The Interactive Effect of Pruning Level and Irrigation Strategy on Grape Berry Ripening and Composition in *Vitis vinifera* L. cv. Shiraz

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A partial rootzone drying (PRD) irrigation technique (0.5 ML/ha) was compared with a standard irrigation treatment (1 ML/ha) at three different pruning levels of 30, 60 and 120 nodes per grapevine in *Vitis vinifera* L. cv Shiraz. Berry size was found to decrease as node number per grapevine increased, but was not significantly altered by the PRD treatment. The influence of these treatments on the accumulation of total soluble solids per berry was investigated during berry ripening and was shown to be reduced at higher node number (120 nodes). There was no effect of PRD on the accumulation of total soluble solids. Juice titratable acidity and the concentration (per g) and content (per berry) of grape anthocyanins and phenolics were compared between treatments at harvest. In one season of the study, juice titratable acidity, anthocyanin and phenolic concentration was unaltered by the PRD treatment. In a further season, juice titratable acidity was decreased in response to the PRD treatment and was associated with increases in grape anthocyanin and phenolic concentration in response to PRD. Where there was a small increase in anthocyanin concentration in response to PRD, this was found to be independent of berry size. In addition, linear regression analysis showed a poor relationship between berry size and anthocyanin concentration, but a significant relationship was found between berry size and anthocyanins per berry.

The application of irrigation to grapevines has generally been found to cause a delay in ripening, as measured by the accumulation of juice total soluble solids (TSS) (Neja *et al*., 1977; Kliewer *et al*., 1983; Bravdo *et al*., 1985; Castellarin *et al*., 2007). This has been proposed to be due to the dilution effect of sugars in an increased fruit volume under irrigation, which is often associated with an increase in fruit weight (Esteban *et al*., 2002). However, this conclusion cannot be drawn by looking at the concentration of TSS in the juice alone. It has been suggested that a more appropriate way to express sugar accumulation in grape berries is on a per berry basis, which factors in the contribution of the sink size (berry size) (Wang *et al*., 2003a, 2003b). The unloading of sugars from the phloem to the berry has been found to be reduced in response to water deficit (Wang *et al*., 2003a, 2003b), which makes consideration of the rate of TSS uptake per berry an important additional factor.

Grape ripening is not only determined by the rate of TSS accumulation, but also characterised by the rate of decline in organic acids. At harvest, irrigated grapevines have been shown to have higher titratable acid (TA) levels when berries are harvested on the same date as those taken from non-irrigated grapevines (Bravdo *et al*., 1985; McCarthy & Coombe, 1999). This initial observation was assumed to be primarily due to the delay in ripening induced in grapevines by irrigation, but was found to persist even when the organic acid levels of irrigated and non-irrigated grapevines were compared at similar juice TSS (Bravdo & Hepner, 1986). The effect was found be due to a decrease in malic acid in non-irrigated grapevines, whereas tartrate was unaffected by the irrigation treatment (Bravdo *et al*., 1985). Therefore, the effect of irrigation on the ripening process may not simply be due to an increase in berry volume, but may be a physiological response to an alteration in plant water status, which in turn impacts the regulation of pathways governing carbon metabolism in the fruit. Apart from studies comparing non-irrigated and irrigated grapevines, the effect of controlled water deficit has also been found to affect the ripening response. A study of early water deficit (pre-véraison) on grapevine water status has been associated with a reduction in malic acid concentration and accelerated sugar accumulation, whereas a late deficit (post-véraison) water deficit has been found to have no effect on ripening by either measure (Matthews & Anderson, 1988; Castellarin *et al*., 2007).

A potential risk with decreasing the leaf area:yield ratio of a grapevine is that it could lead to restriction of carbon allocation to the grape bunches, preventing the crop ripening to desirable TSS levels, as well as reducing its capacity for growth from year to year due to reduced storage reserves. A decreased leaf area:yield

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ratio can result from increased bunch number per grapevine, and has been reported to slow ripening and reduce the final TSS levels attained at harvest (Edson *et al*., 1993; Miller *et al*., 1993; Miller & Howell, 1998). The effect of increased crop load on final grape juice pH and TA can be variable. It either has no significant effect (Edson *et al*., 1993) or is correlated with lower juice pH or higher juice TA (Miller & Howell, 1998). The effect of increased crop load on TSS accumulation in grape berries can be exacerbated by a water deficit. According to the results of a bunch-thinning experiment by Bravdo *et al*. (1985), there is little effect of bunch number on TSS where water supply is adequate, but a 35% reduction in irrigation can cause grapevines with higher bunch number and yield:pruning weight to have reduced TSS. A reduction in pruning weight is associated with a cutback in irrigation (McCarthy & Staniford, 1984; Matthews & Anderson, 1988, 1989; Goodwin & Jerie, 1992), and when this is combined with higher crop load it may cause a significant change in the fruit weight to pruning weight ratio, potentially restricting carbon allocation to the grape bunches.

PRD is a deficit irrigation strategy that has been shown to reduce vegetative growth in grapevines as measured by pruning weight, shoot growth rate and leaf area, without causing a significant change in fruit weight or sugar accumulation (Dry *et al*., 1996; Du Toit *et al*., 2003; Bindon *et al*., 2008). For the measurement of acidity, however, a variable response has been obtained with PRD irrigation. Published data from field-grown grapevines has shown that TA decreased in response to PRD (Dos Santos *et al*., 2003) when PRD was run at 50% of the irrigation level of fully irrigated fruit, even when bunch weight and sugar content were unaffected by the treatment. However, an experiment with potted grapevines under the same irrigation regime showed that PRD did not affect juice pH or TA for a given TSS level (Antolín *et al*., 2006).

The effectiveness of PRD at different yield levels has not yet been determined. In the light of the potential reduction in carbon availability under water deficit at higher yields (Bravdo *et al*., 1985), there may be a threshold for the PRD technique above which grapevines become 'unbalanced' in terms of the source-sink relationship. A primary objective of this study was to determine the effect of PRD irrigation at different bud loads produced by the retention of varying node numbers per grapevine at winter pruning, resulting in differing bunch number and yield categories (Bindon *et al*., 2008). The response of grapevines to the treatments was measured in terms of TSS accumulation, pH and TA at harvest. A second objective of this study was to investigate the response of colour development in grape berries to the abovementioned treatments. Within the wine industry at large there is a commonly held perception that small berry size equates with better wine quality, as measured by wine colour (Matthews & Anderson, 1988; Dry *et al*., 1999; McCarthy, 2000; Ojeda *et al*., 2001). Various studies have explored this question and have shown that natural variation in berry size does not alter the proportions of skin, seed and flesh. This demonstrates a limited role of berry size in determining the concentration of solutes in juice and wines (Roby & Matthews 2004; Roby *et al*., 2004). However, the limitation of berry size in response to water deficit has been shown to significantly affect these proportions, increasing the contribution of skin and seeds to berry mass, primarily through a restriction in the growth of mesocarp tissue (Ojeda *et al*., 2001, 2002; Roby & Matthews, 2004; Roby *et al*., 2004). A water deficit-induced change in berry size therefore has the potential to increase the concentration of anthocyanins and other phenolics in grape berries by altering the skin:flesh ratio. However, the biosynthesis of anthocyanins has also been shown to be affected by water deficit, such that both early (pre-véraison) and late (post-véraison) deficits are associated with increased expression of anthocyanin biosynthetic genes, resulting in increased accumulation of anthocyanins (Castellarin *et al*., 2007). This means that the final anthocyanin content in grape berries may also be directly enhanced as a result of water deficit through altered biosynthesis, rather than by a changed skin:flesh ratio alone.

MATERIALS AND METHODS

Experimental

The vineyard site was at Nuriootpa, in the Barossa Valley, South Australia (34º48'S, 139º14'E, elevation 274 m). The general climate of the region is Mediterranean, warm, with mean January temperatures between 21 and 23°C, and with 1 817 biologically effective degree days. Rainfall is moderate (506 mm), with high summer evaporation and low relative humidity. The soil site was classified as a Light Pass fine sandy loam (Northcote *et al*., 1954).

Irrigation and pruning strategy

The experiment was on 10-year-old Shiraz grapevines on own roots. The experimental design was a split-plot, with six fully randomised treatments, each consisting of five replicates of two-vine plots. Four buffer grapevines were assigned between each consecutive treatment. The trellis type was a permanent bilateral cordon without shoot positioning (sprawled canopy). The row and vine spacing was 3.0 m and 2.25 m respectively, and rows were oriented in an east-west direction. The treatments were: three pruning levels determined by node number at winter pruning of 30, 60 and 120 nodes superimposed over either PRD or a 'control' irrigation strategy. The grapevines were spur-pruned and two-node spurs were used for the 30-node treatment, while a combination of two- and four-node spurs was used for the 60- and 120-node treatments. For the PRD and control treatments, two 4 L/h drippers were set up 45 cm on either side of the grapevine trunk. For PRD, a specially designed dual dripline (Netafim, Adelaide, Australia) was used that allowed for the sides of the irrigation to be switched while preventing the dripper position from shifting. For PRD, only one side of the grapevine's root system received water at any time, whereas both sides of the root system were watered in the standard-irrigated grapevines. The time between PRD cycles was approximately 10 days, and the 'wet' side received an additional irrigation mid-way through a cycle. On average, the length of water application per irrigation was 20 h. The level of irrigation for the control was according to the maximum limit for the Barossa Valley, South Australia, at 1 ML/ha. It was applied in continuous cycles from mid-December (pre-véraison) up to harvest of each growing season and was not adjusted according to rainfall. In the seasons 2000-2001 and 2002-2003, the PRD treatment received half the irrigation water of the control. In 2000-2001, the total water applied was 1.0 ML/Ha and 0.5 ML/Ha for the control and PRD respectively. In 2002-2003, the total water applied was 1.2 ML/Ha and 0.6 ML/Ha for the control and PRD respectively. In 2001-2002, the same amount of irrigation water was applied to both treatments, namely 1.0 ML/Ha.

Determination of sugars, pH and titratable acidity in berry juice

Fruit was collected at weekly intervals post-véraison. Two 50-berry samples were collected and weighed for the determination of berry weight. One 50-berry sample was then immediately frozen at -20°C for later analysis, and juice was extracted by gentle pressure from the second sample. The juice samples were centrifuged for 5 min at 10 000 g and the supernatant was retained. The pH of the juice sample was determined immediately using a pH meter (Activon Scientific Products, Thornleigh, NSW, Australia). TSS was measured as °Brix using a digital refractometer BRX-242 (Erma, Tokyo, Japan). Five mL of the juice sample was then diluted 1:5 with deionised water and frozen at -20°C for later analysis of the TA. The TA of the defrosted juice samples was determined using an autotitrator (Crison Instruments, Barcelona, Spain), with the end-point for the titration against 0.1 N NaOH set at a pH of 8.2.

Extraction of anthocyanins and total phenolics

Whole-berry anthocyanins and phenolics were compared at the same °Brix, either when immature (18 to 20°Brix) or at harvest (23.5 to 24°Brix). The frozen 50-berry sample was defrosted over 30 min and immediately homogenised using an Utra-Turrax T 25 (IKA Labortechnik, Staufen, Germany), ensuring that both the seeds and the flesh were completely crushed. One gram of the homogenate was extracted in 50% ethanol (pH 2 with HCl) – 10 mL per gram berry tissue, according to the method of Iland *et al*. (2000). Extracts were placed on a rotary shaker in the dark for 1 h. Samples were then centrifuged for 5 min at 10 000 g and the supernatant was retained. One mL of the supernatant was diluted in 10 mL 1N HCl and left to stand for a 3-h period, after which the absorbance of the diluted extract was determined at 520 nm and 280 nm. From these values, an estimate of total anthocyanins and phenolics per berry or per gram berry fresh weight was determined.

Statistical analysis

Data were analysed statistically with the Genstat 6 software package, using a split-plot ANOVA to separate the effects of irrigation and pruning type and to observe interactive effects, if any. Linear regression analysis was used to explore the relationships between individual components.

RESULTS AND DISCUSSION

The effect of PRD and node number per grapevine on grape berry ripening

With increasing node number per grapevine, there was a corresponding increase in yield (Bindon *et al*., 2008). Maximum berry weight was reached early in each season of the study, following which berry weight declined (results not shown). The decrease in berry weight, otherwise known as 'shrivel', is commonly observed for Shiraz grapevines (McCarthy, 1999; McCarthy & Coombe, 1999). For the 2000-2001 season, maximum berry weight was reached on 17 February, when the TSS level was 22 to 24°Brix. The accumulation of TSS during ripening is shown per berry (Fig. 1A) and reached a plateau at

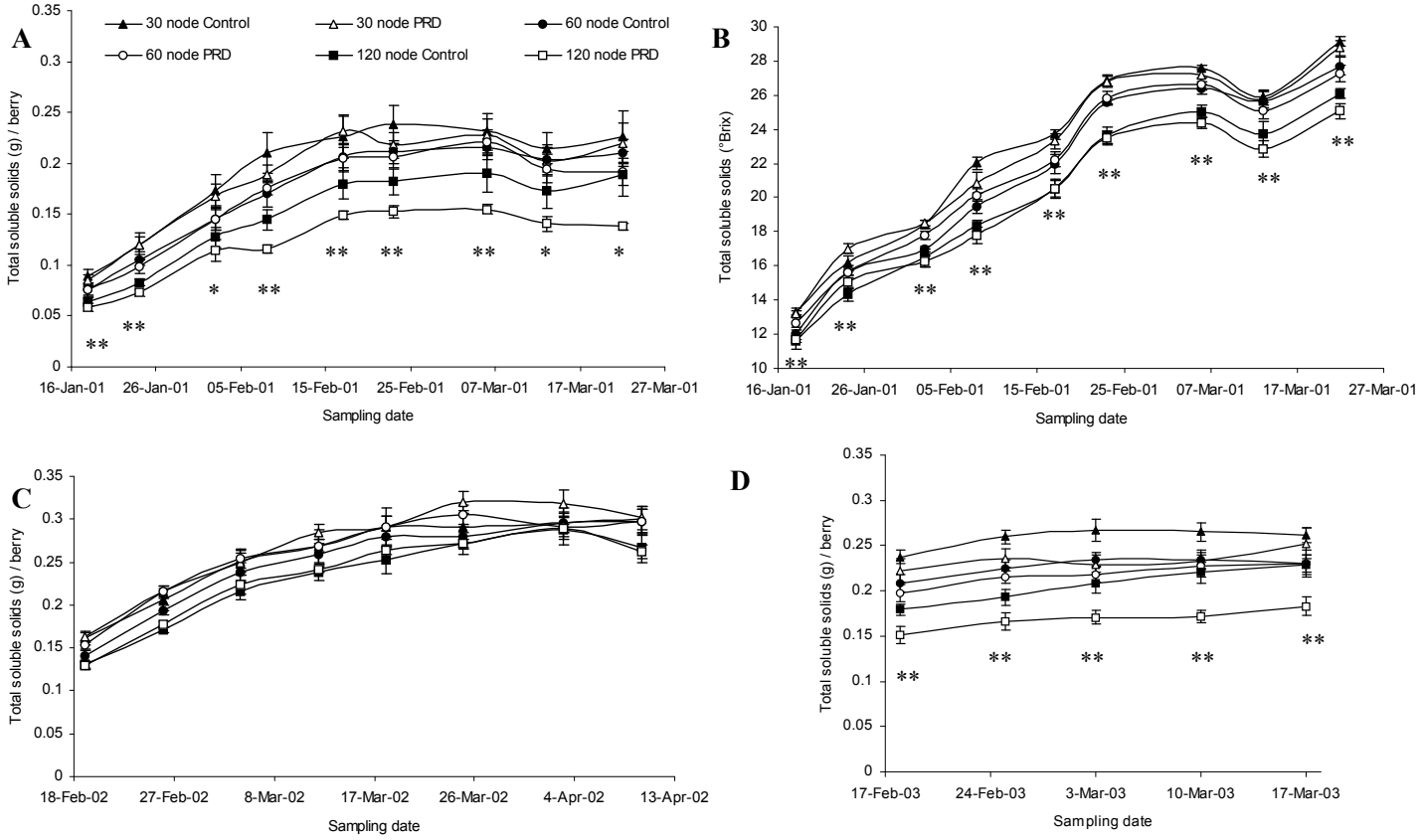

FIGURE 1

Effect of PRD and node number on the accumulation of TSS for A. 2000-2001 season in g TSS per berry; B. 2000-2001 season as °Brix; C. 2001-2002 season in g TSS per berry; D. 2002-2003 season in g TSS per berry. In 2000-2001 and 2002-2003, PRD irrigation was 50% of the control, in 2001-2002 PRD and the control received equal water (data points show mean ± S.E.; ANOVA: n = 30, * and ** represent significant differences between pruning levels only, at $P < 0.05$ and $P < 0.001$ respectively).

TABLE 1

Effect of node number and PRD on pH and TA of Shiraz fruit at 23.5 to 24°Brix pruned to differing node number from three vintages (2001 to 2003). Irrigation level: 2000-2001 and 2002-2003: PRD = 50% of control; 2001-2002: PRD = 100% of control (ANOVA; n = 30; T = irrigation treatment; P = pruning; T x P = interactive effect; ns = not significant).

		30 nodes	60 nodes	120 nodes	P (T)	P (P)	P (TxP)
2001							
pH	Control	3.67	3.73	3.67	ns	ns	ns
	PRD	3.69	3.71	3.64			
TA (g/L)	Control	4.22	4.09	3.89	ns	ns	ns
	PRD	4.39	4.01	4.25			
2002							
pH	Control	3.29	3.27	3.30	ns	ns	ns
	PRD	3.24	3.23	3.30			
TA (g/L)	Control	8.31	7.67	7.82	ns	ns	ns
	PRD	8.67	8.36	8.00			
2003							
pH	Control	3.38	3.44	3.41	< 0.05	ns	< 0.05
	PRD	3.46	3.40	3.48			
TA (g/L)	Control	7.74	7.32	7.14	< 0.01	ns	ns
	PRD	6.82	6.76	6.22			

TABLE 2

Effect of node number and PRD on grape berry anthocyanins in Shiraz in 2000-2001 (ANOVA; n = 30; T = irrigation treatment; P = pruning level; TxP = interactive effect, ns = not significant).

Anthocyanin concentration		30 nodes	60 nodes	120 nodes	P (T)	P (P)	P (TxP)
18°Brix (mg/g)	Control	0.98	0.81	0.99	ns	< 0.05	ns
	PRD	0.97	0.95	1.24			
18°Brix (mg/berry)	Control	0.89	0.70	0.81	ns	ns	ns
	PRD	0.84	0.82	0.84			
24°Brix (mg/g)	Control	1.64	1.62	1.61	ns	ns	ns
	PRD	1.68	1.65	1.70			
24°Brix (mg/berry)	Control	1.58	1.42	1.21	ns	< 0.001	ns
	PRD	1.61	1.36	1.11			

TABLE 3

Effect of node number and PRD on grape berry phenolics in Shiraz in 2000-2001 (ANOVA; n = 30; T = irrigation treatment; P = pruning level; TxP = interactive effect, ns = not significant).

Phenolic concentration		30 nodes	60 nodes	120 nodes	P (T)	P (P)	P (TxP)
18°Brix (A_{280}units/g)	Control	1.21	1.20	1.25	ns	< 0.05	ns
	PRD	1.21	1.22	1.51			
18°Brix (A_{280}units/berry)	Control	1.07	1.00	1.03	ns	ns	ns
	PRD	1.02	1.06	1.00			
24°Brix (A_{280}units/g)	Control	1.28	1.43	1.50	ns	ns	ns
	PRD	1.37	1.39	1.55			
24°Brix (A_{280}units/berry)	Control	1.24	1.27	1.14	ns	ns	ns
	PRD	1.30	1.14	1.01			

17 February for all treatments. The consequence of berry volume decrease after 17 February is reflected in an increase in TSS concentration (Fig. 1B). For TSS accumulation, it is evident that a significant difference was observed between pruning levels, such that lower TSS per berry was obtained from the 120-node pruning treatment (Fig. 1A), which confirms that sugar per berry (sugar loading per berry) is a good indicator of the source-sink relationship. A similar response was found in the 2001-2002 and 2002-2003 seasons (Fig. 1C, 1D), although the effect was smaller in 2001-2002. No significant difference in TSS accumulation per berry was found in response to the irrigation treatment, nor was a significant interactive effect found between irrigation and pruning in any of the three seasons. In each of the three seasons of the study, the point at which maximum berry weight was reached coincided with the point at which TSS per berry ceased to increase (Fig. 1A, 1C, 1D). The point at which this occurred was similar between treatments.

This result is in agreement with previous work, where higher crop loads per grapevine resulted in a limitation in the final sugar level attainable in the fruit at harvest (Edson *et al*., 1993; Miller *et al*., 1993; Miller & Howell, 1998). This phenomenon was also demonstrated by Bravdo *et al*. (1985), who found that water deficit combined with increased crop load led to a significant reduction in the rate of sugar accumulation. This was thought to be due to an increased yield to pruning weight ratio under the deficit irrigation treatment where crop load was high, leading to a reduction in photosynthate available from a limited leaf area for a large crop load. However, there was no significant interactive effect of PRD irrigation on pruning weight (Bindon *et al*., 2008) or TSS accumulation in the current study. This may be due to the increased ability of the grapevine to retain photosynthetic function under water deficit with PRD, despite a consistent reduction in stomatal conductance (Stoll *et al*., 2000; Bindon *et al*., 2008).

The combination of PRD and node number on juice TA and pH was compared at similar TSS levels in order to remove the effect of node number on the rate of ripening (Table 1). There was no significant effect of PRD or node number on the pH and TA of berry juice in 2000-2001 or 2001-2002. However, in 2002-2003 there was an observed increase in juice pH and a decrease in TA in response to PRD that was independent of node number. Large differences in pH and TA were observed between seasons (Table 1), with lower pHs and higher TA found in juice samples for 2001-2002. The 2001-2002 season had milder temperatures than the 2000-2001 and 2002-2003 seasons, which may have led to a reduced rate of malic acid degradation (Bindon *et al*., 2008). In 2000-2001, higher seasonal temperatures may account for the

TABLE 4

Effect of node number and PRD on grape berry anthocyanins in Shiraz in 2002-2003 (ANOVA; n = 30; T = irrigation treatment; P = pruning level; TxP = interactive effect, ns = not significant).

Anthocyanin concentration		30 nodes	60 nodes	120 nodes	P (T)	P (P)	P (TxP)
20°Brix (mg/g)	Control	0.99	0.93	1.04	< 0.001	ns	ns
	PRD	1.18	1.11	1.25			
20°Brix (mg/berry)	Control	1.23	1.01	1.09	ns	< 0.01	ns
	PRD	1.25	1.10	1.09			
24°Brix (mg/g)	Control	1.45	1.43	1.45	< 0.01	ns	ns
	PRD	1.68	1.57	1.55			
24°Brix (mg/berry)	Control	1.56	1.46	1.35	ns	< 0.001	ns
	PRD	1.75	1.49	1.21			

TABLE 5

Effect of node number and PRD on grape berry phenolics in Shiraz in 2002-2003 (ANOVA; n = 30; T = irrigation treatment; P = pruning level; TxP = interactive effect, ns = not significant).

Phenolic concentration		30 nodes	60 nodes	120 nodes	P (T)	P (P)	P (TxP)
20°Brix (A_{280}units/g)	Control	1.09	1.08	1.07	< 0.001	ns	ns
	PRD	1.25	1.19	1.25			
20°Brix (A_{280}units/berry)	Control	1.36	1.18	1.13	ns	< 0.001	ns
	PRD	1.32	1.19	1.07			
24°Brix (A_{280}units/g)	Control	1.33	1.29	1.36	< 0.01	ns	ns
	PRD	1.45	1.41	1.47			
24°Brix (A_{280}units/berry)	Control	1.43	1.32	1.27	ns	< 0.01	ns
	PRD	1.51	1.33	1.15			

increase in pH and drop in TA observed across all the treatments at the same TSS level (Kliewer, 1971; Bindon *et al*., 2008).

Units of expression for anthocyanin and phenolic data

The effect of pruning level and PRD on grape berry anthocyanins and phenolics was studied over two seasons (2000-2001 and 2002-2003). The data collected in 2001-2002 are not included here due to the difference in the irrigation treatments applied in that season. In 2000-2001 and 2002-2003, the three pruning levels produced three different categories of yield and berry weight, with the 30-node grapevines having the highest berry weight and lowest yield (Bindon *et al*., 2008). The effect of node number and PRD on the resulting anthocyanin and phenolic composition was investigated at two stages of maturity: pre-harvest (18 or 20°Brix) and harvest (24°Brix). In 2000-2001, the pruning level resulted in significant differences in grape anthocyanin and phenolic concentration at 18°Brix, such that the 120-node PRD treatment had the highest concentration of anthocyanin and phenolics (Tables 2 and 3). However, when anthocyanin and phenolic content were expressed per berry, no effect of either the pruning or irrigation treatments was observed, because berry size was not significantly different between the treatments at this stage. By harvest in 2000-2001, the effect of pruning on anthocyanin and phenolic concentration was no longer observed. However, when anthocyanin content was expressed per berry, it was found to be significantly decreased as node number per grapevine increased, which was due to the smaller berry size in the 120-node treatments at 24°Brix (Bindon *et al*., 2008). Phenolic content per berry, on the other hand, showed no significant response to either treatment.

In 2002-2003 there was no significant effect of pruning on anthocyanin and phenolic concentration at either 20°Brix or 24°Brix (Tables 4 and 5). However, in the 2000-2001 season there was a clear effect of pruning on anthocyanin and phenolic content per berry at both maturity levels. This indicates that reduced berry size resulting from increased bunch number per grapevine does not significantly alter the measurement of anthocyanin and phenolics in fruit tissue expressed as concentration (per g). This also means that there is not likely to be a strong surface area to volume relationship in the expression of grape colour. Instead, it appears that, with increasing berry size, the total content of anthocyanin and phenolics per berry is increased due to a higher surface area and thus larger skin area over which anthocyanins are concentrated. Thus, the change in surface area to volume ratio with larger berries did not affect the concentration of anthocyanin per gram, since total anthocyanin content was increased.

This hypothesis was explored further through linear regression analysis of anthocyanins and phenolics, expressed as concentration per gram fresh weight or as content per berry, plotted against berry weight (Fig. 2). For both seasons of the study, anthocyanin concentration at harvest showed a poor relationship with berry weight, as demonstrated by low R^2 values (Fig. 2A, 2B) relative to higher R^2 values calculated for the relationship between anthocyanin content per berry and berry weight (Fig. 2C, 2D). For the analysis of both anthocyanin concentration and content, the derived P-value indicated a significant fit of the data to the regression curve in each case. Linear regression analysis of phenolic concentration and content versus berry weight showed a similar response to that seen for anthocyanins (data not shown).

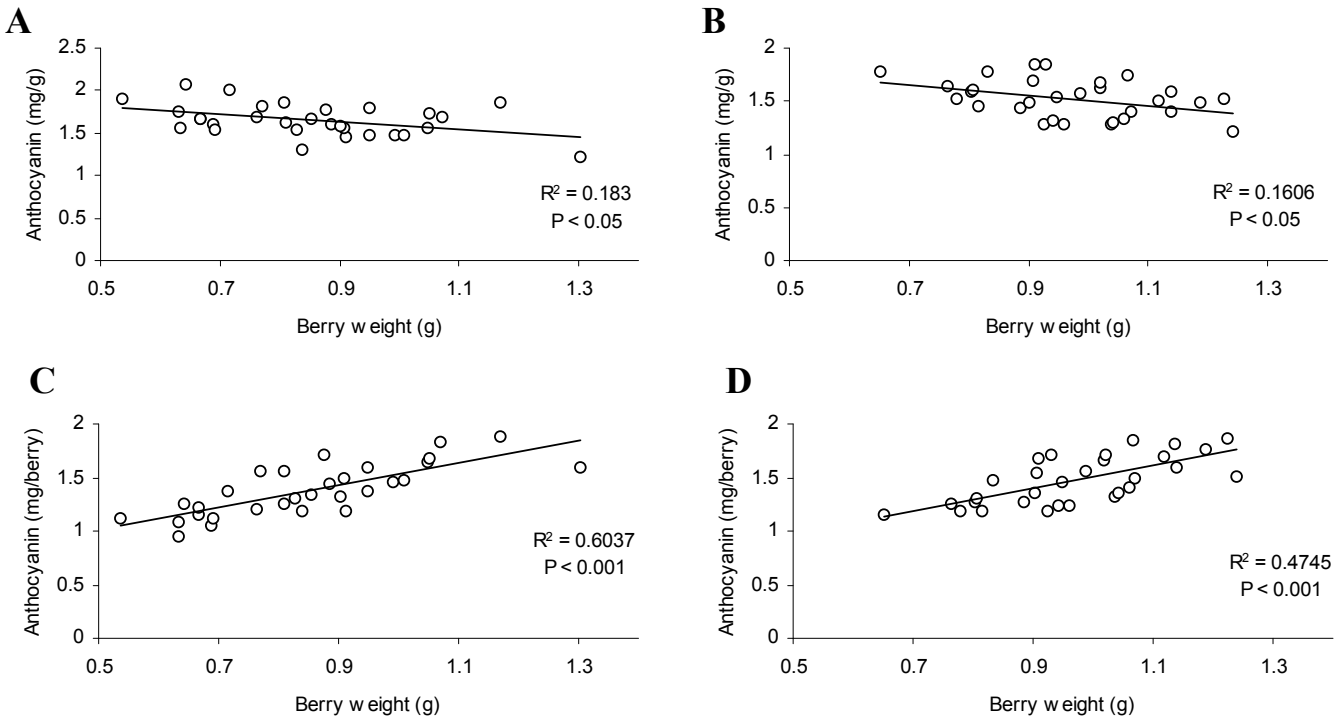

FIGURE 2

Linear regression of anthocyanin versus berry weight for pooled berry samples from PRD-irrigated and standard-irrigated Shiraz vines pruned to 30, 60 and 120 nodes **A**. 2000-2001 mg/g **B**. 2002-2003 mg/g; **C**. 2000-2001 mg/berry; **D**. 2002-2003 mg/berry (P value indicates significance of fit, n = 30)

The effect of PRD on the concentration of anthocyanins and phenolics differed between seasons. In 2000-2001, only a slight, non-significant increase in these components was observed in the 120-node PRD treatment (Tables 2 and 3). In 2002-2003, the concentration of anthocyanins and phenolics was increased by the PRD treatment and was independent of node number per grapevine in the intensity of the response (Tables 3 and 4). Pooled over pruning level, PRD increased the concentration of anthocyanins and phenolics by 11% and 9% respectively for fruit at 24ºBrix in 2002-2003. The response was observed at both fruit maturity levels studied. Since berry weight was not significantly affected by PRD in that season, it can be concluded that the increase was not brought about by an increase in the skin:flesh ratio.

PRD has been shown to cause minimal or no change in berry weight in other experiments (Dry, 1997; Stoll, 2000). Consequently, previous observations of an increase in anthocyanin concentration under PRD irrigation were due to an increase in anthocyanin content per berry, as berry weight was unchanged (Dry, 1997; Stoll, 2000). It was proposed that there was an underlying biochemical response of the anthocyanin metabolic pathway to the induced stress signals caused by PRD, increasing the final concentration of the anthocyanin product. In previous studies, correlation analysis of potential factors contributing to altered metabolism in the anthocyanin pathway showed that colour was negatively correlated with vigour indices, canopy density and stomatal conductance, and positively correlated with light penetration into the canopy (Dry, 1997; Stoll, 2000). This shows that changes in grapevine physiology and bunch microclimate are likely to influence secondary metabolism where berry size remains constant. In this study, the variability in berry weight between treatments prevented the comparison of anthocyanin content on a per berry basis. However, the results indicate that, where PRD does cause an increase in fruit anthocyanin and phenolic concentration, this is more likely to be due to a physiological response to the treatment and not a berry size effect. A similar observation was made by Antolín *et al*. (2006) for potted Tempranillo grapevines, whom has shown that a PRD treatment brought about an increase in anthocyanin concentration in the berry skin alone. This was done relative to a control treatment where irrigation was doubled and a deficit treatment run at the same level as the PRD treatment. In that study, PRD did not alter bunch weight relative to the control, while the deficit irrigation treatment showed decreased bunch weight. This observation warrants speculation as to how signalling within the grapevine in response to the partial drying of the root system can alter berry metabolism and, potentially, anthocyanin biosynthesis. Antolín *et al*. (2006) observed earlier ABA accumulation (véraison) in the berry in response to PRD and proposed that this may be one of the underlying physiological factors that control this response.

CONCLUSIONS

The current study has shown that higher crop loads can be carried by Shiraz grapevines grown in the Barossa region, causing only a delay in ripening and reduced sugar per berry, but with no deleterious effect on fruit composition as measured by pH, TA, or the concentration of anthocyanins and phenolics. However, there does appear to be a threshold crop load level (120 nodes per grapevine) at which the bunches will not be able to reach target ripeness levels due to a restricted carbon allocation from the canopy, as shown by the sugar load in the berries (Wang *et al*., 2003a, 2003b). PRD had a variable effect on grape composition between seasons and did not cause consistent increases in the concentration of anthocyanins or phenolics. Where increases in these compounds were observed, this was associated with decreased TA in the grape juice. Nevertheless, it was shown that the PRD technique can be used to obtain increased grapevine water use efficiency with no penalty either in yield, ripeness or the concentration of anthocyanins and phenolics, particularly at lower node numbers per grapevine (30 and 60 nodes). Furthermore, the current results provide an important basis for a discussion of the influence of berry size on grape secondary metabolite concentration and the implications for colour extraction into wines. The results have shown a weak relationship between secondary metabolite concentration and berry size, drawn from a wide range of berry sizes produced by varying node numbers at winter pruning.

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