Effect of water deficits and season on berry development and composition of Cabernet Sauvignon (*Vitis vinifera* L.) grown in a hot climate

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Keywords: *anthocyanin, berry maturation, organic acids, RDI, Vitis vinifera*

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/ajgw.12274](https://doi.org/10.1111/ajgw.12274)

Abstract

Background and Aims: The effect of season and deficit irrigation treatments was monitored on berry development and composition, yield and wine composition of *Vitis vinifera* L. cv. Cabernet Sauvignon grown in a hot climate during 2003–2005).

Methods and Results: The treatments were regulated deficit irrigation (RDI) and prolonged deficit irrigation (PD), compared with a standard industry irrigation (Control) treatment. Compared to the Control, RDI berries were smaller from a reduction in mesocarp (flesh) rather than in exocarp (skin) or seed mass but insufficient to significantly impact on yield. The PD treatment reduced berry size driven by mesocarp reduction resulting in a lower yield. Season 2005 had a significantly larger yield mainly a result of increased berry fresh mass, exocarp and seed mass compared to that of either 2003 or 2004. Grape juice TSS and pH were not significantly affected by water deficit but each varied between seasons. The malic acid concentration of grape juice was reduced by the RDI and PD treatments resulting in enhancement of the tartaric: malic acid ratio. Grape anthocyanin at harvest was unaffected by irrigation treatment but significantly impacted by season. Wine colour density, anthocyanin, ionised anthocyanin and phenolic substances were increased by RDI and PD and impacted by season.

Conclusions: Wine attributes were improved further by PD relative to RDI. Seasonal effects were shown to have a impact greater than the within-season water deficit treatments on most aspects of berry development and composition.

Significance of the Study: The study has demonstrated the utility of the PD treatment as an irrigation tool, where water availability is significantly limited and / or where improved spectral properties are required for wines.

Introduction

There is a rising trend to impose deficit irrigation in vineyards of the irrigated inland growing districts of Australia to reduce vigour and improve the quality of winegrapes (Kriedemann and Goodwin 2003). The most common approach is the application of regulated deficit irrigation (RDI) where the irrigation amount is reduced, generally during the period between berry set and veraison. Reductions to irrigation at this particular phenological stage slow canopy development and result in reduction of berry size (McCarthy 1997, McCarthy et al. 2002). As seasonal variation can significantly alter the relationship between source characteristics (leaf area, net canopy photosynthesis) and sink carbohydrate partitioning into berries, stems, trunk and root, the degree of soil water deficit can have a potentially significant impact on carbon assimilation and carbohydrate partitioning to the various sinks (Pellegrino et al. 2014).

Leaf water potential is reduced when grapevines are subjected to water stress (Williams and Aroujo 2002, Bota et al. 2004, Cifre et al. 2005). Such reduction often results in reduced stomatal conductance (g_s) (Escalona et al. 1999), transpiration (T), photosynthesis (A) (Rodrigues et al. 1993, Flexas et al. 1999, Iacono and Sommer 2000, Bota et al. 2004) and sugar accumulation by the grape berry (Smart and Coombe 1983,

Quick et al. 1992, Schultz 1996). The responses can vary depending on the level of reduction in leaf water potential, the cultivar (Gomez-Del-Campo et al. 2004, Cifre et al. 2005, de Souza et al. 2005) and seasonal effects (Moutinho-Pereira et al. 2004).

High temperature has also been shown to inhibit g_s and A (Sinclair et al. 1984, Edwards et al. 2011). In glasshouse studies, Edwards et al. (2011) found that short term effects of high temperature on leaf physiology were more severe in deficit irrigated vines, a problem compounded by leaf loss.

The effect of water deficit on sugar accumulation by the grape berry appears to be further exacerbated in years with a high crop (Poni et al. 1994), although crop load does not always appear to be the sole cause of the difference in maturation between similar cultivars. For example, in a hot irrigated region, such as the Sunraysia region of Australia (Clingeleffer et al. 2001), maturation of well watered Cabernet Franc vines was severely impaired during the 1998/99 growing season, beginning shortly after veraison (Sommer 2000). In this case, heat-induced, transient water stress combined with a heavier than average crop may have contributed to the temporary arrest of colour and sugar accumulation (Sommer 2000).

The concentration of anthocyanin in grape berries increases in response to water deficit (Pirie and Mullins 1977, Freeman and Kliewer 1983, Matthews and Anderson 1988, McCarthy et al. 1996), although Ashley et al. (2006) and Bindon et al. (2008) found partial rootzone drying had no effect on the concentration of berry

anthocyanin which has also been shown to depend on the degree of bunch shading and resultant berry temperature (Haselgrove et al. 2000).

Seasonal consistency of wine composition is considered important in the region where this research was conducted which is characterised by relatively high cropping and mechanical pruning and harvesting. Accordingly, seasonal impacts along with irrigation strategies were explored. The irrigation strategies used in this research consisted of two deficit irrigation treatments comparing impacts of soil water deficit on berry development, bunch mass, berry mass, berry structure, berry composition and final yield relative to an industry standard (Control) irrigation treatment. Grape berry TSS, anthocyanin, phenolic substances, titratable acidity (TA) and berry texture were monitored. The research aimed to increase our understanding of the maturation process in response to deficit irrigation treatments and, with that understanding, to achieve greater seasonal consistency of yield and berry composition. Evapotranspiration (ET) can be high post fruitset when decisions on timing and amount of irrigation are important and the impact of such decisions on yield and berry composition can be critical. Therefore both a regulated deficit irrigation treatment and a prolonged deficit irrigation treatment were compared. The study aimed to provide valuable insights into best practice water management strategies for improving wine composition from mechanically hedged, high yielding vines in a hot irrigated region.

Materials and methods

Cabernet Sauvignon (*Vitis vinifera* L.) vines on own roots were planted in 1995 at a density of approximately 1389 vines/ha (2.4 m within the row and 3 m between rows) as part of a 5 ha commercial vineyard located in the Sunraysia region of Victoria, Australia. The soil was a calcarosol (Isbell 1996) consisting of a sand to loamy sand increasing to a sandy loam (to approximately 80 cm) above a carbonate layer (containing <30% calcrete fragments) leading to a restrictive clay base at (> 170 cm) (Edwards and Clingeleffer 2015). Vines were trained in east–west running rows on a two-wire vertical trellis with wires at 1.5 and 1.8 m. Vines retained approximately 150 buds after mechanical hedging.

Three irrigation treatments consisting of: (i) an irrigated Control; (ii) a regulated deficit irrigation (RDI) treatment; and (iii) a more severe form of RDI, termed prolonged deficit (PD), were initiated after fruitset each season (2002/03, 2003/04, 2004/05), starting in November 2002 (Table 1). Drip irrigation was applied using drippers spaced at 0.6 m intervals and delivering 1.6 L/h. Table 1 shows irrigation applied and rainfall received for each treatment and season totals for each year. A water table was present at around 1.40 m. Soil moisture in all treatments was monitored throughout the season by nine neutron moisture meter access tubes located 15 cm within the inter-row space. Neutron probe readings (Hydroprobe model 503DR, CPN Martinez, CA, USA) were taken at 10 cm intervals to a depth of 1.2 m bi-weekly throughout the irrigation season. At the same trial site, the performance of the neutron

probe has been compared with frequency domain reflectometry (FDR) (Edwards and Clingeleffer 2013). Analysis of the underlying data from that study showed a strong linear relationship between the neutron probe readings and the FDR readings ($Y = 49.13x + 15.70$, $R^2 = 0.79$) in the 0–30 cm zone. This also revealed, however, an overestimation of soil moisture in dry soil (e.g. a reading of 23.1% compared to 15% volumetric water content determined by FDR, a 54% overestimate by neutron probe) and a slight underestimation in wet soil (e.g. 33% compared to 35% determined by FDR). Hence, soil moisture data for the RDI and PD treatments are presented as a proportion relative to the Control treatment (Figure 1). The mean volumetric soil moisture content for the 0–100 cm zone of the Control treatment was 29.5, 28.7 and 24.3% for seasons 2002/03, 2003/04 and 2004/05, respectively.

The irrigated Control treatment was established by using two standard drip lines that delivered twice as much water as that delivered to RDI and PD vines during the RDI stress period. Irrigation volume applied for each treatment was determined based on valve flow rate and irrigation duration for the experimental block taking into account the number and flow rate of drippers and use of in-line taps to regulate water supply to PD treatment vines. The Control was maintained at close to field capacity throughout the season by soil water replenishment based on neutron-probe data readings (CPN Probe for Windows Version 1.0.210 and with set points determined from soil water use data of previous seasons [hereafter referred to as 100% of the estimated evapotranspiration (ET) based on soil water extraction]).

The RDI treatment received 100 % ET until fruitset (typically during November but the date varied each season) at which time water applied was halved by reducing irrigation duration (hereafter referred to as 50% ET). The RDI period of 50% ET commenced at fruitset and continued for 17–38 days until canopy indicators, associated with excessive vine stress were visible. These included brittle tendrils (yellow colour), changes to the appearance of shoots (from light bright green to dull green) and most importantly, a reduction in canopy due to leaf loss (McCarthy et al. 2002) which was monitored to ensure there was adequate cover to sustain the crop load for that season. Canopy indicators were assessed bi-weekly along with soil water potential profiles. After the RDI period the irrigation (in the RDI treatment) was returned to 100% ET until harvest.

The PD treatment received the same RDI treatment from fruitset (mid- to late November) until canopy indicators were visible. This was followed by a period without irrigation (0% ET) until veraison (early to mid-January) or late January, at which time it was returned to 100% ET until harvest. In the first season, 2002/03, the PD period was interrupted by a significant rainfall event of 66 mm on 31 December (Table 1). Compared to the Control, high relative soil moisture values, recorded for the PD treatment in 2003/04 and both deficit treatments in 2004/05, can be attributed to the development of smaller canopies and reduced total transpiration (Edwards and Clingeffer 2013).

The duration of the RDI period and subsequent PD stress period varied each year as the canopy stress indicators were subjected to seasonal variation. For example, the vegetative cover in season 2002/03 was smaller than that of seasons 2003/04 and 2004/05 (data not shown).

Irrigation treatments were arranged in a fully randomised block design across and down the rows (blocks). The experimental design consisted of 12 vines per irrigation treatment, three irrigation treatments per row and a total of 12 irrigation treatment rows. Thirty-six rows were used in the experiment with two buffer rows placed between each treatment row.

Sampling protocols

Grape berry maturation in season 2002/03 was monitored from the onset of veraison, defined here as the beginning of a change in colour of the berries from green to red (E-L 35), through to harvest (E-L 38) (Coombe 1995). Harvest date was determined when a sample of berries from each of the treatments reached as close as possible to TSS 24.5°Brix as determined by sampling across all treatment replicates on a twice-weekly basis. During season 2003/04, berry maturation was monitored from pre-veraison (E-L 33–34) through to harvest. During season 2004/05 it was monitored from an earlier stage, from E-L 29–30 until harvest.

Of the 12 vines established with each irrigation treatment, two were established as buffer vines and ten were selected for sampling. Vines within each treatment block were sampled alternately to minimise potential changes in carbohydrate partitioning due to the reduction in bunch numbers. Five bunches per vine were randomly collected weekly as described by Krstic et al. (2003). Bunches were placed in plastic bags and stored on ice during transfer from the field to the laboratory. Bunch and berry fresh mass was recorded. The total fresh mass of 100 berries randomly selected from the five bunches was recorded, and juice samples were extracted by pressing (pestle and mortar) into a pulp and then straining the sample through a 1 mm² mesh.

Grape juice TSS, TA, pH and organic acids

Total soluble solids was determined with a temperature compensating digital refractometer (Atago, Tokyo, Japan). Titratable acidity and pH were determined using an auto-titrator (VIT90 Video Titrator, Radiometer, Willich, Germany, with SAC 80 Sample Changer and ABU91 Autoburette). In each case, a 5 mL sample with acid equivalents was titrated to an end point of pH 8.2. The organic acids of berry juice were determined using HPLC (see below for details).

Spectral properties of berries

A further sample of 100 berries randomly selected from each replicate vine was placed in a self-seal plastic bag and immediately placed at -18°C for storage until pigment

analysis (approximately 1 week to 3 months). The concentration of anthocyanin (colour) and phenolic substances in macerated whole berries was measured by the UV-VIS spectrophotometric method developed by Somers and Evans (1977) and modified by Iland et al. (1996) with the homogenised extraction occurring over 1 h with 50% ethanol thus enabling red-free glycosyl glucose determination spectrophotometrically. Sample pre-processing procedures are described by Cooley et al. (2006). Measurements of non-degraded and degraded anthocyanin were based on the pH-differential method developed in cranberry juice by Fuleki and Francis (1968) and adapted for wine grapes.

The colour of the skin of each whole berry at harvest during seasons 2004 and 2005 was analysed, usually within 5 h of collection, on a sample of ten berries randomly selected from each replicate vine. Each berry was measured three times using a Minolta colour meter (Minolta data processor, DP – 301, Chroma Meter CR -300 Series, Minolta Camera, Osaka, Japan). The meter measures reflective colour of surfaces as L^* , a^* and b^* values from which chroma, theta (intermediate used to calculate colour hue) and hue (H°_{ab}) can be calculated. The measuring area was reduced from 8 mm to 6.3 mm to allow for small berry surfaces. A Minolta calibration plate (CR – 200 / CR– 300) was employed for calibration at the beginning of each set of measurements or whenever the power supply was interrupted. Exocarp (skin) colour measurements were made at three different points around the midriff of each berry.

Berry texture analysis

Following measurements with the Minolta colour meter, berries were transferred to a texture analyser (TA.XT plus Texture Analyser, Arrow Scientific, Lane Cove, NSW, Australia, incorporating the Texture Expert Exponent software with a P/2 probe).

Texture analysis is a technique claimed to be an indicator of quality in winegrapes (Segade et al. 2008, Rolle et al. 2012). The resistance, which is measured as force applied, along with the distance the probe travelled, was recorded at 3 and 10 s. The force applied was set at a rate of 10.2 mm per min distorting the berry surface at a set distance of 2.0 mm. Mean values of berry texture data were calculated from 120 samples. Both the resistance measured and distance the probe moved into the berry exocarp (skin) are indications of the berry surface strength and therefore berry development. Berries were subsequently separated into exocarp, mesocarp (flesh) and seed, for which dry mass was obtained after 5 days at 80°C resulting in constant mass.

Small scale winemaking

Grape bunches were collected for small scale wine production immediately prior to the commercial harvest of the block. Harvest dates were 11 March 2003, 15 March 2004 and 2 March 2005 (E–L 38). Total soluble solids reached 24.5°Brix during the ripening phase of berry development. Grape bunches (15–20) from each vine (located in a 1 m wide transect) were randomly selected and mixed to make one wine replicate. Each wine replicate consisted of about 12 kg of fruit. Three wine replicates for each

irrigation treatment were made. Fruit was fermented based on the method of Becker and Kerridge (1972). Grape bunches were processed through a de-stemmer/crusher (Amos, Heilbronn, Germany) into 10 L polyethylene containers to which both 0.1 g/L sulfur dioxide (SO₂) (10% w/v sodium metabisulfite) and 0.75 g/L diammonium hydrogen orthophosphate (10% w/v) were added. The pH was adjusted into the range of 3.40 to 3.55 by addition of tartaric acid. Lalvin 245D yeast (Lallemand, Montréal, QC, Canada) was rehydrated and added at the rate of 2 g/L before the must was placed in a 25°C room. Must was plunged twice daily and then pressed with a 30 L Willmes air-bag (Willmes, Bensheim, Germany) and stored at 25°C for 3 days. Must was transferred to CO₂-filled gas bottles to continue fermenting at 25°C. When the sugar concentration of the wine was less than 0.25%, the wine was racked, adjusted to 20 mg/L SO₂ (10% w/v sodium metabisulfite) and stored for 14 days at 15°C. A second racking occurred and further SO₂ adjustments were made. Wine was cold stabilised at 4°C for 30 days and sterile filtered into 375 mL bottles. The spectral properties of the wines were assessed as developed by Somers and Evans (1977) and modified by Iland et al. (1996).

Analysis of organic acids

Organic acids were determined by HPLC (GBC Scientific Equipment, Dandenong, Vic., Australia). The system consisted of an LC1610 auto-sampler, LC1150 pump with in-line degasser, LC1210 UV/UV-VIS detector, refractive index (RI) detector and operated via the WinChrom chromatography manager software. The sample injection volume

was 20 μ L and sample concentration was determined using peak area. A Supelcosil LC18 RP (column dimensions 5 μ m, 25 cm * 4.6 mm) stationary phase (C₁₈ RP) protected by a C18 guard column (Supelcosil, Sigma-Aldrich, Castle Hill, NSW, Australia) was run at 40°C. Organic acids (malic, tartaric, citric and lactic) were transported to the column in the mobile phase 0.25% v/v acetic acid at pH 3. Organic acids were separated according to their Pka and physical size by passing through the stationary phase at 0.6 mL/min (total run time was 15 min). Compounds were detected at 195 nm (Escobal et al. 1997).

Statistics

Mean values were calculated and treatment differences were tested by two-way ANOVA (GenStat, 6th edition, VSN International, Hemel Hempstead, England). The main effects were irrigation treatment by season and the blocking was defined by columns (down the row). Where a significant difference was found, mean values were separated using Fisher's LSD test ($P = 0.05$). Curve fitting functions were employed by using Microsoft EXCEL Solver non-linear equation optimisation algorithm GRG2 (Pellegrino et al. 2006, Kemmer and Keller 2010).

Results

Regulated deficit irrigation treatment (RDI) and PD treatment were imposed across three seasons (Table 1, Figure 1) and compared to the Control which was the industry

standard for the winegrape growing region (Alexander and Potter 2004, Pink 2008, Toll et al. 2008, McClymont et al. 2012). Differences in seasonal ambient temperature were observed (Figure 2). Based on thermal time (degree days after flowering), season 2002/03 was hotter than the other seasons (Figure 2b). Contributing to this was a relatively hot December 2002, January and February 2003 (Figure 2a). Season 2003/04 had a hot December 2003, followed by a cooler January and hot February (Figure 2a). Season 2004/05 was characterised by a cool December, followed by a hot January and cool February (Figure 2). Berry development was characterised by three phases, the first from fruitset to the start of the lag phase (phase I), lag phase to 50% veraison (phase II) and finally veraison to berry ripening based on TSS determination.

Effect of irrigation treatments on bunch and berry development characteristics and yield

Bunch fresh mass was not significantly affected by deficit irrigation treatment (Table 2). Berry development was monitored during phase III in season 2002/03, during phases II and III in season 2003/04 and in all phases in season 2004/05 (Figure 3). Berry dry mass development for all treatments across all seasons was calculated to fit a continuous function of time and where a typical exponential relationship with growth was found (solver equation function, EXCEL, calculations for all treatments in season 2004/05 are illustrated in Figure 3). A significant reduction in berry dry mass was observed at harvest for both deficit irrigation treatments when compared to that of the Control ($P < 0.001$, Table 2), and this reduction was shown to occur as early as phase I berry

development during season 2004/05 ($P < \mathbf{0.001}$, Figure 3). Similarly, a significant reduction to berry fresh mass at both deficit irrigation treatments compared to that of the Control was observed at harvest (Table 2) and during berry development, where time was recorded as thermal time after flowering ($P < \mathbf{0.05}$, Figure 4) compared to that of the Control treatment. Where time was recorded as day of year or days after flowering, reduction in berry mass for both the RDI and PD treatments was also observed (data not shown). The significantly reduced fresh and dry berry mass in the deficit treatments did not impact on the berry fresh mass to dry mass ratio at harvest (Table 2).

No significant effect on exocarp (skin) or seed dry mass was found with the deficit treatments (Table 2) compared to that of the Control treatment. A significant reduction was found in mesocarp (flesh) dry mass with deficit treatments ($P < 0.05$), but there was no significant effect on berry midriff diameter compared to that of the Control (Table 2).

A reduction in berry mass did not affect the yield in the RDI treatment, but it was associated with a reduction in yield in the PD treatment (Table 2). Irrigation treatment did not significantly alter water use efficiency (WUE) when expressed as irrigation water use index [yield per volume of water applied (Table 2)].

Season effect on bunch and berry development characteristics and yield

Bunch fresh mass at harvest was not significantly different over the three seasons of this trial (Table 2). Berry fresh mass at harvest in season 2005 was significantly higher than

that in seasons 2003 and 2004 ($P < 0.001$, Table 2). Where time was calculated as degree days after flowering, a seasonal difference in berry fresh mass was observed (Figure 4, $P < 0.05$) with earlier and bigger berry development in 2005 (the coolest season) suggesting that seasonal fluctuations in temperature (Figure 2) are impacting on berry development (Figure 4). Berry dry mass at harvest was similar in 2003 and 2004 but significantly higher in season 2005 ($P < 0.001$, Table 2).

Berry diameter was larger in season 2005 compared with that in season 2004 (not measured in season 2003) (Table 2). The berry fresh to dry mass ratio varied with season (Table 2). Lighter exocarp mass was found during season 2004 but there was no significant change in mesocarp mass across seasons (Table 2). Seed dry mass was highest in season 2005 (Table 2). No change in seed number per berry was found across seasons (data not shown). Crop yield in season 2005 was 55% higher than that in season 2003 and 37% higher than that in season 2004 (Table 2). Water use efficiency expressed as irrigation water use index (IWUI) varied with each season studied and ranged from 10.4 t/ML applied (2002/03) to the low efficiency of 3.7 t/ML in season 2003/04.

Irrigation treatment and season effects on berry skin strength

The strength of the surface of each berry can be used as an indication of berry development/quality (Segade et al. 2008, Rolle et al. 2012). During phase I of berry development, the exocarp is structurally stronger than in phase III when berry softening occurs (Winkler et al. 1962). There was no significant effect of deficit irrigation

treatments on berry surface strength, measured at harvest as distance (of skin) moved during a 10 s period using a texture analyser compared to that of the Control treatment (mean value for the Control, RDI and PD treatments, -4.4 mm). When resistance was measured as force applied, the PD treatment exerted a greater force at 10 s [0.65 N (newtons)] compared to the Control (0.60 N) or RDI treatments (0.58 N) ($P < 0.01$). This indicates that at harvest the surface of the PD berries was stronger than the berry surface of either the RDI or Control treatments. Zsofi et al. (2014) demonstrated that the mechanical properties of grape skin are dependent on skin structure rather than its thickness. The skin structure is largely composed of cellulose, phenolic substances and anthocyanin. This work was supported by research conducted by Fernandes et al. (2015) where it was demonstrated that berry texture can indicate changes in specific properties that may be influenced by the amount of phenolic substances. This may impact not only the grape skin quality but on the crushing process as well.

Berry surface texture at harvest during season 2005 exhibited a higher resistance when measured as force applied per berry, a value of 0.67 N at 10 s compared with 0.55 N for season 2004 ($P < 0.001$), or when calculated as the distance the probe travelled, a less negative value of -4.3 mm at 10 s compared with -4.5 in season 2004 ($P < 0.05$), suggesting increased resistance and a stronger exocarp in 2005.

Effect of irrigation and season on berry composition

The accumulation of TSS in grape juice of berries plotted against day of year was non-linear with a similar accumulation rate for all treatments for all seasons (Figure 5) and

similar maturity levels at harvest (Table 3). When plotted against degree days after flowering, there appeared to be some seasonal differences, specifically earlier commencement of sugar accumulation in 2005 than in 2004 (initial data for 2003 not available) and earlier maturation in 2004 and 2005 relative to that in 2003. Ripening also appeared to be slower in 2003 (Figure 5). When TSS (in grape juice), however, is expressed as sugar content per berry, an impact of irrigation treatment was observed, with significant reduction ($P < 0.001$) in the PD treatment compared to that of the Control and RDI treatments during berry development (Figure 5) and at harvest (Table 3).

Grape juice pH increased linearly through the season with all irrigation treatments (Figure 6). At harvest, pH was similar between the irrigation treatments but varied between the seasons, being highest in 2005 and lowest in 2003 (Table 3).

There was an exponential decrease in grape juice TA for all irrigation treatments with increase in degree days after flowering (Figure 6). Grape juice TA at harvest was similar between the irrigation treatments but significantly higher in seasons 2003 and 2005 than in season 2004 (Table 3).

The concentration of tartaric acid and citric acid in juice of berries at harvest was not affected by irrigation treatment but the concentration of malic acid was 24% lower in RDI and 28% lower in PD relative to that in the Control (Table 3, $P = 0.008$) leading to a higher tartaric to malic acid ratio for the deficit irrigation treatments. Furthermore, malic acid concentration was significantly lower in 2004 than in 2003 and 2005 (Table

3, $P < 0.001$). **Citric acid concentration** was significantly higher in 2005 than in the other seasons (Table 3, $P < 0.05$)

Anthocyanin concentration in macerated whole berry samples plotted against degree days after flowering was reduced in 2003 compared to that in 2004 and 2005 (Figure 7) including at harvest (Table 3). There was no effect of irrigation treatment on the concentration of berry anthocyanin at harvest (Table 3). The concentration of non-degraded anthocyanin in macerated whole berry samples at harvest was not significantly affected by irrigation treatment (Table 3), however, there was a significant seasonal effect, with a lower concentration in 2003 relative to the other two seasons (Figure 7) including at harvest (Table 3). The concentration of degraded anthocyanin in macerated whole berries increased throughout phase III of berry development (Figure 7). At harvest, mean values for degraded anthocyanin were significantly higher in the RDI and PD treatments compared to that of the Control treatment (Table 3); concentration was also higher in 2005 than in 2003 and 2004.

The concentration of phenolic substances in macerated whole berry samples in two of the three seasons tended to decrease during the middle stages of berry development before stabilising or increasing towards harvest. The pattern was similar in seasons 2004 and 2005 but less evident in 2003 (Figure 7). The concentration of phenolic substances was similar between the irrigation treatments at harvest (Table 3), however, it was sensitive to seasonal variation, with the concentration at harvest highest in season 2004 and lowest in season 2005 (Table 3).

No significant effect was found with irrigation treatments on exocarp (skin) colour at harvest, measured as L (lightness), A (redness), B (yellowness), chroma or hue values, in either season 2004 or 2005 (data not shown). At harvest in season 2004, berry exocarp was lighter, had increased yellowness, higher chroma and hue values ($L = 37$, $B = -2.1$, chroma = 2.2, hue = 265) compared to season 2005 ($L = 33$, $B = -0.6$ $P < 0.001$, chroma = 1.04 or hue = 211), indicating that berry exocarp colour was at a higher level in season 2005 compared to that in season 2004.

Effect of irrigation and season on wine attributes

Wine made from vines treated with PD had lower pH and lower colour hue relative to that of wine from Control vines (Table 4). Wine colour density, and concentration of anthocyanin and ionised anthocyanin were in the order PD > RDI > Control, while the concentration of wine phenolic substances was similar between RDI and PD but was higher than in wines made from Control vines (Table 4, P values ranged from $P < 0.001$ to $P < 0.05$). There was also a strong seasonal effect, with pH and TA in season 2005 > 2004 > 2003 and with highest colour density and concentration of ionised anthocyanin in 2004, highest anthocyanin and phenolic substances in 2003 and lower wine colour hue in 2004 relative to 2003, with similar values for 2004 and 2005 (Table 4).

Discussion

Berry development

Compared to the Control, deficit irrigation treatments (RDI and PD), respectively, had a lower yield (by 7 and 15%) and reduced berry size (by 9 and 12% fresh mass, 14 and 18% dry mass) with no influence on inflorescence number (mean of 126 ± 5 per vine, results not shown). Berry size and yield reduction have been recorded in earlier studies on the effect of water deficit on grapevines, with timing of water deficit a key determinant of the extent of berry size and yield reduction (Hardie and Considine 1976, McCarthy 1997). In this study, the deficit irrigation treatments had reduced the mass of berry flesh (mesocarp) (11% for RDI and 17% for PD) but had no significant effect on berry skin (exocarp) mass, seed mass or fresh to dry mass ratio of berries (Table 2). The exocarp to mesocarp ratio increased but the increase was not significantly different between the treatments (Table 2). Other studies (Bravdo et al. 1985, Kennedy et al. 2002) have reported an increase in the ratio of skin to flesh in smaller berries of vines subjected to water deficit. The absence of a change in ratio in this study may indicate compensatory changes in the exocarp. The compositional and physical changes leading to this outcome were not identified.

Ojeda et al. (2001) plotted berry development traits against degree days after flowering and found that water deficit modified both the onset and duration of the three phases of berry development. Phase I and II were found to be shorter for the water deficit treatments compared to that of the Control (Ojeda et al. 2001). Our study provided no clear evidence for this effect (Figure 3). Temporary reduction in berry

growth due to water stress (Matthews et al. 1987, Ojeda et al. 2001) and a reduction in cell volume with early water deficit, which were not reversed by return to normal irrigation (Ojeda et al. 2001), have also been reported.

When our results are compared with those of Sadras and Petrie (2011), we were unable to determine change in early berry development due to temperature impact as observed by season. Early berry development responses have been found where the mean seasonal temperature ranges from 15.5 to 20.5°C (Sadras and Petrie 2011), whereas in our study, average temperature from budburst to harvest was hotter (at 22.0°C in 2002/03, 22.2°C in 2003/04 and 21.8°C in 2004/05).

Berry TSS

The slower maturation of berries in 2003, identified in the plot of berry TSS against degree days after flowering, may have been related to the hot 2002/03 season (Figure 2) relative to the 2003/04 and 2004/05 seasons despite having the lowest yield. The total carbohydrate pool may have been limited during 2002/03 and not in the other years and/or that other environmental impacts such as the high seasonal temperature in 2002/03 confounded the water stress effect. Transient high temperature events are known to cause greater reduction in stomatal conductance and net photosynthesis in vines experiencing water stress than in well-watered vines (Edwards et al. 2011). The effect of water deficit on sugar accumulation in grape berries appears to depend on the timing of the water deficit, its intensity and the variety (Chaves et al. 2010). For example, the impact on sugar accumulation is greater if the water deficit is imposed pre-

veraison (Keller et al. 2006). There are also genotype effects, as demonstrated in the study of Deluc et al. (2009), where water deficit increased accumulation of berry sugar of Cabernet Sauvignon, but not of Chardonnay.

Sadras and McCarthy (2007) using allometric modelling concluded that during phase II of berry ripening, sugar acclimation (as determined by sugar per berry) increased at a rate greater than the accumulation of berry mass. The maximum for berry mass accumulation reported here was in the same range stated by Sadras and McCarthy but our measurements were ceased at 24°Brix and we did not follow berry mass postharvest. In our study, there was no deficit irrigation treatment effect on berry water content at harvest, since all treatments were the same at 70%. This indicates no berry desiccation effect due to deficit irrigation treatment.

Anthocyanin

Several studies that have investigated the impact of deficit irrigation treatments and seasonal impacts on anthocyanin concentration in grape (Roby et al. 2004) and wine (Chalmers et al. 2010, Costello and Patterson 2012, Edwards and Clingeleffer 2013,) or both (Ashley et al. 2006, Bindon et al. 2008). Only limited research, however, has been conducted on temporal changes in the accumulation of grape berry anthocyanin, for example, by Downey et al. (2004). In our study, the temporal pattern of the accumulation of anthocyanin and non-degraded anthocyanin in the berry differed between seasons with a lower concentration at harvest in 2003 than in 2004 and 2005.

This was in contrast with the impact of water deficit on spectral parameters of wine, where for colour density, anthocyanin and ionised anthocyanin, the concentration was in the order PD > RDI > Control. These observations are similar to those by Ashley et al. (2006) and Bindon et al. (2008) who found no difference in anthocyanin in grape berry homogenates from Control and a partial rootzone drying treatment of Shiraz and Cabernet Sauvignon, respectively, but found significantly higher colour density and polymeric pigment in wines from the deficit treatments.

Degraded anthocyanin or polymeric pigments formed by condensation of anthocyanin with other phenolic substances are linked with polymeric colour and browning (Wrolstad et al. 2005), including in wine (Somers and Evans 1974). Degraded anthocyanin increased throughout phase III of berry development, and at harvest was higher in macerated berries of the deficit irrigation treatments than in the Control treatment and were lower in the cooler 2004/05 season relative to seasons 2002/03 and 2003/04.

Seasonal differences in berry anthocyanin concentration may also reflect seasonal differences in temperature, since higher temperature during berry development of Cabernet Sauvignon is known to be associated with reduced berry colour (Mori et al. 2007). While the hottest season in this study (2002/03) was associated with a lower concentration of anthocyanin in whole berry samples for all irrigation treatments, the wine anthocyanin concentration was highest in the following order, 2003 > 2005 > 2004. Berry exocarp colour was higher in 2005 than in 2004, which compared well

with the concentration of wine anthocyanin for those years. The lack of agreement between whole berry and wine anthocyanin concentration in 2003 and 2005 may relate to changes in polymeric pigment inter-relationships between whole berries and wine in those years (Boulton 2001).

Organic acids

Deficit irrigation treatment reduced the concentration of malic acid in grape juice and enhanced the tartrate to malate ratio during the ripening period, similar to observations made by Matthews and Anderson (1989). These responses may be associated with reduced shoot growth which has been found with lighter pruning (Clingeleffer 1993). Smaller leaf area index values observed for the water deficit treatments in our study in 2004 and 2005 (data not shown) are indicative of reduced canopy growth which may have resulted in more direct sun exposure within the canopy and greater exposure of fruit to light. Low vigour rootstocks are known to reduce malic acid concentration in grape berries (Clingeleffer et al. 2001). High temperature can result in lower malic acid concentration in berries at maturity (Kliewer 1965), because malic acid can be degraded by NADP –dependent malic enzyme or because of an increase in CO₂ solubility that suppresses decarboxylation reactions (Lakso and Kliewer 1975), or by degradation of malic acid during respiration (Coombe 1987). While the 2002/03 season was hotter than the other seasons in this study (Figure 2) the concentration of malic acid was not significantly impacted (Table 3). Cautious interpretation is required, however, since the optimum accumulation temperature for malic acid is 20°C (Lakso and Kliewer 1975)

and the average daily maximum temperature for December to January for the region (Figure 2) may not be the best indicator for malic acid reduction. In addition, the concentration reported here is low.

Berry texture

Zsofi et al. (2014) demonstrated that grape skin mechanical properties are dependent on skin structure rather than its thickness. The skin structure is largely composed of cellulose, phenolic substances and anthocyanin. The current study has demonstrated a strong correlation (r greater than 0.85) between berry texture and a range of berry physical and compositional traits, for example, strong negative correlations between berry texture and berry surface colour (Minolta measurements L, $r = -0.87$, chroma, $r = -0.91$ and hue, $r = -0.82$, with b an exception, where there was a strong positive correlation of $r = 0.89$ with berry texture). Strong negative correlations were found between berry texture and berry anthocyanin composition (anthocyanin, $r = -0.91$ and non-degraded anthocyanin, $r = -0.94$) and between berry texture and berry mass ($r = -0.87$). Strong positive correlations were obtained between berry texture and berry skin characteristics (skin fresh mass $r = 0.81$ and skin: total whole berry mass ratio $r = 0.82$). Berry texture has also been shown to indicate changes in specific properties that may be influenced by the amount of phenolic substances (Fernandes et al. 2015). Further, texture changes may impact on the grape crushing process. Texture analyser data showed that PD led to greater strength of berry exocarp at harvest than that for the Control and RDI treatments. Reasons for the additional strength of PD exocarp are

unclear but may include compositional and physical factors including skin thickness (Salmanov and Magomedov 1999). Boyer (1988) suggested that water deficits can modify the composition of cell walls in leaves and the physical properties of cell wall extensibility. It has been shown that leaf cell wall synthesis, in particular synthesis of cell wall polysaccharides, is sensitive to water deficit (Sweet et al. 1990, Schultz and Matthews 1993). During water stress the precursors of cell wall components (like glucose) are incorporated differently in growing and non-growing tissues (Sweet et al 1990). Iraki et al. (1989) showed that cellulose synthesis was sensitive to water stress, and it may be possible that changes to cellulose synthesis impact more on exocarp strength than on skin thickness.

Seasonal impacts

While the imposed irrigation treatments (RDI and PD) produced different soil moisture deficits, which compared to that of the Control had a significant effect on berry development, physiology (Glenn et al. 2010) and composition, the effect of season was generally greater than the effect of the irrigation treatments on these attributes. Major differences between seasons were determined for yield (range 36%); maturity (up to 2 weeks difference between seasons); fresh berry mass, dry berry mass and the ratio of fresh to dry mass (i.e. 18, 35 and 29%, respectively); exocarp, mesocarp and seed dry mass (i.e. 17, 8 and 26%, respectively); juice pH (3.3–3.6), despite there being only a small difference in TA (5.0 –5.3 g/L), TSS (24.1–25.6 °Brix) and sugar per berry (0.19–

0.21g); berry phenolic substances (1.4–1.9 mg/g), anthocyanin (1.3–1.9 mg/g), non-degraded anthocyanin (1.1–1.7 mg/g), degraded anthocyanin (0.10–0.22 mg/g) and wine spectral properties, that is colour density (9.4–16.1 a.u.), hue (0.51–0.54), anthocyanin (678–944 mg/L), ionised anthocyanin (85–125 mg/L) and phenolic substances (55–73 mg/L).

Seasonal comparisons based on daily thermal time over the period of berry development (i.e. from flowering to harvest) provided a valuable tool to compare seasonal effects on berry development and fruit maturation. Despite the adjustment to a common denominator based on daily thermal time, there were large seasonal differences in berry development, sugar accumulation (on a concentration and per berry basis) and berry colour development. Such results indicate that the final stages in berry development and their impact on sugar concentration are influenced by other factors not controlled by heat summation.

Relating changes in berry compositional traits to physiological indicators of stress

In a companion study conducted at the same site, water deficits were shown to affect vine physiology, leading to reduced photosynthesis, leaf water potential and turgor, transpiration and stomatal conductance and increased leaf temperature (Glenn et al. 2010) up to 3 weeks after the end of the stress period. Water deficits had a long term impact on shaded leaf water potential for up to 2 months after return to normal irrigation during fruit ripening (Glenn et al. 2010). Furthermore, post-stress measurements of soil

moisture indicate that vines subjected to deficit treatments adapted to the conditions over time and had a lower requirement for water during ripening and postharvest periods in later seasons. This may be a result of the development of vines with smaller canopies carrying smaller crops and possibly reduced root system size and/or function. Reduced photosynthetic rates (Glenn et al. 2010) would potentially have impacted on berry development, maturation and the partitioning and storage of carbohydrate (Cooley et al. 2006) with implications for long term sustainability of production. Edwards and Clingeleffer (2013) showed that the effect of these deficit irrigation treatments on yield was not compounded over multiple seasons. Maximum berry mass occurred about 2 weeks after 50% veraison and then remained relatively constant regardless of season until harvest 1 month later. Grape juice TSS increased rapidly for 2 weeks after 50% veraison, reaching 20°Brix, followed by a slower rate of increase in concentration until harvest (Figure 5), where limited movements of sugar into the berry were offset by water loss as berry mass remained relatively constant (Figure 4). Water content of berries at harvest was the same for all treatments (70%). The effects of season or water deficits on the pattern of change in TSS in grape berries were small. It is likely that reduction in total sugar accumulation attributed to water deficit, particularly PD, is most likely due to the limited supply of carbohydrates around veraison (Cooley et al. 2006).

Summary

Prolonged deficit irrigation has potential as an additional technique for managing soil and plant water status to assist in meeting set specifications for grape composition and

to improve the consistency of wine quality. While yield and berry mass of PD treated vines were reduced by approximately 15%, along with a reduction in mesocarp dry mass and sugar per berry, there was no change to exocarp (skin) to mesocarp (flesh) ratio. There was, however, an increase in exocarp strength, which could potentially result in less berry damage during harvesting. Wine colour density and the concentration of anthocyanin and ionised anthocyanin were higher than that for the Control and RDI, despite that anthocyanin concentration in macerated whole berry samples was similar between the irrigation treatments, indicating changes in polymeric pigment inter-relationships between whole berries and wine. Prolonged deficit irrigation also had a beneficial effect on the acid composition of grape juice, with lower malic acid concentration and higher tartaric to malic acid ratio than that for the Control.

The significant seasonal effects on berry parameters, such as fresh and dry mass and fresh to dry mass ratio at harvest, yield, concentration of whole berry anthocyanin and of non-degraded anthocyanin and grape juice organic acid composition, may be due to interactions that occur when water stress and high ambient temperature coincide. For example, with colour, the hottest season (2002/03) reduced grape anthocyanin and non-degraded anthocyanin in all irrigation treatments but wine anthocyanin (averaged across all irrigation treatments) was highest in that season. Such apparent interactive effects between irrigation treatment and season have potential to significantly minimise seasonal impacts with regard to meeting wine style requirements.

Acknowledgements

The research was supported in part by the Commonwealth Cooperative Research Centre program through the Cooperative Research Centre for Viticulture with support from the Australian grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation (now Wine Australia) with matching funds from the Federal Government. Thanks also to CSIRO (former Plant Industry).

We are grateful to our industry collaborators, Wingara Wine Group Pty Ltd, particularly Mr Justin McPhee, Mr Jeff Milne and Mr Craig Thornton. Thanks kindly for the technical support from Mr Damian Watson (principle technical input), Dr Rachel Hanlin, Mr Peter Lo Iacono, Ms Sonja Needs, Mr Chad Farrell, Ms Melissa Craig, Mr Troy Fry and winery casual support staff. Thanks kindly to Dr Anne Pellegrino and Ms Deidre Blackmore for the dates of budburst and flowering monitored during season 2005 and also for the dry mass data collected during phase I of berry development (2005). The authors are indebted to the valued input by Dr Rachel Ashley and Dr Paul Petrie. Ms Deidre Blackmore kindly assisted with figure presentation and Dr Everard Edwards provided helpful advice with manuscript preparation.

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Figure 1. Relative soil moisture content in the 0–100 cm soil zone of the RDI (□) and PD (●) treatments for (a) 2002/03, (b) 2003/04 and (c) 2004/05 expressed as a proportion of the Control treatment. Periods of treatment are shown as RDI and PD. In season 2003/04, the end of RDI and start of PD occurred on the same day. Readings were measured using a neutron probe at 20 cm intervals. Readings were acquired on a bi-weekly basis between 0600 and 1100. The mean volumetric soil moisture content (0–100 cm zone) of the Control treatment was 29.5, 28.7 and 24.3% for 2002/03, 2003/04 and 2004/05, respectively.

Figure 2. Seasonal temperature variations presented as (a) mean daily maximum temperature for December, January and February in each year of the study and (b) time after flowering during berry development, for 2002/03 (●), 2003//04 (△) and 2004/05 (◆).

Figure 3. Effect of Control (◆), regulated deficit irrigation (RDI) (□) and prolonged deficit irrigation (PD) (●) treatments during 2004/05 on berry dry mass. Average berry dry mass for all treatments was calculated ($n = 12$ replicate samples containing 100 berries). EXCEL solver equations were employed to fit a continuous function of time (t) as day of year, $GROdw(t) = M/[1 + \exp[-a.(t - t_i)]]$, where GROdw is berry growth (dry mass), t_i is initial time and M and a are parameters estimated by non-linear fitting. Goodness of fit R^2 values were Control = 0.98, RDI = 0.98 and PD = 0.99 and *, $P < 0.05$ and **, $P < 0.01$ indicate significant differences (ANOVA).

Figure 4. Effect of (a) Control, (b) regulated deficit irrigation (RDI) and (c) prolonged deficit irrigation (PD) treatments within 2002/03 (○), 2003/04 (▲) and 2004/05 (■) on berry development. Mean berry growth ($n = 12$) for treatment and season was calculated using an EXCEL solver equation to fit a continuous function of thermal time (t) as degree days after flowering, $GROfw(t) = M/[1 + \exp[-a.(t - ti)]]$, where $GROfw$ is berry growth (fresh mass), ti is initial thermal time and M and a are parameters estimated by non-linear fitting. Goodness of fit R^2 values range: Control, 0.97 – 0.99; RDI = 0.98 – 0.99; and PD = 0.97 – 0.98. Conduct of ANOVA across the full data set, from 50% veraison to harvest, showed significant effects of treatment ($P < 0.001$), year ($P < 0.001$) and the treatment x year interaction ($P < 0.001$) on berry mass with respective least significant difference values ($P = 0.05$) of 0.02, 0.02 and 0.03.

Figure 5. Effect of (a, d, g) Control, (b, e, h) regulated deficit irrigation (RDI) and (c, f, i) prolonged deficit irrigation (PD) treatments on mean berry juice TSS ($n = 12$) and mean berry juice TSS per berry ($n = 12$) for 2002/03 (○), 2003/04 (▲) and 2004/05 (■) (a, b, c) Time (t), a continuous function, was observed as day of year and (d, e, f, g, h, i) thermal time as degree days after flowering. $TSS(t) = M.[LN(x)-a]$ and $TSS/berry(t) = M.x^2 + (a.x) - ti$ were calculated using EXCEL solver equations where ti is initial time and M and a are parameters estimated by non-linear fitting. Goodness of fit R^2 values range from 0.89 to 0.90 for TSS and 0.96 – 0.99 for TSS/berry. Conduct of ANOVA across the full data set, from 50% veraison to harvest, showed significant

effects of year ($P < 0.001$) on TSS with a LSD value ($P = 0.05$) of 0.53 and treatment ($P < 0.001$) and year ($P < 0.001$) on TSS per berry with respective LSD values ($P = 0.05$) of 0.01 and 0.01.

Figure 6. Effect of Control (a, d), regulated deficit irrigation (RDI) (b, e) and prolonged deficit irrigation (PD) (c, f) treatments on berry juice pH and titratable acidity (TA) for 2002/03 (○), 2003/04 (▲) and 2004/05 (■), ; where the mean per treatment was calculated ($n = 12$). Thermal time (t) was observed as degree days after flowering. The relationship between pH and time is linear $pH(t) = Mx + c$ with a goodness of fit R^2 values range between 0.98 and 0.99, while the acidity relationship is a power function $Acidity(t) = M \cdot x^a$, with a goodness of fit R^2 values range between 0.94 – 0.99. M, a and c are parameters estimated by non-linear fitting. Conduct of ANOVA across the full data set, from 50% veraison to harvest, showed significant effects of year ($P < 0.001$) on pH with a LSD value ($P = 0.05$) of 0.03 and significant effects of treatment ($P = 0.012$) and year ($P < 0.001$) on TA with respective LSD values ($P = 0.05$) of 0.56 and 0.59.

Figure 7. Effect of (a, d, g, j) Control, (b, e, h, k) regulated deficit irrigation (RDI) and (c, f, i, l) prolonged deficit irrigation (PD) treatments on the concentration of anthocyanin (A), non-degraded NDA) and degraded anthocyanins (DA) and phenolic substances (P) during berry development for 2002/03 (○), 2003/04 (▲) and 2004/05 (■). Values are treatment means ($n = 12$). Relationships were calculated using solver

equation functions (EXCEL) as described by the following formulae with goodness of fit R^2 values in brackets: for A and DA, TA and $DA(t) = M / (1 + \exp^{-a^{(x-ti)}})$ (0.86 - 0.98); for NDA, 2003 and 2005 $NDA(t) = a.(1 + \exp^{-M^{(x-ti)}}) - a.(1 + \exp^{-b^{(x-ts)}})$ (0.93–0.95) and 2004 $NDA(t) = M \ln x + a$ (0.60– 0.86); and P, $P(t) = a.(1 + \exp^{-M^{(x-ti)}}) - a.(1 + \exp^{-b^{(x-ts)}})$ (0.85–0.98). Relationships are presented as a continuous function of thermal time (t) as degree days after flowering, t_i = initial thermal time, t_s = spaced time series, and parameters M , a and b were estimated by non-linear fitting. Conduct of ANOVA across the full data set, from 50% veraison to harvest, showed significant effects of treatment ($P = 0.039$) and the treatment x year interaction ($P = 0.001$) on A with LSD values ($P = 0.05$) of 0.09 and 0.99, respectively; significant effects of year ($P < 0.001$) and the treatment x year interaction ($P = 0.003$) on NDA with respective LSD values ($P = 0.05$) of 0.10 and 0.17; significant effects of treatment ($P = 0.002$), year ($P < 0.001$) and the treatment x year interaction ($P = 0.002$) on DA with respective LSD values ($P = 0.05$) of 0.01 0.01 and 0.02; and, significant effects of treatment ($P < 0.001$), year ($P < 0.001$) and the treatment x year interaction ($P < 0.001$) on PS with respective LSD values ($P = 0.05$) of 0.04, 0.04 and 0.08.

Table 1. Irrigation treatment durations, water volumes applied and rainfall received during managed stress periods and total seasonal irrigation applications to Cabernet Sauvignon grapevines treated with Control (industry standard), regulated deficit irrigation and prolonged deficit irrigation treatments over three seasons.

Season	Treatment	RDI period			PD period			Total applied (ML/ha)	
		ET [†] (%)	I (mm)	R (mm)	ET [†] (%)	I (mm)	R (mm)	I	R‡
2002/03		21/11/2002–3/12/2002			24/12/2002–29/01/2003				
	Control	100	50	8	100	25	66	4.5	1.3
	RDI	50	25	8	100	25	66	4.3	1.3
	PD	50	25	8	0	0	66	4.0	1.3
2003/04		25/11/2003–2/01/2004			02/01/2004–18/01/2004				
	Control	100	134	13	100	42	3	6.8	1.6
	RDI	50	67	13	100	42	3	5.0	1.6
	PD	50	67	13	0	0	3	4.6	1.6
2004/05		22/11/2004–9/12/2004			20/12/2004–13/01/2005				
	Control	100	34	13	100	50	0	4.5	0.9
	RDI	50	18	13	100	50	0	4.4	0.9
	PD	50	18	13	0	0	0	3.4	0.9

[†]ET was based on soil water, as described in the Materials and methods. Rainfall recorded by the Bureau of Meteorology, Mildura, Vic. weather station during the RDI and PD period and over the ‡growing season (October–February). Flowering dates (50%) were 29 October 2002, 7 November 2003 and 7 November 2004. Veraison dates (50%) were 4 February 2003, 19 January 2004 and 14 January 2005. ET, evapotranspiration; I, irrigation applied; PD, prolonged deficit irrigation; R, rainfall; RDI, regulated deficit irrigation. .

Table 2. Effect of irrigation treatments and season on bunch mass, berry growth characteristics and yield at harvest, and irrigation water use index.

	Irrigation treatment			<i>P</i> -value
	Control	RDI	PD	
Bunch FM (g)	70.4	66.1	63.9	ns
Berry FM (g)	0.90 a	0.82 b	0.79 b	**
Berry DM (g)	0.23 a	0.21 b	0.20 b	**
Berry FM DM ratio	3.9	3.9	3.9	ns
Exocarp DM (g)	0.094	0.088	0.084	ns
Mesocarp DM (g)	0.081 a	0.072 b	0.067 b	**
Seed DM (g)	0.052	0.050	0.047	ns
Exocarp: mesocarp	1.24	1.28	1.35	ns
Diameter (mm) [†]	10.8	10.7	10.6	ns
Yield (t/ha)	23.7 a	22.1 ab	20.2 b	**
Irrigation water use index (t/ML applied)	7.2	7.1	6.9	ns
Season				
	2003	2004	2005	
Bunch FM (g)	66	65.5	69	ns
Berry FM (g)	0.77 a	0.81 a	0.94 b	***
Berry DM (g)	0.20 a	0.20 a	0.23 a	*
Berry FM: DM ratio	3.85 b	4.0 a	4.01 c	***
Exocarp DM (g)	0.091 a	0.081 b	0.098 a	**
Mesocarp DM (g)	0.073	0.070	0.077	ns
Seed DM (g)	0.043 a	0.048 a	0.058 b	***
Exocarp: mesocarp	1.31	1.23	1.33	ns
Diameter (mm) [†]	-	10.3 a	11.2 b	***
Yield (t/ha)	17.9 a	20.3 a	27.8 b	***
Irrigation water use index (t/ML applied)	10.4 a	3.7 c	7.0 b	***

Significant treatment and seasonal effects are indicated: *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$. Treatment ($n = 12$) and season means followed by different letters are significantly different. No significant interaction of treatment by season was found (data not shown). [†]Berry diameter was not recorded in season 2003. Mean fruit maturity (TSS) at harvest across the irrigation treatments was 24.2, 24.7 and 24.3°Brix (Table 3). DM, dry mass; FM, fresh mass; ns, not significant.

Table 3. Effect of irrigation treatments and season on berry composition at harvest.

	Irrigation treatment			<i>P</i> -value
	Control	RDI	PD	
TSS (°Brix)	24.2	24.7	24.3	ns
Sugar per berry (g)	0.22 a	0.21 a	0.19 b	***
Total acidity (g/L)	5.27	5.05	5.10	ns
pH	3.47	3.50	3.51	ns
Anthocyanin (mg/g)	1.65	1.68	1.69	ns
Non-degraded anthocyanin (mg/g)	1.43	1.49	1.55	ns
Degraded anthocyanin (mg/g)	0.16 a	0.18 b	0.18 b	*
Phenolic substances (mg/g)	1.57	1.65	1.72	ns
Tartaric acid (g/L)	5.78	5.62	5.89	ns
Malic acid (g/L)	1.78 a	1.34 b	1.28 b	**
Citric acid (g/L)	0.54	0.57	0.64	ns
Tartaric: malic acid ratio	3.3 a	4.1 b	5.9 b	**
Season				
	2003	2004	2005	
TSS (°Brix)	24.5 a	24.1 b	25.6 a	**
Sugar per berry (g)	0.21 a	0.19 b	0.21 a	**
Total acidity (g/L)	5.20 a	4.95 b	5.27 a	*
pH	3.34 a	3.52 b	3.62 c	***
Anthocyanin (mg/g)	1.31 a	1.86 b	1.76 b	***
Non-degraded anthocyanin (mg/g)	1.11 a	1.64 b	1.73 b	**
Degraded anthocyanin (mg/g)	0.20 a	0.22 a	0.10 b	***
Phenolic substances (mg/g)	1.69 a	1.93 c	1.40 b	***
Tartaric acid (g/L)	5.84	5.81	5.64	ns
Malic acid (g/L)	1.61 a	0.93 b	1.88 a	***
Citric acid (g/L)	0.34 a	0.25 a	1.15 b	***
Tartaric: malic acid ratio	3.6 a	6.3 b	3.0 b	***

Significant treatment and seasonal effects are indicated: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Treatment ($n = 12$) and season means followed by different letters are significantly different. Except for degraded anthocyanins, no significant interaction of treatment by season was found (data not shown). †Berry composition parameters were determined on juice except for the concentration of anthocyanin and phenolic substances which were determined on macerated berries. ns, not significant.

Table 4. Effect of irrigation treatments and season on wine attributes.

	Irrigation treatment			<i>P</i> -value
	Control	RDI	PD	
Wine				
Titratable acidity (g/L)	7.81	7.86	7.86	ns
pH	3.53 a	3.50 a	3.47 b	***
Colour density (AU)	11.0 a	12.1 b	14.0 c	***
Colour hue (AU ₄₂₀ /AU ₅₂₀)	0.54 a	0.53 ab	0.51 b	**
Anthocyanin (mg/L)	686 a	784 b	895 c	***
Ionised anthocyanin (mg/L)	91 a	102 b	126 c	***
Phenolic substances (mg/L)	57 a	64 b	65 b	*
	Season			
	2003	2004	2005	
Wine				
Titratable acidity (g/L)	7.34 a	7.55 b	8.4 c	***
pH	3.55 a	3.45 b	3.50 c	***
Colour density (AU)	9.4 a	16.1 c	11.7 b	***
Colour hue	0.54 a	0.51 b	0.52 ab	*
Anthocyanin (mg/L)	944 a	678 c	755 b	***
Ionised anthocyanin (mg/L)	85 a	125 c	109 b	***
Phenolic substances (mg/L)	73 a	62 b	55 c	***

Significant treatment and seasonal effects are indicated: *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$. Treatment ($n = 3$) and season means followed by different letters are significantly different. No significant interaction of treatment by season was found (data not shown). AU, absorbance units; ns = not significant.

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Title:

Effect of water deficits and season on berry development and composition of Cabernet Sauvignon (*Vitis vinifera* L.) grown in a hot climate

Date:

2017-06-01

Citation:

Cooley, N. M., Clingeleffer, P. R. & Walker, R. R. (2017). Effect of water deficits and season on berry development and composition of Cabernet Sauvignon (*Vitis vinifera* L.) grown in a hot climate. AUSTRALIAN JOURNAL OF GRAPE AND WINE RESEARCH, 23 (2), pp.260-272. <https://doi.org/10.1111/ajgw.12274>.

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