
Modified wine colour density	9.3	10.3	11	<0.001
Modified wine colour hue	0.59	0.58	-1	ns
Modified degree of red pigment colouration (%)	30.6	32.6	6	<0.05
Total phenolics (A_{280} HCl)	34.4	37.3	8	<0.001
Copigmentation results	Control	PRD	%	P
Anthocyanins in copigment complex (mg/L)	30.6	34.1	11	<0.001
Anthocyanins in free monomeric form (mg/L)	247.2	248.7	1	ns
Colour of anthocyanins in polymeric form (A_{520}units)	2.0	2.2	12	<0.001
Total Colour	9.4	10.4	10	<0.001
% Colour due to copigment complex	64.1	65.0	1	<0.01
% Colour due to anthocyanins	14.9	13.6	-10	<0.001
% Colour due to polymer	21.0	21.5	2	ns

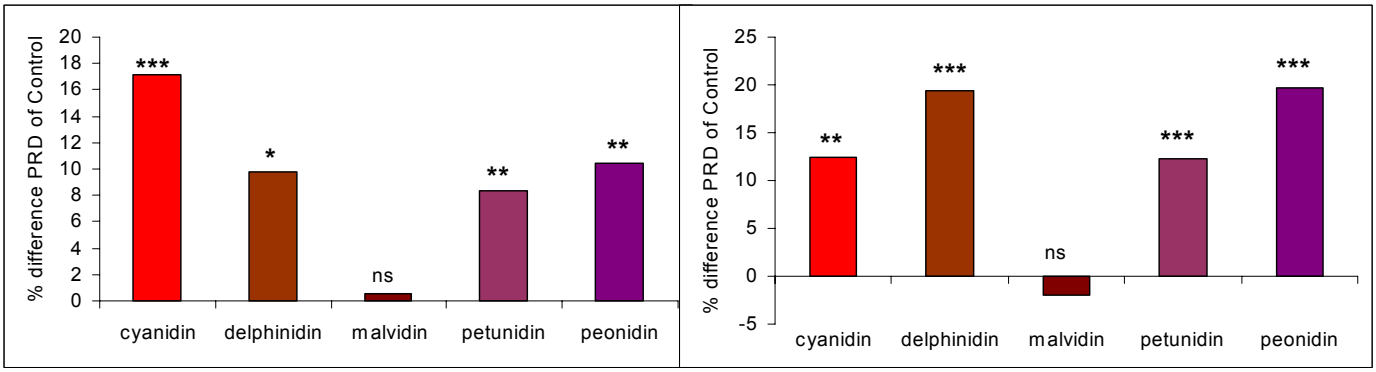
6.3 Bunch exposure

A second key question addressed in the current study was the importance of light interception by the fruiting zone of grapevines in determining final levels of secondary metabolites in harvested fruit. From a range of measures of bunch exposure within the fruiting zone of PRD-irrigated and standard-irrigated vines in the current study, it was evident that PRD irrigation has the potential to significantly increase light incidence within the canopy, most likely due to a decrease in lateral leaf area (Dry 1997; Stoll 2000). From the literature, it would be expected that increasing levels of bunch exposure may potentially increase the concentration of anthocyanins, and certain C_{13}-norisoprenoids (TDN) in grape berries (Bergqvist *et al*. 2001, Marais *et al.* 1992), although carotenoids have been shown to degrade under high light intensities (Marais *et al.* 1991).

6.3.1 Anthocyanins

In the case of the anthocyanins, total shade was shown to significantly reduce anthocyanin concentration in grape berries relative to fruit that developed within the vine canopy. When the shading treatment was superimposed over PRD and standard irrigation treatments in the Cabernet Sauvignon experiment at Langhorne Creek, the increase in anthocyanin concentration, which was obtained in sun-exposed PRD-treated fruit, was not found for shaded fruit. This suggested that the increase in anthocyanins under the PRD treatment was mediated, at least in part, by higher levels of bunch exposure in those vines. However, comparison of fruit sampled from PRD- and control-treated Cabernet Sauvignon grown in pots where berries had the same level of exposure to light showed a large (27%) increase in the anthocyanin content of berry skins, which suggested a direct response of the anthocyanin metabolic pathway to the PRD treatment that was independent of bunch exposure. Closer examination of the composition of anthocyanins, in terms of the relative proportion of anthocyanidin types, and the pattern of glycosidation showed that the response of the anthocyanins to the PRD treatment was not typical of that expected for increased exposure to sunlight. Instead of showing a decrease in the proportion of the coumarate forms of anthocyanins, a response previously shown for fruit under increased irradiation (Haselgrove *et al.* 2000), anthocyanins under the PRD treatment showed a shift in the relative contribution of anthocyanidin types in field-grown vines. Non-malvidin anthocyanidins were significantly increased by PRD at the expense of malvidin, although the effect on malvidin was small (Figure 5). This effect was evident in both grape and wine samples. A conclusion from this result was that the response of grape berry anthocyanins to PRD requires sunlight, as demonstrated by the shading experiment, but is not exclusively mediated by increased levels of sunlight reaching PRD-treated fruit. Rather, signals produced by the PRD treatment appear to have a direct effect on anthocyanin metabolism, which can occur even where berries receive equivalent levels of sunlight during their development. In the presence of high levels of sun exposure in the case of field-grown vines, it appears that the distribution of the anthocyanins into a diverse range of anthocyanidin types would increase the effectiveness of the berry skin to photo-protection by absorption of a variety of wavelengths of radiation, but also appears to increase the radical scavenging capacity of the fruit in the protection against oxidative stress. This appears to be a response to increased potential for oxidative damage within the fruit under high levels of light incidence, which would be exacerbated by the oxidative conditions caused by an additional water deficit brought in the PRD treatment.

Figure 5: The effect of PRD on the relative contribution of the five component anthocyanidin types in A. grapes (ANOVA, n = 42) and B. wines (ANOVA, n = 12).



6.3.2 Precursors to aroma compounds

The current study has shed some light on our current thinking about the response of C_{13}-norisoprenoids and carotenoids to sunlight. In Shiraz fruit grown at Nuriootpa, it became evident that total shading of bunches from berry set produced grapes and wines with very low levels of C_{13}-norisoprenoids. However, only a small amount of sunlight penetration to the canopy interior was required to restore C_{13}-norisoprenoid levels. This was shown through a canopy-management experiment where three different levels of bunch exposure were compared. There was little difference in the levels of the C_{13}-norisoprenoids β-damascenone and β-ionone in fruit sampled from shaded canopies and highly exposed canopies. However, TDN showed a significant response to higher levels of irradiation, which has been shown in previous studies (Marais *et al*. 1992). The aim of the current study was to explore the influence of irrigation strategy on C_{13}-norisoprenoid metabolism, and to explore the potential contribution of bunch exposure to the observed response. Interestingly, a deficit irrigation treatment on Shiraz—samples were obtained from another experiment (Ristic 2003)—significantly increased levels of both β-damascenone and TDN, even though bunch exposure was equivalent to levels observed for a moderately-exposed canopy.

This response shows that these C_{13}-norisoprenoids can be affected by a metabolic response to water stress alone, and are not necessarily regulated by external factors such as sunlight. The PRD irrigation experiment on Cabernet Sauvignon at Langhorne Creek additionally showed that over two seasons, C_{13}-norisoprenoid concentration could be increased in grape berries (Table 2). In the light of the results of the canopy trial on Shiraz, this response is not

likely to be due to the slight increase (30%) in sunlight incidence of the fruiting zone of PRD vines. Rather, there appears to be a metabolic response of the isoprenoid pathway to the PRD treatment, due to changes in either hydraulic or non-hydraulic signals or both produced in response to soil drying.

Table 2: Total C_{13}-norisoprenoids in PRD-treated Cabernet Sauvignon grown at Langhorne Creek (ANOVA; n=42)

	2002 Vintage				2003 Vintage			
	Control	PRD	% PRD> Control	P	Control	PRD	% PRD> Control	P
β-damascenone (ng/g)	30.44	34.94	12.9	<0.05	34.80	45.60	23.7	<0.01
β-ionone (ng/g)	17.11	19.53	12.4	ns	12.57	15.51	19.0	<0.05
TDN (ng/g)	5.90	7.46	20.9	<0.05	4.19	6.25	33.0	<0.01

An interesting finding was that carotenoid concentration increased in response to the PRD treatment. This was reflected in increases in the most abundant grape berry carotenoids lutein and β-carotene at different stages of berry maturity (Figures 6 and 7). Traditionally, increases in C_{13}-norisoprenoid concentration have been associated with increased degradation of carotenoid precursors, leading to a reduction in the total pool of carotenoids (Marais *et al.* 1991, Baumes *et al.* 2002). However, an increase in the pool size of carotenoid precursor in response to water limitation was recently shown by Oliveira *et al.* (2003), where non-irrigated treatment caused 60% increase in carotenoid concentration compared with an irrigated control. This finding is in agreement with the results of the current study, indicating that water stress can result in an increase in the pool size of carotenoid precursors to the C_{13}-norisoprenoids, and may in part account for the increase in certain C_{13}-norisoprenoids in response to PRD. This result, in conjunction with the findings of the current study shows that deficit irrigation has the potential to increase flux in the isoprenoid pathway, presumably toward increased synthesis of the stress hormone ABA via the carotenoids (Lichtenthaler, 1999). This response to stress conditions experienced by the vine has the potential to increase the pool size of precursors to carotenoid-derived flavour compounds, which include the C_{13}-norisoprenoids. This has important implications for

flavour development in grapes and wines, and deficit irrigation may become a significant tool to manipulate wine quality in the future. It is important to note that this increase in wine quality is independent of any change in berry size, or indeed yield per vine.

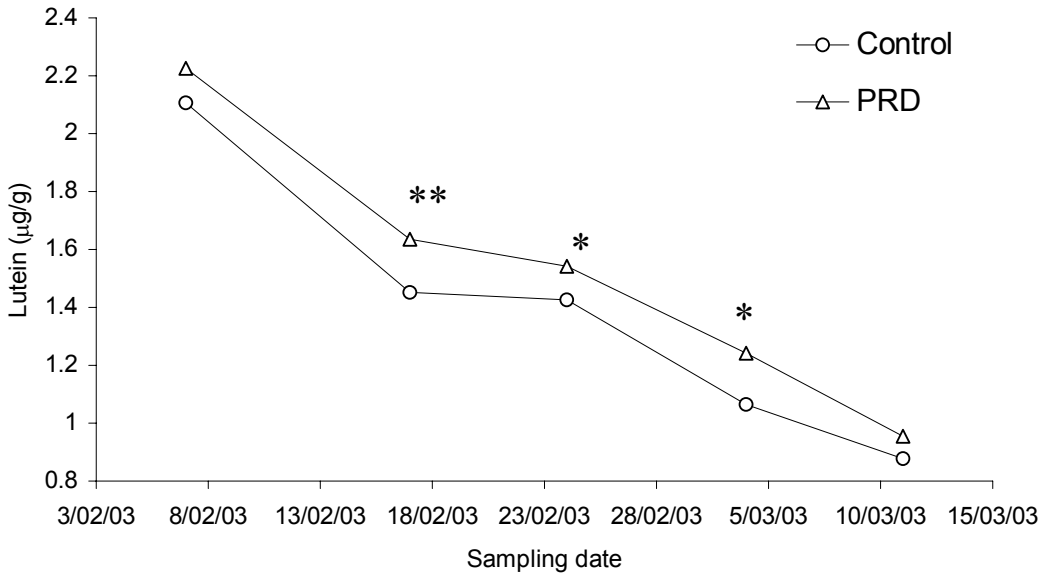


Figure 6: Effect of PRD on the concentration of lutein over berry development for the 2002 vintage (ANOVA; n=42; *=P<0.05; **=P<0.01)

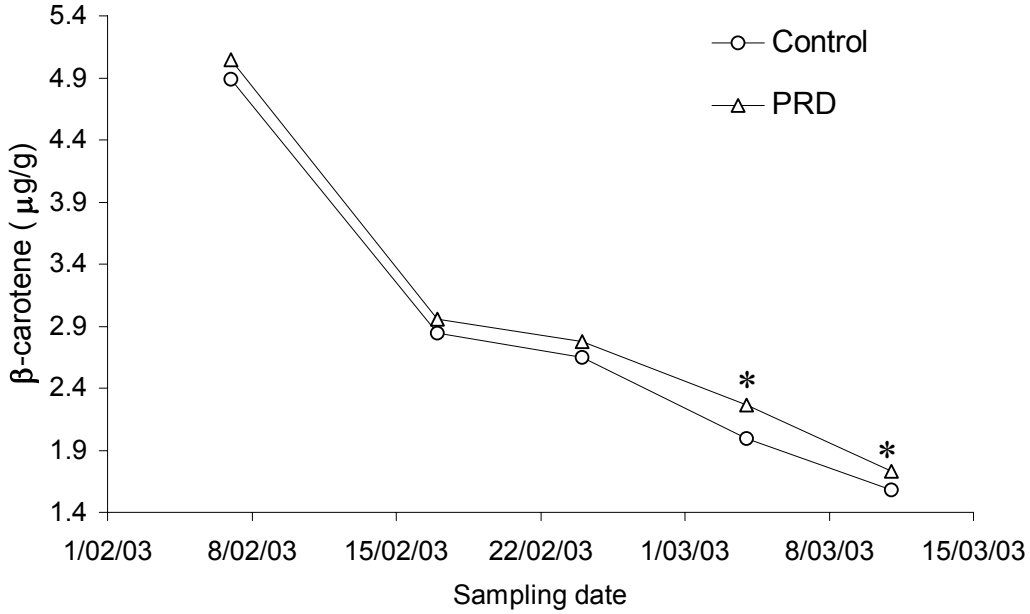


Figure 7: Effect of PRD on the concentration of β-carotene over berry development for the 2002 vintage (ANOVA; n=42; *=P<0.05)

6.4 Yield versus quality

A further objective in the current study was to explore the limitation of the PRD technique in terms of crop load and water-use efficiency. For previous studies of the application of PRD in different grape varieties and in different localities, it was shown that PRD could be implemented where irrigation was reduced up to 50% relative to conventional practice, without a significant yield penalty (Dry *et al.* 2000). The current study has shown that there are a number of factors that may influence the response of vines to a PRD-type irrigation strategy, which may affect the final yield and quality of the crop at harvest.

6.4.1 Soil type, location and irrigation cycling

The responses to the PRD irrigation strategy shown in the current study bring to light some important questions surrounding the application of PRD as a deficit irrigation strategy. It was shown that the PRD treatment resulted in a hydraulic water deficit relative to the control treatment, where the PRD treatment had half the irrigation volume applied compared with the control treatment. This result is different from what has previously been found for the technique, where no change in leaf water potential was observed in response to PRD (Dry, 1997; Stoll, 2000; Stoll *et al.* 2000). This was associated with a yield penalty in some instances of up to 25% relative to the control treatment, mainly caused by a reduction in berry size. As the effectiveness of PRD as an irrigation strategy is largely dependent upon soil type, this will become an increasingly important consideration in the choice of irrigation strategy by grapegrowers in the future (Kriedemann and Goodwin, 2003). In the current study, PRD irrigation was implemented in medium to light textured soils at both the Cabernet Sauvignon (Langhorne Creek) and Shiraz (Nuriootpa) experiments; such soils have been found to be the best medium for PRD irrigation strategy (Kriedemann and Goodwin, 2003). Despite this, a 'classic' PRD response was not achieved at either site, even where irrigation scheduling was rigorous and planned according to closely-monitored levels of soil moisture. The question that needs to be addressed is the length of the PRD cycle. The potential exists that soil water content may decrease more rapidly in sandy soils than in soils of a high clay content. This would be due to increased drainage of irrigation water in sandy soils together with more rapid use of available soil water by roots in sandy soils compared with high-clay soils. Thus, under these conditions,

an extended PRD cycle time may lead to excessively dry soil on the 'dry' side of the PRD treatment, such that the vines experience an hydraulically-mediated water deficit. As this holds the potential to reduce yield and berry size in addition to vine canopy size, the applicability of PRD must be considered as a 'cost-benefit' scenario. PRD has the potential to increase the water use efficiency (WUE) of vines, as has been shown in the current study, but if there is a significant yield penalty associated with the treatment, there would be no net gain in WUE.

6.4.2 Node number per vine

The comparison of three different pruning levels superimposed on standard and PRD irrigation strategies allowed for the limitations of irrigation techniques at different cropping levels to be explored. As would be expected, increasing node number per vine resulted in an increase in yield. Where PRD received half the irrigation volume of the control treatment, a general observation was that yield and berry size were not altered by the PRD irrigation strategy when node number (and thus bunch number) per vine was low, i.e. at 30 and 60 nodes per vine. However, when node number per vine was increased to 120 nodes, the vines showed increased sensitivity to PRD irrigation, in terms of both reproductive and vegetative growth. Thus, there was a reduction in both yield and vegetative growth in PRD vines at the higher node number, although this was not statistically significant when analysed across the three pruning levels.

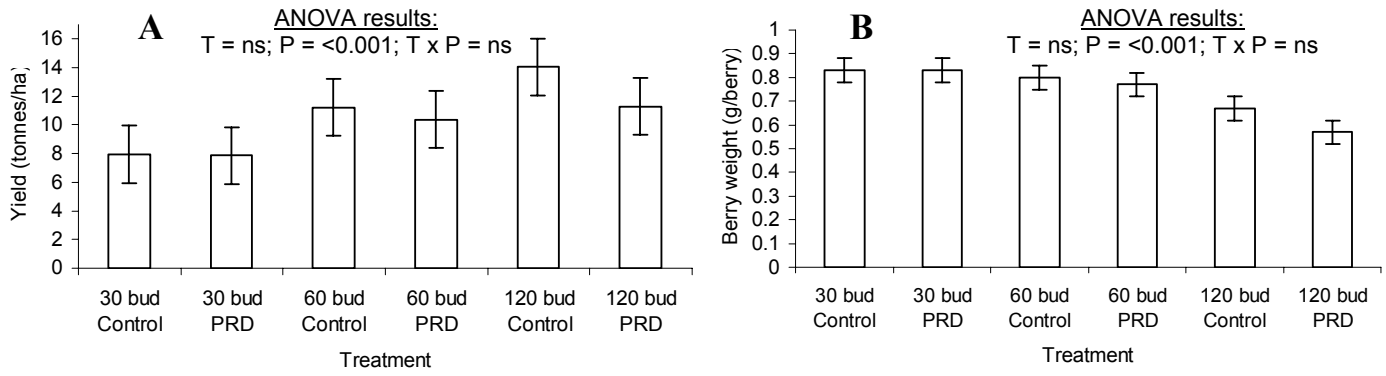


Figure 9: A. Yield (tonnes/ha) and **B.** berry weight (g) of Shiraz vines pruned to three different node numbers in the 2001 season. PRD and standard irrigation strategies were implemented where PRD received half the irrigation water of the control treatment (ANOVA; n=30; ns = not significant; T = treatment, P =pruning; T x P = interactive effect).

No change in fruit secondary metabolites was observed at higher node number per vine, although in one season, PRD significantly improved anthocyanin and phenolic concentration across all pruning levels. This means that using the PRD technique, growers might expect to see an improvement in quality, provided that vines are not over-cropped. Where PRD is combined with a large bunch number per vine, e.g. in the case of minimal pruning, growers might expect to see a yield penalty, but see little change in fruit quality compared with PRD-irrigated vines pruned to lower node numbers. It appears that 'vine balance', i.e. the partitioning of structural carbohydrate between vegetative and reproductive growth, became problematic where node number per vine was high. Thus, there was a limitation of carbon resources for partitioning over a large reproductive sink, which was exacerbated under PRD irrigation, such that a reduction in both vegetative and reproductive growth occurred.

7. Outcomes/Conclusions

The current study has shown that the application of a mild water deficit to grapevines has the potential to alter the metabolism secondary compounds in fruit. As many of these secondary compounds are important to the determination of final wine quality, this may demonstrate that growers have the potential to enhance the quality of fruit and wines through deficit irrigation strategies, including PRD. Despite the potential limitations surrounding the application of PRD in different regions, soil types and at different cropping levels (Kriedemann and Goodwin 2003), the technique still holds the advantage of providing a continuous and mild water stress to vines, and additionally has the potential to improve fruit quality (Dry *et al*. 2000).

Using PRD would allow growers to avoid the potential deleterious effect of other deficit irrigation strategies such as RDI, which may result in a devastating yield penalty if incorrectly managed. Furthermore, if a yield penalty is associated with increased fruit quality, the financial trade-off of reduced yield may not be as severe, and may actually result in increased productivity through the production of 'premium' wines of greater marketability. This is an important consideration where there is currently an increased drive within the Australian wine industry to produce fruit of improved quality. This drive is set against a background of a rapidly expanding wine production base, which could potentially lead to a glut of low-quality, low-profit wines if viticultural practices are not improved in the immediate future.

This study has compared PRD and conventional irrigation where both irrigation strategies have used water volumes at the lowest limit (approximately 1 ML/ha) for the viticultural regions where the experiments were based. Thus, the margin for the improvement of grape and wine quality through the effect of deficit irrigation on secondary metabolites was small. However, in regions where far higher irrigation volumes are used e.g. 5 to 6 ML/ha in Riverland, Sunraysia, Riverina, the potential to restrict water use and boost grape and wine quality may be far greater than the levels shown in the current study. This question will become increasingly important in the future, in the light of recent restrictions imposed on water use.

8. Recommendations

A number of key issues have arisen from the results of the current study:

1. A very important question is the role of ABA in the regulation of secondary pathways. Are the responses to due increased ABA in grape berries throughout fruit growth and development; or is there a potential 'switching on' of certain secondary pathways by increased ABA at certain key developmental stages? We know ABA is increased in fruit experiencing water deficit from recent reports (Antolin *et al*. 2003) but as yet, the timing and potential alteration of its accumulation pattern in fruit in response to water deficit remains unsolved. Within this question, a separation of the effects of water deficit versus a 'classic' PRD-type response involving solely non-hydraulic signals needs to be achieved. Both hydraulic and non-hydraulic plant signals are mediated to some extent through the metabolism of ABA, but the effects of these respective signals on grape berry ABA levels remains unclear.

2. A key finding in the current study was that wine colour could be improved using the PRD irrigation technique, even where anthocyanin levels in fruit and wines were unchanged. Increases in the contribution of 'total phenolics' to wine colour, possibly through increased intermolecular co-pigmentation and polymerisation of coloured pigments appears to be responsible for this phenomenon. The concept of 'total phenolics' is currently based on a measure of wine absorbance at 280 nm, and therefore includes anthocyanins, simple phenolics, flavonols, flavan-3-ols and condensed tannins. Each component of this diverse group of phenolic compounds has the potential to contribute to the co-pigmentation effect observed in wines at different levels. Future research of this topic should include a detailed analysis of which phenolic compounds are increased by the PRD irrigation technique. The current study showed that seed tannins can be increased in fruit, but as yet, their potential contribution as wine co-pigments is poorly understood.

3. A third issue which needs to be addressed is the limitation of region and soil type on the application of the PRD technique. Before PRD is presented to the wine industry as a possible tool for the improvement of grape and wine quality, its limitations in

different winegrowing regions of Australia needs to be explored on an ongoing basis. A range of studies has been conducted in different regions of Australia in recent years, and some have not demonstrated the expected response to PRD irrigation (Kriedemann and Goodwin, 2003; S. Pudney, pers comm.). Despite this, the technique holds strong potential for the manipulation of grape and wine quality, together with an increasing need for improved water use efficiency of a limited water supply within the wine industry. Thus, research into the applicability of PRD as an irrigation strategy should continue, perhaps in conjunction with other deficit irrigation strategies like RDI.

Appendix 1: Communication

- CRC for Viticulture Program 2 reviews: Adelaide, 2000; Mildura, 2002; Adelaide, 2003
- Poster presented at the Australian Wine Industry Technical Conference, Convention Centre, Adelaide, October 2001, "Optimising PRD for improved grape and wine quality."
- Paper presented by K. Bindon at the South African Society for Enology and Viticulture (SASEV) convention in Somerset West, November 2002, "Optimising grape and wine quality with PRD". Received award for 'Best Student Presentation' at the SASEV convention.
- Bindon, K., Dry, P.R. and Loveys, B.R. (2002) Optimising winegrape quality with partial rootzone drying. PRD Workshop, Adelaide, October 2002.
- Paper presented by K. Bindon at the 2nd Australian Grapevine Physiology Workshop, October 2003, Melbourne "Effect of PRD on grape secondary metabolites."
- PhD thesis: "Optimising partial rootzone drying (PRD) for improved grape and wine quality", University of Adelaide. Submitted April 2004.

Appendix 2: Intellectual Property

Information developed during the course of the project is in the public domain and will subsequently be published in international refereed journals.

Appendix 3: References

Antolin, M.C., Baigorri, H., de Luis, I., Aquirrezabal, F., Geny, L., Broquedis, M. and Sanchez-Diaz, M. (2003). ABA during reproductive development in non-irrigated grapevines (*Vitis vinifera* L. cv. Tempranillo) *Australian Journal of Grape and Wine Research* **9**:169-176.

Baumes, R., Wirth, J., Bureau, S., Gunata, Y. and Razungles, A. (2002). Biogeneration of C_{13}-norisoprenoid compounds:experiments supportive for an apo-carotenoid pathway in grapevines. *Analytica Chimica Acta* **458:** 3-14

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):1-7.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Americal Journal of Enology and Viticulture* **52**(2):67-87.

Dry, P.R. (1997). Response of grapevines to partial drying of the root system. PhD Thesis. University of Adelaide.

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

Freeman, B.M. (1983). Effects of irrigation and pruning of Shiraz grapevines on subsequent red wine pigments. *American Journal of Enology and Viticulture* **34**(1):23-26.

Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000). *Techniques for chemical analysis and quality monitoring during winemaking*. Patrick Iland Wine Promotions, Campbelltown, South Australia, pp. 64-79.

Haselgrove, L., Botting, D., van Heeswijk, R., Høj, P., Ford, C. and Iland, P. (2000). Canopy micoclimate 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.

Kriedemann, P.E. and Goodwin, I. (2003). Regulated deficit irrigation and partial rootzone drying: an overview of principles and applications. *Irrigation Insights* **No. 4**, Land and Water Australia, Canberra

Lichtenthaler, H.K. (1999). The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**:47-65.

Oliveira, C., Silva Ferreira, A.C., Mendes Pinto, M., Hogg, T., Alves, F and Guedes de Pinho, P. (2003). Carotenoid compounds in grapes and their relationship to plant water status. *Journal of Agricultural and Food Chemistry* **51**:5967-5971.

Marais, J., van Wyk, C., Rapp, A. (1991). Carotenoid levels in maturing grapes as affected by climatic regions, sunlight and shade. *South African Journal of Enology and Viticulture* **12**(2) 64-69.

Marais, J., van Wyk, C., Rapp, A. (1992). Effect of sunlight and shade on norisoprenoids levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *South African Journal of Enology and Viticulture* **13**(1) 23-32

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

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

Appendix 4: Staff

- Assoc. Prof. Peter Dry, University of Adelaide
- Affil. Prof. Brian Loveys, CSIRO, Plant Industry, Adelaide
- Mr Peter Mansfield and Ms Libby Boschen, Winetac, The Cooperative Research Centre for Viticulture (CRCV)
- Ms Fiona Brooks, casual technical support
- Ms Sue Maffei, CSIRO, Plant Industry, Adelaide
- Dr Mike McCarthy and Ms Shannon Pudney, PIRSA/SARDI, Nuriootpa
- Mr John Kennedy and Mr Matt Partridge, Orlando-Wyndham, Langhorne Creek.
- Mr Stephen Clarke and Mr Chris Day, University of Adelaide winery