

# Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins

GASPAR ROBY, JAMES F. HARBERTSON, DOUGLAS A. ADAMS and MARK A. MATTHEWS<sup>1</sup>

Department of Viticulture and Enology, University of California, Davis, CA 95616-8749, USA

<sup>1</sup> Corresponding author: Professor Mark A. Matthews, facsimile +1 530 752 0382, email mamatthews@ucdavis.edu

## Abstract

Soluble solids, seed tannin, skin tannin, and skin anthocyanin were measured in fruit from Cabernet Sauvignon vines that had experienced either High, Control or Low water status during ripening. Berries from each treatment were segregated into 6 size categories at harvest in order to test independently for relationships due to size compared with those due to water deficits. Berry content of all solutes increased approximately in proportion to the increase in berry size. Deviations from proportionality caused °Brix and anthocyanin concentration (mg per unit berry fresh mass) to decrease, and the concentration of skin tannin to remain unchanged or decrease slightly with increasing berry size. The concentration of seed tannin did not decrease and appeared to increase with berry size in multiple-seeded berries. In comparison with skin tannin or anthocyanin content, seed tannin content varied more with berry size and less with vine water status. In addition to decreasing berry size, water deficits increased the amount of skin tannin and anthocyanin per berry and the concentrations of skin tannin and anthocyanins, but did not significantly affect the content or concentration of seed tannin. The results show that there are effects of vine water status on fruit composition that arise independently of the resultant differences in fruit size. The effect of vine water status on the concentration of skin tannin and anthocyanin was greater than the effect of fruit size on those same variables. However, the increases in skin tannin and anthocyanin that accompanied water deficits appear to result more from differential growth sensitivity of inner mesocarp and exocarp than direct effects on phenolic biosynthesis.

**Keywords:** *Vitis vinifera L., Cabernet Sauvignon, berry size, irrigation, water potential, water stress, berry growth, berry composition, sugar, tannin, anthocyanin*

## Introduction

Berry size is widely acknowledged as a factor determining winegrape quality. Vine water deficits generally lead to smaller berries and changes in fruit and wine composition (e.g. Bravdo et al. 1985, Kennedy et al. 2002, Matthews et al. 1990). Hence, the question arises whether changes in fruit and wine composition that develop in response to drought or irrigation arise simply from resultant changes in berry size.

The implicit mechanism of this concept is that surface area:volume of the approximately spherical berries decreases with berry size according to  $3/\text{radius}$ . Anthocyanins and other phenolic compounds that contribute to red wines their unique characteristics accumulate in the skin (Coombe et al. 1987). It is generally assumed that larger berries have a relatively greater solvent (inner mesocarp cell sap) to solute (secondary metabolites in exocarp (skin)) than smaller berries because of smaller surface:volume ratio (Galdstones 1992, Hardie et al. 1997, Singleton 1972). However, there are few measurements to substantiate these assumptions. Several early studies showed some decrease in °Brix with increasing berry size (e.g. Muller-Thurgau 1898), but sugars in ripe berries are not localised in the skin (Coombe 1987, Possner and Kliever 1985). Nevertheless, Dry et al. (1999) speculated that

changes in fruit composition caused by water deficits could be attributed to changes in fruit size, and Matthews and Anderson (1988) implied as much. The most important experimental results are probably those from the indirect approach of Singleton (1972). In that study fruit was crushed, treated with SO<sub>2</sub>, and inoculated with yeast before some juice was removed from replicate fermentations and added to others to simulate differences in berry size. Measurements conducted on filtered wine samples showed that flavanoid and total anthocyanin concentrations were altered by about 6–7% in Cabernet Sauvignon wines made with the addition or removal of 10% of the free run juice.

However, adding and removing juice is not strictly comparable to changing berry size. Both skin and seed masses are functions of both berry size and vine water status (Roby and Matthews 2004). Accordingly, this study was conducted to evaluate the roles of fruit size and water deficits on these aspects of fruit composition that are of immediate interest in winemaking.

## Material and methods

### *Grape cultivation and irrigation treatments*

Irrigation treatments were imposed during 1999 in a commercial, drip-irrigated vineyard (*Vitis vinifera* cv. Cabernet

Sauvignon grafted onto 110 Richter rootstock) in Oakville, CA, USA (latitude 38° 27' N; longitude: 122° 25' W; elevation: 65m). Vine cultivation and water status were described previously (Roby and Matthews 2004). Briefly, treatments were established in a randomised block design replicated five times by supplying different volumes of irrigation: control (C), 32 litres/vine/week; high irrigated (H), 64 litres/vine/week; and low irrigated (L), 32 litres/vine/week, not irrigated until midday leaf water potential ( $\psi$ ) was  $-1.5$  MPa. The C and H treatments were irrigated when vine water status reached approximately  $-1.0$  MPa, on 20 July 1998 and 25 June, 1999. Vine water status was monitored approximately weekly from the first irrigation until harvest by measuring midday leaf water potential with the pressure chamber technique as previously described (Matthews et al. 1987).

#### Berry sampling and processing

Three bunches from each replication were sampled at harvest. Berries were separated from the pedicel, weighed, and separated into six size categories:  $< 0.5$  g,  $0.51-0.75$  g,  $0.76-1$  g,  $1.01-1.25$  g,  $1.26-1.5$  g,  $> 1.5$  g. Note that the four intermediate size categories contained over 90% of the fruit (Roby and Matthews 2004). From each of the five replicates, 10–20 berries were randomly selected from each size category, crushed together in a hand press through 2 layers of cheesecloth, and soluble solids were determined on a small aliquot of the juice with a hand-held refractometer.

#### Tannin and anthocyanin assays

From each size category within each replicate, 20 berries were randomly sampled for analysis of tannins and anthocyanins using an extraction described by Adams and Harbertson (1999). Berries were sliced in half with a razor blade. Skin was obtained by carefully removing seeds and mesocarp from each berry-half using a small metal spatula and avoiding rupturing of pigmented hypodermal cells. The seeds were carefully separated from remnants of flesh by hand. Both skin and seed were rinsed in de-ionised water and weighed after blotting excess water. Skin and seed samples were stored at  $-20^{\circ}\text{C}$  until thawing for phenolic assays. Skin and seed solutes were extracted in 2 mL/berry of 66% (v/v) acetone solution overnight on an orbital shaker. Acetone was completely removed under vacuum using a rotary evaporator in a warm bath at  $40^{\circ}\text{C}$ . Volume was adjusted to 1 mL/berry with de-ionised water.

Tannin concentrations were determined in the skin and seed extracts using a protein precipitation assay from Hagerman and Butler (1978) as modified and described by Harbertson et al. (2002). Briefly, extracts were diluted into a model wine solution containing 12% ethanol (v/v) with 5 g/L potassium bitartrate adjusted to pH 3.3 with HCl. A 500 mL aliquot of diluted extract was added to a 1.5 mL microfuge tube containing 1.0 mL of buffer (0.2 M acetic acid and 0.17 M NaCl, adjusted to pH 4.9 with NaOH) to which BSA was added to give 1 mg/mL of protein. The samples were vortexed and allowed to incubate on a shaker (50 rpm) for 15 min. After centrifugation at  $13,500 \times g$  for 5 min, the supernatant was discarded, and

the surface of the pellet and the walls of the tubes carefully washed with 500  $\mu\text{L}$  of acetate/NaCl buffer without BSA. Samples were centrifuged again at  $13,500 \times g$  for 1 min, supernatant was discarded, and the tannin/protein precipitate was dissolved in 875  $\mu\text{L}$  of a buffer containing 5% (v/v) triethanolamine and 10% sodium dodecyl sulfate, adjusted to pH 9.4 with HCl. Samples were let to stand for 10 min before measuring  $A_{510}$  (background absorbance). Then, 125  $\mu\text{L}$  of a ferric chloride solution (0.01 M  $\text{FeCl}_3$  in 0.01 M HCl) was added to each sample, and after a 10 min incubation,  $A_{510}$  was measured again. After background absorbance was subtracted, tannin concentrations were calculated from a standard curve that was generated with Chardonnay seed tannin, and values are reported in catechin equivalents.

Total anthocyanins were determined spectrophotometrically (Hewlett Packard 8453 Spectrophotometer fitted with a sipper system, autosampler, UV-visible detector, and HPCChemstation software) as  $A_{520}$  of extracts adjusted to pH  $< 1$  (Somers and Evans 1977).

Values for tannin and anthocyanin are reported as 'content' (mg per berry) or 'concentration' (mg per unit of berry fresh mass).

#### Results

The irrigation treatments created significant differences in the vine water status among H, C, and L vines, but differences in berry fresh mass only between L and the other treatments (Table 1). Midday leaf water potential of C vines was about 0.2 MPa less than H vines and about 0.2 MPa greater than L vines. The mean berry sizes for each treatment within a size category were similar. Therefore, any treatment differences that arose within a size category were not due to treatment differences in berry size within that category.

#### Soluble solids

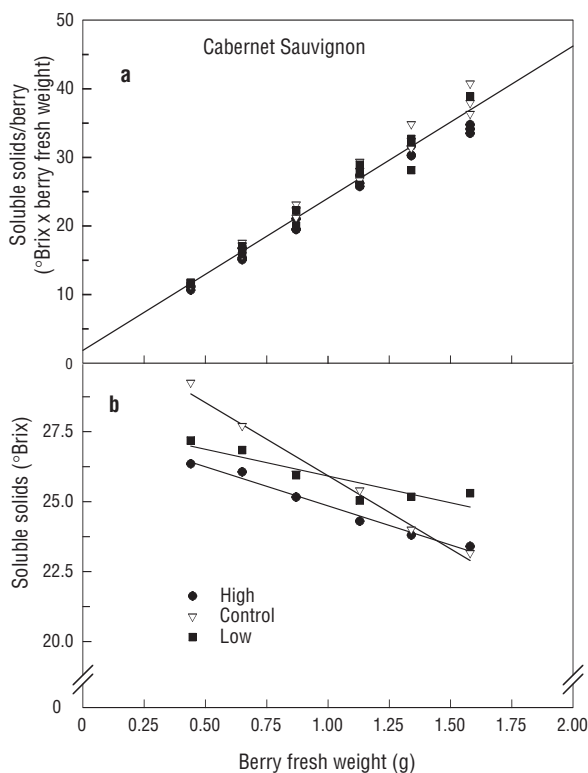
The accumulation of soluble solids depended upon berry size in two respects. Total soluble solids per berry ( $^{\circ}\text{Brix} \times \text{berry size}$ ) increased approximately linearly with berry size (Figure 1a). However, the increase in total soluble solids per berry was less than the approximately 250% increase in size from the smallest (0.45 g) to the largest (1.6 g) size category. Thus, the concentration of soluble solids in each berry ( $^{\circ}\text{Brix}$ ) was also dependent upon size, in this case exhibiting a negative function of berry size (Figure 1b). Although the correlation coefficients were not

**Table 1.** Midday leaf water potential and berry size at harvest of vines exposed to High, Control, and Low irrigation regimes (from Roby and Matthews 2004).

Treatment	Leaf water potential (MPa)	Berry size (g)
High	$-1.16 \pm 0.03^{\text{a}}$	0.95
Control	$-1.36 \pm 0.06$	0.95
Low	$-1.59 \pm 0.06$	0.78 <sup>b</sup>

<sup>a</sup>  $\pm$  s.e.m.; n = 5

<sup>b</sup> Different at  $P < 0.01$



**Figure 1.** The relationship between soluble solids (°Brix) and berry size for Cabernet Sauvignon fruit from vines that had been exposed to different irrigation treatments and sorted into berry mass categories. Data are plotted at the mean mass for each category. **a.** Total soluble solids per berry (product of °Brix and fresh mass of each berry). Linear regression coefficients were 0.97–0.99 for each of the three treatments and 0.97 for all treatments combined (regression curve shown). **b.** Concentration of soluble solids (treatment means) and corresponding linear regression lines.

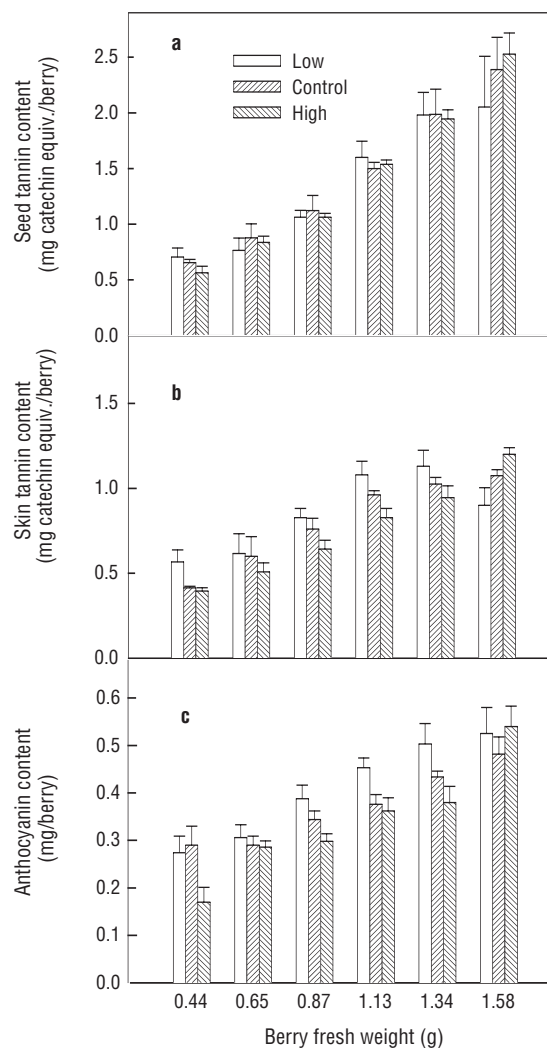
high, they were all significant ( $P \leq 0.05$ ). The °Brix of H berries in each size category was consistently lower than that of the L berries, and generally lower than that of C berries.

#### Seed tannin

Seed tannin content (mg/berry) also increased with berry size in all treatments (Figure 2a). In general, seed tannin content increased over 200%, from about 0.6 to 2.1 mg/berry. For H berries, the increase in seed tannin content from small to large berries was over 300%. Seed tannin content increased with berry size in all size categories of H fruit but reached a maximum at the next-to-largest category in L fruit. There was less variation in seed tannin content among small berries compared to large berries. Vine water status had no clear effect on seed tannin content.

Seed tannin content was a linear function of both seed number and seed mass/berry (Figures 3 and 4). However, seed tannin content was better correlated with seed mass ( $r^2 = 0.95$ ) than with seed number ( $r^2 = 0.90$ ). The difference was apparently due to variability in extracted seed tannin of one-seeded berries that was explained by accounting for variation in seed mass of one-seeded berries (cf. lower end of curves in Figures 3 and 4).

The relationship between berry size and concentration of seed tannin (based upon berry fresh weight) suggests two phases, with seed tannin concentration initially

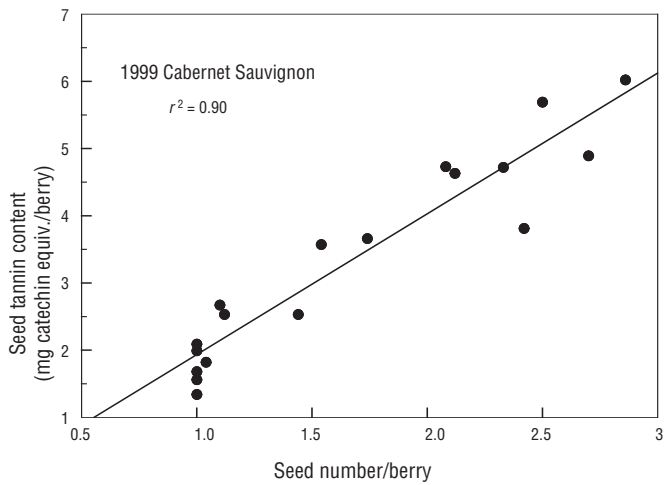


**Figure 2.** Content of seed tannin (**a**), skin tannin (**b**), and anthocyanin (**c**) in Cabernet Sauvignon berries harvested from vines that were exposed to high, control, and low irrigation. Error bars represent  $\pm$  s.e.m. ( $n = 5$ ).

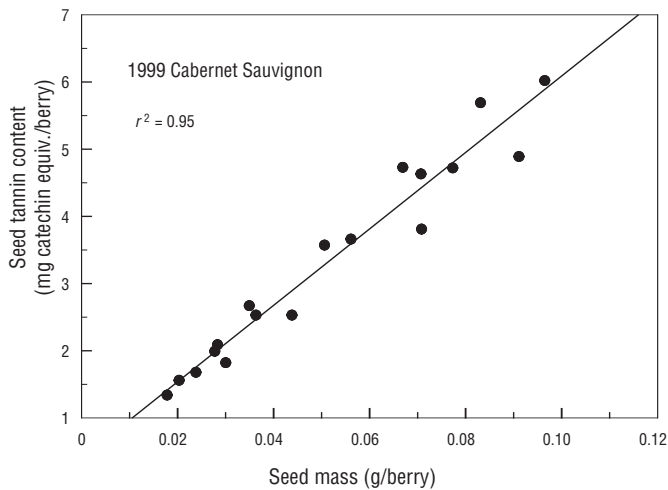
decreasing and then increasing as berry size increases (Figure 5a). This pattern would correspond with the smaller berry sizes that have one seed and the larger berry sizes that have two seeds (Roby and Matthews 2004). However, there were insufficient data to confirm two relationships among the size categories. The means of seed tannin concentration increased with berry size for the intermediate size categories that contained most of the berries. There were no significant differences in seed tannin concentration among irrigation treatments within size categories.

#### Skin tannin

In general, skin tannin content increased with berry size from approximately 0.4 to 1.0 mg/berry (Figure 2b). As with seed tannin, the skin tannin content of H fruit increased through all size categories, but only through the first four categories for L fruit. Skin tannin content in L berries was the highest in all but the largest berry size category. In the intermediate size categories, L berries had between 17 and 30% greater skin tannin contents than H berries, and those differences were significant ( $P < 0.05$ ).



**Figure 3.** Content of seed tannin in Cabernet Sauvignon berries that contained various numbers of seed. Seed tannin data are from Figure 2a of this study and seed number data are from Figure 7 of Roby and Matthews (2004).

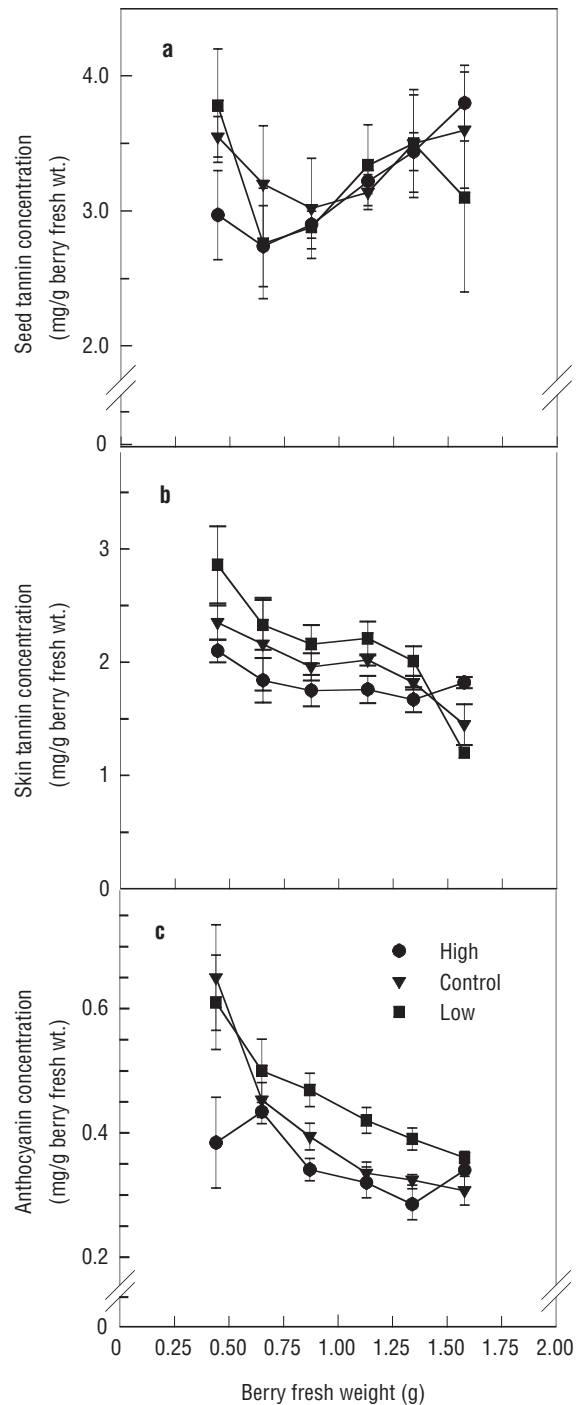


**Figure 4.** Content of seed tannin in Cabernet Sauvignon berries that contained various amounts of seed mass. Seed tannin data are from Figure 2a of this study and seed mass data are from Figure 8 of Roby and Matthews (2004).

The increase in skin tannin content with berry size was similar to the increase in berry size for all treatments. Consequently, the concentration of skin tannin was relatively constant among the intermediate size categories, although the data do exhibit a slight decrease (approximately 10%) from the smallest to the largest berries (Figure 5b). The concentration of skin tannin was about 0.5 mg/g fresh weight (or 30%) greater ( $P < 0.05$ ) in L than in H fruit for all except the largest berries.

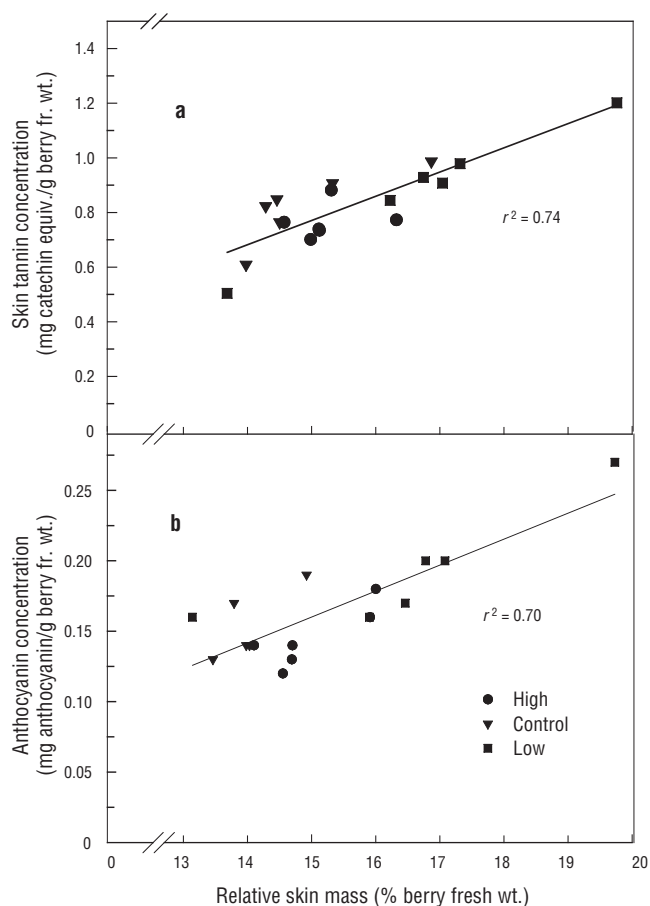
*Anthocyanins*

Anthocyanin content per berry was positively correlated with berry size (Figure 2c) but did not increase as much as seed or skin tannin content. When averaged across treatments, anthocyanin content increased about 100% from 0.25 mg/berry in the smallest berries to about 0.5 mg/berry in the largest ones. L berries had the highest anthocyanin content in the four intermediate size categories (Figure 2c). These size categories accounted for more than 95% of the berries in each treatment (Roby and Matthews 2004).



**Figure 5.** Concentration of seed tannin (a), skin tannin (b), and anthocyanin (c) in Cabernet Sauvignon berries harvested from vines that were exposed to high, control, and low irrigation. Error bars represent  $\pm$  s.e.m. ( $n = 5$ ).

Because the increase in anthocyanin content was less than the corresponding increase in berry fresh weight, the concentration of anthocyanins decreased with berry size at an average rate of 0.27 mg/g fresh weight for all treatments (Figure 5c). Correlation coefficients were 0.50, 0.79, and 0.92 for H, C, and L berries, respectively. There were no significant differences between C and H berries, except for the smallest size category. L berries had the highest mean concentration in most size categories. For three of the four largest size categories, the approximately



**Figure 6.** Concentration of skin tannin (a) and anthocyanin (b) in Cabernet Sauvignon berries with various relative skin masses. Data are means of five replications. Symbols as in Figure 5.

0.1 mg/g fresh weight (or 30%) higher concentration of anthocyanin in L than in H fruit was significant ( $P < 0.01$ ). For those same size categories, this increase was larger than the increase due to berry size found among all size categories within an irrigation treatment.

When skin tannin (Figure 6a) or anthocyanin (Figure 6b) concentrations were regressed on relative skin mass, approximately 70% of the variation in skin phenolic concentrations was explained by relative skin mass. The increased concentrations in L fruit based upon berry fresh weight were no longer evident. However, in both cases, the regressions were driven primarily by the L values.

## Discussion

Varying amounts of sugars, skin tannin, seed tannin and anthocyanins in Cabernet Sauvignon berries at maturity showed that the *content* of each of those constituents per berry was, as a first approximation, proportional to the size of the berry sampled. Small but potentially important variations in that approximation caused the *concentration* of sugars and anthocyanins to decrease with increasing berry size. However, the concentration of skin tannin was relatively insensitive to berry size, and the concentration of seed tannin generally increased with berry size. Taken together, these observations indicate that the composition of mature berries is not dependent in a simple way on the final size attained by the berry.

### Berry size and soluble solids

The increase in sugar content with fresh mass was not sufficient to prevent lower °Brix in larger berries. An inverse relation between °Brix and berry mass has been observed (e.g. Scienza et al. 1978, Cawthon and Morris 1982), but recently Glynn and Boulton (2001) reported no relation in Cabernet Sauvignon. Consistent with a weak relationship between °Brix and berry fresh mass, Kasimatis et al. (1977) reported in a series of regression analyses of fruit from several vineyards that the correlation between °Brix and berry size in Sultana was as often positive as it was negative. The negative relationship observed here is probably not a consequence of the surface:volume ratio of berries, because the sugars that dominate the °Brix assay accumulate in the flesh as much or more than in the skin (Coombe 1987).

Moderate water deficits also have often, but not always, been observed to increase °Brix, but the question has remained whether that was solely a consequence of reduced berry size. The lower concentration of soluble solids in H berries of any size demonstrates clearly that water deficits cause increased sugar accumulation. The increase in sugar accumulation may reflect a partitioning response to water status. For example, Yakushiji et al. (1998) found that moderate water deficits increased the allocation of recent photosynthate to developing Satsuma fruit, and that this effect was diminished with severe water deficits. That °Brix was not highest in the L fruit may indicate that the severity of the water deficit was sufficient to inhibit photosynthesis and translocation to fruit. This suggests an optimum midday leaf water potential for sugar accumulation between  $-1.2$  and  $-1.4$  MPa.

### Seed tannin

Seed tannin content increased approximately in proportion with berry size, seed number, and total seed mass per berry. Seed tannin content was better correlated with total seed mass than seed number, presumably because the correlation of seed tannin content with seed number could not account for the variation in seed tannin resulting from variable seed size in one-seeded berries (Roby and Matthews 2004). Except for the smallest berries, the concentration of seed tannin appeared to increase with berry size, but the high variability among samples obscured the relationship.

The results indicated no independent effect of vine water status on the concentration of seed tannin. However, this may mask an effect of vine water status on seed tannin metabolism, because seed mass for most berry sizes was greater in L fruit, and relative seed mass (fraction of berry fresh weight that is seed) in L berries was as much as 25% greater than in other treatments (Roby and Matthews 2004). These results indicate that the amount of seed tannin per seed mass was less in L berries than in H or C berries. Thus, water deficits either inhibited tannin accumulation or accelerated the rate of decline during fruit ripening. Recent results (outlined below) indicate that a decline during ripening is more likely.

Both flavan-3-ol monomers and seed tannin attain a maximum near veraison and decrease thereafter (Har-

bertson et al. 2002, Kennedy et al. 2000, Kennedy et al. 2001). Thus, the water deficits in the present study probably developed after tannin accumulation was complete or nearly so. It is clear from the different rates of postveraison decrease in the concentration of seed tannin among seasons (Harbertson et al. 2002) that there are important environmental factors determining that rate. In experiments that were similar to the present study, seed tannin content declined by as much as 57 to 65% within the first month after veraison (Kennedy et al. 2000). In that study, postveraison water deficits did not alter seed tannin content significantly, but did slow the postveraison decrease in monomers and tannin. The water deficits also reduced seed tannin per unit seed mass (cf. Figure 3b and 4b in Kennedy et al. 2000) in berries at harvest and lowered the concentrations of flavan-3-ol monomers at veraison.

#### *Skin tannins and anthocyanins*

As with seed tannin, skin tannin and anthocyanin content increased with berry size. However, the increase of skin tannin was less than that of seed tannin, and the increase of anthocyanin content considerably less than that of skin tannin. Consequently, the concentration of skin tannin was essentially unchanged with berry size, but the concentration of anthocyanin decreased with berry size, approximately 20% from small to large. The decrease in the concentration of anthocyanin is in accordance with results obtained by Hardie et al. (1997) and Ummarino and Di Stefano (1996, 1997).

Water deficits increased both the content per berry and the concentration of skin phenolics. When skin phenolics were analysed in berries of the same size from vines that had been exposed to different irrigation regimes, both skin tannin and anthocyanin contents and concentrations were greater in L than H and C fruit for all except the largest berries. Thus, the concentrations of skin tannin and anthocyanin were increased by an effect of vine water status that was independent of the role of water status in berry size because all berries in these comparisons were of similar size.

The effects of water deficits on the concentrations of skin tannin and anthocyanin differed in that the effects on anthocyanin concentration were twofold. There was a subtle difference between skin tannins (phenolics) and skin anthocyanins in their response to grapevine water deficit. For a given berry size category, both phenolics and anthocyanins showed a greater concentration in the skin of berries from grapevines that had experienced water deficit. However, with respect to anthocyanins, there was an additional effect that derived from a shift in the distribution of berry size towards smaller berries (Roby and Matthews 2004) that have higher concentrations of anthocyanins. The latter phenomenon was not observed for skin tannin, for which the concentration in most size categories was similar. Furthermore, the effect of vine water status on anthocyanin concentration for each berry size was greater than the effect of fruit size. For any intermediate berry size, the difference in concentration of anthocyanin between L and H berries was greater than the difference between small and large berries.

The primary mechanism by which water deficits increased the concentrations of skin tannin and anthocyanin is probably the differential growth responses of skin and inner mesocarp tissue to water deficits (Roby and Matthews 2004), although there may also be a direct stimulation of biosynthesis. This difference in growth sensitivity resulted in greater skin mass and relative skin mass per berry for most berry sizes in L fruit than in the other treatments, and thus, without invoking any other mechanism, greater amounts of skin-localised solutes. Treatment differences largely disappeared when the concentrations of skin tannin and anthocyanin were evaluated on the basis of relative skin mass per berry. Also, L skins were heavier in the intermediate size categories only (Roby and Matthews 2004). The similar skin mass of large L and H berries suggests that when growth was not restricted, a similar skin mass developed. These observations imply little effect of vine water status on synthesis of tannins or anthocyanins in berry skin when water status is altered after veraison.

It may be important to note however, that the concentrations of both solutes generally corresponded to vine water status. Thus, the concentrations of skin phenolics was generally greater in C fruit than in H fruit, although there were no growth differences between C and H fruit. The concentration of both solutes remained lowest in the H berries for the intermediate berry sizes. The concentrations remained low for H fruit and high for C fruit when expressed on a skin mass basis. And the regression analysis (Figure 6) indicated that skin mass accounted for about 70% of the variation in concentration of skin phenolics, leaving 30% unexplained. Thus, there may have been some positive effect of water deficit on phenolic metabolism in skins.

Hardie and Considine (1976) suggested that water deficit-induced shrivelling may increase sugar concentrations in the skin, and the resultant osmotic effect would enhance anthocyanin synthesis. The accumulation of anthocyanin has been closely correlated with sugar concentration (e.g. Pirie and Mullins 1977). Increasing the osmotic potential of the medium with sucrose or mannitol from  $-0.5$  MPa to  $-0.9$  MPa increased accumulation of anthocyanins in cultured cells (Do and Cormier 1990). However, Hardie and Considine (1976) reported decreased colour with water deficits, and in most cases the fruit with low colour was harvested at lower °Brix than the controls. The correlation was present in this study as well in that the highest concentration of anthocyanin per unit skin mass occurred in C berries with the highest °Brix.

However, Greenspan et al. (1996) showed that the Cabernet Sauvignon berry is resistant to water deficit-induced contraction when water deficits were imposed after veraison. There was no visible evidence of berry shrivelling in this experiment, and data from previous seasons also indicated no contraction at and even well beyond harvest (Kennedy et al. 2000). Thus, berry shrivelling was not a likely cause of increased sugar or other solute concentrations, but altered allocation patterns (discussed above) may have been. Hardie et al. (1997) described developmental changes in plastids of pericarp

cells that are important in polyphenol metabolism. It would be interesting to determine whether plastid development is altered by vine water deficits.

It should perhaps also be noted that water deficits might have effectively packed more cells into a similar volume, but that would require a proliferation of cells in response to water deficits. Although there has been some evidence of increased cell layers on other systems, the more general observation is that both cell division and, especially, cell expansion are inhibited by water deficits. Moreover, present water deficits developed after the main phase of cell division in berry growth would have ended.

The results of this study have clear implications for the role of vine water deficits and berry size in wine composition and sensory attributes. For example, at the mean berry size of the Control irrigation (C) and High irrigation (H) treatments (0.95 g), the Low irrigation (L) water deficits per se increased the concentration of anthocyanins about 30%, from 0.33 to 0.43 mg/g fresh weight. The further increase in the concentration of anthocyanins resulting from reduced berry size can be estimated from the concentration of anthocyanin in L berries at the mean size for that treatment (0.78 g), 0.48 mg/g fresh weight, or an additional 15% above the concentration in the larger H berries. Thus, the gain in concentration of anthocyanin in L fruit was approximately 45% over that in H fruit, but the corresponding loss in yield due to reduced berry growth was less than 20%. A similar analysis would indicate that seed tannin concentration decreased with berry size and solely because of berry size. In contrast, the concentration of skin tannin increased with water deficits, but due almost exclusively to water deficits per se, because berry size was a relatively insignificant factor.

If the extraction of each solute is independent of size and water status, wine made from larger berries would contain lower concentrations of anthocyanin, and probably higher concentrations of seed tannin. The ratio of seed:skin tannin would increase as the size of the source berries increases, because seed tannin increases while the contribution of skin tannin remains essentially unchanged. However, the extractions in this study were both comprehensive and into acetone, compared with low concentrations of alcohol as would prevail during fermentation.

On balance, present results indicate that the source(s) of variation in berry size are more important in determining composition than size per se. Further evidence of this principle can be found in the published literature for growth and composition responses to various environmental factors. For example, high yield reduces the weight of individual fruit, but generally causes lower rather than higher concentrations of solutes (e.g. Bravdo et al. 1985). And there are many examples of increased light exposure increasing both berry size and solute concentrations (e.g. Dokoozlian and Kliewer 1996). Finally, the timing of the water deficit is clearly important in determining fruit and wine composition (Matthews et al. 1990). Water deficits that develop prior to veraison may have considerably different consequences for berry development than those observed here.

## Acknowledgements

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