

The Impact of Water Deficit and High Temperature on  
Berry Biophysical Traits and Berry and Wine Chemical  
and Sensory Traits

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## **Abstract**

Warming and drought associated with climate change are major concerns in grape production worldwide. Our current understanding on the effects of temperature on berries and wines has been indirectly gained from comparisons of thermally contrasting seasons or sites, or from experiments in controlled conditions. Indirect methods, however, cannot prove cause and effect relationships, and extrapolation from controlled environments to field is not warranted. A comprehensive comparison of these methods is discussed. Furthermore, interactions are the main cause of complexity in field experiments; nevertheless, predictions about warmer and drier environments are based on studies that separately addressed these factors. Using direct manipulation of temperature on field growing vines with open-top chambers, the current work is the first combining water and temperature regimes in realistic vineyard conditions. The aims of this research were: (i) to critically assess methods to investigate thermal effect in viticulture; (ii) to measure the effects of elevated temperature on berry shrivelling (BS) and berry mesocarp cell death (MCD) in Shiraz and Chardonnay (exp. 1); and to evaluate the single and combined effects of temperature and water deficit on (iii) BS, MCD and on (iv) berry and wine chemical and sensorial composition in Shiraz (exp. 2).

An increment in background temperature increased both MCD and BS in Shiraz, and increased MCD but had not impact on BS in Chardonnay; MCD seems necessary but not sufficient to explain BS. Similarly, transient water deficit post-veraison increased MCD and BS at harvest in Shiraz. MCD response to temperature was primarily explained by the advance in onset, while the effect of water deficit was traced back to the increment in the rate of MCD post-onset. An interaction between water deficit and temperature was found whereby the onset of berry net water loss was advanced by high temperature under water deficit but not in the irrigated treatments.

MCD during berry senescence has been proposed to enhance berry flavour and aroma. The association between MCD and grape sensory balance was investigated in exp. 2. The balance of berry sensory traits was quantified in terms of offset, which accounts for delay or advance in ripening, and decoupling, which measures the scatter in the response of the different traits. Sensory traits typical of ripened berries were associated with higher MCD; however, warming and water deficit advanced ripening and decoupled berry sensory traits. Thermal effects were larger than water effects; the large decoupling caused by high temperature was mainly associated with differences within

berry parts, whereas water-driven decoupling was mostly associated with a differential response between seed and other berry parts.

The extraction of the major phenolics classes in fully ripe fruit and their contribution to the final wine chromatic characteristics, phenolic composition and sensory attributes were determined in exp. 2. The effect of temperature on berry composition was larger than the effect of water but no interactions were found between these factors. Significant, previously unrecorded interactions were found for grape and wine phenolics, and wine sensory traits. Wines from control temperature and water deficit treatments had higher total phenolics, tannin concentration, colour density, non-bleachable coloured compounds and a higher proportion of polymeric pigments than the other combinations of temperature and water regimes. These wines were also characterised by attributes such as cooked fruit flavour, berry flavour, tannin structure and higher red tones and colour saturation. Therefore, the effect of water deficit leading to colourful, flavoursome and phenolic-rich wines may not hold under high temperature.

Scientifically, this thesis provides unequivocal answers to questions of berry physiology under elevated temperature in contrast to indirect methods and accounts for previously unknown interactions with water deficit in realistic vineyard conditions. From the perspective of the industry, this study represents a novel contribution as it answers the question of how warmer and drier conditions during ripening would affect grape and wine attributes and established the bases for new research aiming at counteracting the effects of climate change.

## Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certified that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Marcos Bonada

## **Publications**

This thesis is a collection of manuscripts that were published or submitted for publication in refereed journals, and has been prepared according to the University of Adelaide's specifications for 'Thesis by publication' format.

Each manuscript is displayed as a separate chapter in the thesis in the published or submitted format required by the specific journal. References for the submitted manuscripts, and the introduction and general discussion sections, have been incorporated into a single consolidated reference list at the rear of the thesis. A Statement of Authorship listing individual contributions and signatures of authors precedes each chapter.

The following peer-reviewed publications form the basis of the thesis:

Chapter 2. Bonada, M. and Sadras, V.O. (2014) Critical appraisal of methods to investigate temperature effects on grapevine berry composition. *Australian Journal of Grape and Wine Research* [Accepted].

Chapter 3. Bonada, M., Sadras, V.O. and Fuentes, S. (2013) Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Australian Journal of Grape and Wine Research* 19, 87–94.

Chapter 4. Bonada, M., Sadras, V.O., Moran, M.A. and Fuentes, S. (2013) Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrigation Science* 31, 1317–1331.

Chapter 5. Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A. and Sadras, V.O. (2014) Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Australian Journal of Grape and Wine Research* [Submitted manuscript].



## **Related Publications and Communications Arising During Candidature**

### *Publications in peer-reviewed journals*

Fuentes, S., Mahadevan, M., Bonada, M., Skewes, M.A. and Cox, J.W. (2013) Night-time sap flow is parabolically linked to midday water potential for field-grown almond trees. *Irrigation Science* 31, 1265-1276.

Sadras, V.O., Moran, M.A. and Bonada, M. (2013) Effects of elevated temperature in grapevine. I Berry sensory traits. *Australian Journal of Grape and Wine Research* 19, 95–106.

### *Publications in industry journals*

Bonada, M. (2013) Grapevine research leads PhD candidate to South America. *Australian & New Zealand Grapegrower and Winemaker* September, p. 38.

Bonada, M., Sadras, V.O. and Fuentes, S. (2013) Effects of elevated temperature on mesocarp cell death and shrivelling in Shiraz and Chardonnay berries. *Australian & New Zealand Grapegrower and Winemaker* February, p. 35-36.

### *Conferences and seminars*

Bonada, M. Maintaining wine quality under elevated temperatures. SARDI Waite Seminar Series, 22 August 2013 Adelaide, Australia. [Oral presentation].

Moran, M.A., Sadras, V.O., Petrie, P.R. and Bonada, M. Effect of elevated temperature on vine phenology, physiology and berry composition. The Australian Wine Industry Trade Exhibition (WineTech), 13-18 July 2013 Sydney, Australia. [Poster].

Bonada, M., Sadras, V.O., Moran, M.A. and Fuentes, S. Elevated temperature and water deficit accelerated berry mesocarp cell death and shrivelling, and decoupled sensory traits in Shiraz berries. IX International Symposium on Grapevine Physiology and Biotechnology, 21-26 April 2013 La Serena, Chile. [Oral presentation].

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## **Dedication**

To my mom, Silvia, for her integrity and her remarkable strength. To my grandma, Paula, for feeding me with all her sweetness and delightful meals. To my partner in this journey, Celina, for her patience, common sense, intelligence and, by far, for being the great mother that you are. My small achievements would not be possible without you, thanks!



# **Chapter 1**

## **Introduction and Literature Review**

## 1.1 Overview

The call to ensure quality grape production, which will help maintain the profitability of the wine industry, has never been more urgent. Limited water resources and uncertainties associated with climate change emerge as keystone concerns for production of quality grapes (Duchene et al. 2005, Giorgi et al. 2008, Webb et al. 2013, Webb et al. 2007). Effective adaptive strategies and practices that are environmentally safe and climate-resilient require a deep understanding of the trade-off between quality traits and environmental factors.

The effect of water deficit on grape composition has been widely investigated (Chaves et al. 2010, Sadras et al. 2012e); moderate water deficit has been associated with berry quality in red wine cultivars (Basile et al. 2011, Iland et al. 2011b, Intrigliolo et al. 2012, Kennedy et al. 2002, Roby et al. 2004b). Likewise, the effect of temperature has attracted increasing attention, but results have been mainly indirectly gained from natural gradient of temperature in space or time (Cozzolino et al. 2010, Duchene and Schneider 2005, Jones et al. 2000, Petrie et al. 2008, Sadras et al. 2007b, Soar et al. 2008, Urhausen et al. 2011, Vrsic et al. 2012). The absence of factorial-design experiments, where heated vines are directly compared against unheated control vines (Sadras et al. 2012a, Sadras et al. 2009, Spayd et al. 2002), makes the interpretations from these studies rather speculative. Furthermore, interactions among factors are the main source of complexity in field growing conditions (Mooney et al. 1991, Wootton 2002); nevertheless, much of the understanding about water and temperature effects has been independently gained from controlled environments (Basile et al. 2011, Buttrose et al. 1971, Girona et al. 2009, Kliewer 1977b, Kliewer et al. 1972, Mori et al. 2007, Mori et al. 2005a, Yamane et al. 2006a) that do not necessary replicate field conditions or are biased by experimental artefacts (de Langre 2008, Marion et al. 1997, Passioura 2002, 2006, Sadras et al. 2013, Sadras and Soar 2009, Soar et al. 2009, Tarara et al. 2000).

Against the background of partial understanding of the effect of temperature and water deficit on berry physiology, and berry and wine attributes, and with the main goal to provide the industry with indications of shifts in production, the aims of this project are to (i) critically assess methods to investigate thermal effect in viticulture; (ii) determine cultivar differences between mesocarp cell death (MCD) and shrivelling (BS) under two thermal regimes, for the most widely planted and economically important red and white cultivars (Shiraz, Chardonnay) in Australia (ABS 2012); (iii) evaluate the single and combined effects of temperature and water deficit on the dynamic of MCD and BS in Shiraz; and (iv) determine relationships between grape and wine sensory traits and

changes in berry biophysical traits as driven by warming and water deficit in Shiraz. The effects of elevated temperature, by cultivars (experiment 1) and its interaction with water (experiment 2), are investigated using open-top heating chambers under realistic vineyard conditions.

## **1.2 Greenhouse Gas Emission and Climate Change**

The role of greenhouse emissions on climate change and the impact of climate change in viticulture have been extensively reviewed (de Orduña 2010, Intergovernmental Panel on Climate Change 2007, Keller 2010a, Schultz 2000); hence the aim of this section is to provide a high-level background to the thesis rather than duplicate current, in-depth revisions. Briefly, the atmospheric concentration of greenhouse gasses (carbon dioxide, methane and nitrous oxide) has increased without precedent since the beginning of the industrial period (Intergovernmental Panel on Climate Change 2007). Anthropogenic causes such as an accelerated use of fossil fuels and changes in the use of land have been likely contributors to this process (Intergovernmental Panel on Climate Change 2007). Indeed, carbon dioxide (CO<sub>2</sub>) concentration, one of the main greenhouse gases, has reached near 370 ppm, which represents an increase of approximately 20% over the last 50 years (Schultz 2000). This atmospheric-CO<sub>2</sub> enrichment has been accompanied by a rapid increase in global temperature and more frequent and widespread droughts (Dai 2011). During the period 1950-2008 most land areas around the globe have warmed up by 1-3 °C (Dai 2011). Arid regions during the same period have expanded either by a decrease in precipitation, and increase in evaporation or both (Dai 2012).

Modelling suggests a worsening in climate conditions during the 21<sup>st</sup> century in some grape growing regions worldwide. Based on projections of CO<sub>2</sub> emissions, average temperatures in Australia, for example, have been predicted to increase in relation to 2006 by 0.2 to 1.1 °C in 2030, and by 0.4 to 2.6 °C in 2050 (Webb 2006). Similar projections estimate an average warming of 1.7 °C by 2050 in grape growing regions of the United States (Jones et al. 2005). Modelling also suggests increased severity of soil moisture deficit in some agricultural regions by the end of the 21<sup>st</sup> century (Dai 2011).

Assessed and predicted change in climate reveals discrepancies in warming and drought on both space and time scales in important grape growing regions around the world (Webb et al. 2013). Warming has been projected for all regions, although patterns show predominant warming in northern areas and in lower latitude inland regions or coastal

areas where topographical barriers block sea breeze penetration (Grace et al. 2009, Webb et al. 2013). Similarly, at smaller scale, higher temperatures are expected at lower altitudes in topographically diverse regions (Webb et al. 2013). Beyond the long-term rise in average temperatures, projections made for 23 grape growing regions show an asymmetric effect of warming on seasonal and diurnal temperatures (Webb et al. 2013). Projections of annual precipitation indicate variations with latitude, ranging from wetter climate at higher latitude regions to drier at mid latitude regions (Webb et al. 2013). Overall, the impact of climate change will represent a profound change to growing conditions, spanning from a long-term trend in climate at a global scale to diurnal variation in temperature within a region. While it is expected that warming may initially benefit cool areas, long-term increases in average temperatures will sequentially intensify vintage variability, affect wine style and, finally, compromise the sustainability of major wine production regions (Jones et al. 2005). A rising threat for production in currently warm areas is the risk of more frequent extreme temperatures and the limited availability of quality water for supplementary irrigation (Jones et al. 2005).

There are several aspects of warming that are relevant to grape and wine production. On the one hand, there is an increase in background average temperature that affects vines during the whole growing cycle, notwithstanding seasonal and daily asymmetries. Seasonal asymmetries indicate that warming during winter may be less marked than warming during summer in the course of berry ripening (Webb et al. 2013). Daily asymmetries involve stronger increases in minimum as compared with maximum temperature (Easterling et al. 1997). On the other hand, increased intensity and duration of heat waves (Intergovernmental Panel on Climate Change 2007) is relevant for production of quality fruit and wine (Webb et al. 2010). Despite the close association between these diverse aspects of temperature, isolation of their effects is important to understand and manipulate the agricultural system against warming.

### **1.3 Berry Growth and Development**

Grape berry growth is characterised by a double sigmoid pattern that defines two main phases of formation and ripening, being separated by a lag phase (Coombe 1992, Coombe et al. 2000) (Figure 1). The first formation stage takes place between flowering and veraison. When the fruit has been set, pericarp cells divide and expand rapidly (Coombe and McCarthy 2000). During this period, berry water uptake exceeds losses by transpiration and increases in berry volume and weight are associated with intense water flows into the berry with a progressive accumulation of organic acids (McCarthy



et al. 1999) and proanthocyanidins<sup>1</sup> in berry skin and seeds (Downey et al. 2003); both xylem and phloem contribute to these flows. Both malic and tartaric acid are produced early in berry development and accumulated in skin and mesocarp cells; however, while malic acid undergoes post-veraison metabolism, tartaric acid content remains constant and reduction in the concentration is partially due to dilution by water uptake (Iland et al. 2011a, Keller 2010b).

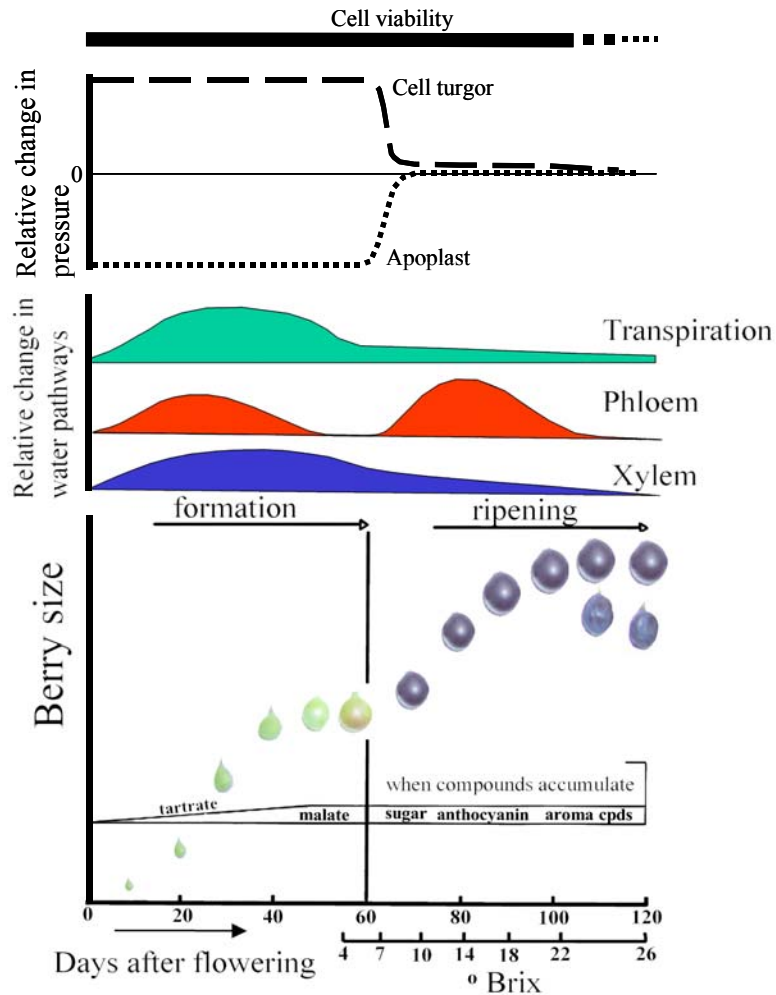


Figure 1: Biophysical and compositional traits during berry ontogeny and associated changes in the contribution of xylem, phloem and transpiration to berry water balance. Timing of accumulation of various berry compounds are indicated along berry growth. Veraison (vertical line) defines the main stages of formation and ripening; major changes in pericarp cell turgor and apoplastic tension (negative pressure) are defined around veraison. The onset of shrivelling matches the net berry water loss, the onset of mesocarp cell death and the development of aroma compounds in the berry. Source: Tilbrook and Tyerman (2006).

<sup>1</sup> The terms proanthocyanidins and tannins are considered synonyms and therefore are used interchangeably in the present document.

The second stage, or ripening, is characterised by progressive cell expansion and simultaneous increase in berry sugar content, skin coloration by accumulation of anthocyanins and reduction in acidity (Coombe and McCarthy 2000) (Figure 1). At this stage berry transpiration rate decreases, and water and solute flow is restricted to the phloem (Greenspan et al. 1994). Apparent loss of xylem functionality after veraison was first attributed to blockage by stretching or breaking of tracheary elements in the brush zone (Findlay et al. 1987). However, later studies showed that xylem remains functional and accounts for water backflows to the vine in some cultivars (Bondada et al. 2005, Keller et al. 2006, Tilbrook et al. 2009, Tyerman et al. 2004). Moreover, at the onset of veraison, phloem becomes apoplastically loaded and facilitates bulk flow of assimilates into the berries (Zhang et al. 2006). This shift seems responsible for subsequent increases in apoplastic solute concentration (Wada et al. 2009) and reduction in the turgor pressure of mesocarp cells (Matthews et al. 2009, Thomas et al. 2008) (Figure 1); possibly allowing a gradual loading of the berry and reducing the risk of splitting (Wada et al. 2008). This finding contrasts with the early model of loss of membrane integrity (Land et al. 1991), which was proposed to explain the drop in cell turgor during the beginning of the ripening stage.

Indirectly, new research supports the theory of an increase in apoplastic solute concentration by demonstrating that cell integrity is maintained until late ripening, in contrast to what was initially assumed (Krasnow et al. 2008, Tilbrook et al. 2008, Tilbrook and Tyerman 2009). Additionally, the increase in apoplastic solute concentration has been linked with the change on xylem pressure from negative to positive in some cultivars at veraison (Tilbrook and Tyerman 2008) (Figure 1); as phloem sap continues and berries keep high levels of assimilates (i.e. sugar), surplus phloem water may be recycled from the berry to the plant through the xylem, if this pathway is maintained (Keller et al. 2006). The drop in turgor pressure is reflected in the progressive berry softening and increasing berry deformability during ripening (Tyerman et al. 2004).

A third phase of berry growth was recently proposed to account for the physiological processes involved in the onset of berry weight loss observed in some grape cultivars (Sadras et al. 2007a, Tilbrook and Tyerman 2008) (Figure 1). At the time that the berry reaches maximum weight, influx to the berry through phloem falls drastically and the subsequent increase in solute accumulation is explained by shrinkage (McCarthy and Coombe 1999). This stage has also been related to the development of berry aromas and flavours (Coombe et al. 1997, Tilbrook and Tyerman 2008). From this point,

subsequent weight losses are a consequence of cultivar-dependent water relations in berries (Fuentes et al. 2010, Tilbrook and Tyerman 2008).

#### **1.4 Berry Shrivelling**

The four more frequent berry shrivel disorders have been recently described in the literature (Bondada et al. 2012, Krasnow et al. 2010, Krasnow et al. 2009). Unlike normal developing berries, the collapsing pericarp of shrivelling berries causes a progressive reduction in berry volume and fresh weight. Nevertheless, although shrivelling symptoms appear to be identical between disorders, they have some distinguishing features. Comparatively, “sunburn” affected berries are characterised by a shifting in skin coloration (browning) and cracking depending on the stage of development, the severity and the cultivar. Commonly found in sunlight expose bunches, this disorder is caused by extreme temperature, UV radiation, or a combination of both. “Bunch-stem necrosis” is characterised by necrotic tissue (black spots) in the pedicel of affected bunches. No specific causes have yet been attributed to “bunch stem necrosis”; necrosis may develop in earlier stages (around anthesis) or after veraison, more presumably blocking the inflow into the berry and preventing accumulation of photoassimilates. Berries with “sugar accumulation disorder” are characterised by lower final berry weight and pH, reduced sugar and anthocyanin accumulation, and an overall delay in berry growth during ripening. Unlike “sunburn” symptoms, “sugar accumulation disorder” and “bunch-stem necrosis” are often confounded. The particular appearance of necrotic lesions on the rachis does not allow for distinguishing between these disorders; in fact, necrotic lesions occur indistinctly on the rachis of bunches displacing both disorders (Hall et al. 2011). However, while symptoms of “bunch-stem necrosis” can appear at any time before or after veraison, symptoms of “sugar accumulation disorder” usually appear in the last stages of berry ripening, from weeks to days prior harvest.

The most frequent symptom of shrivel that appears in Shiraz is “late-season dehydration”<sup>2</sup>. This disorder develops late in ripening and is normally associated with a delay in harvest time (McCarthy and Coombe 1999). While it is more common for Shiraz, it has also been reported in other cultivars such as Carignane and Cabernet Sauvignon (McCarthy and Coombe 1999). Causes of “late-season dehydration” are manifold and in close relation with environmental conditions (Sadras and McCarthy

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<sup>2</sup> The term late-season dehydration is referred indistinctly as berry shrivel or shrivelling in the present document.

2007a); it has been proposed to be consequence of the combined effects of berry water net loss by: (i) transpiration (Greenspan et al. 1994, Greer et al. 2009, McCarthy and Coombe 1999), (ii) a reduction of phloem influx (Rogiers et al. 2006) and a (iii) loss of water as a consequence of a back flow to the plant through the xylem (Keller et al. 2006, Tilbrook and Tyerman 2009, Tyerman et al. 2004).

In Australia, where nearly half of the total red wine production (362,000 tonnes) is Shiraz, “late-season dehydration” is particularly important for grape production (ABS 2012). Probably the more prominent characteristic associated with this disorder is the significant reduction in yield; losses of up to 30% have been reported in some grape growing regions (Rogiers et al. 2006). Nevertheless, from a winemaking and marketing perspective, the more prevalent characteristic is its effect on grape quality. The concentrating effect on berry sugar associated with “late-season dehydration” in Shiraz may lead to wines with higher alcohol concentrations, which is arguably one of the main concerns for the Australian wine industry nowadays.

### **1.5 Mesocarp Cell Death and Shrivelling – a Linking Hypothesis**

Programmed cell death (PCD) is a common process during plant ontogeny (Danon et al. 2000, Greenberg 1996, Love et al. 2008, Thomas et al. 2009, Thomas et al. 2001). Recent studies suggest that PCD in the mesocarp of grapevines is a likely process during berry development (Krasnow et al. 2008, Tilbrook and Tyerman 2008). While MCD is common in normally developing berries of some cultivars, indirect studies suggest that it could be modulated by biotic and abiotic stresses (De Pinto et al. 2012, Maccarrone et al. 2001). Modulation of cell death by water deficit and high temperature has been widely documented, particularly from monocarpic species (Bandurska et al. 2013, Chauhan et al. 2009, Zuppini et al. 2006). However, water deficit and high temperature effects on the dynamics of berry MCD and its relation with BS are largely unknown (Chapters 3 and 4)

The process of BS is partially related to MCD (Tilbrook and Tyerman 2008). Recent studies reported cultivar-dependent correlations between MCD and BS (Fuentes et al. 2010); some cultivars such as Chardonnay, for example, showed a high level of MCD with minimal BS while other cultivars such as Shiraz and Cabernet Sauvignon showed moderate MCD and BS (Fuentes et al. 2010). A mechanistic hypothesis of BS accounting for differences in berry hydraulic conductance through the xylem and MCD between cultivars was proposed (Tilbrook and Tyerman 2008). When berries have reached maximum weight at the beginning of the third stage of growth, in cultivars such

as Chardonnay and Shiraz, mesocarp cells start losing membrane integrity (Figure 1). As membrane semi-permeability is lost, mesocarp cells are no longer effective in balancing xylem tension generated by leaf transpiration and, in cultivars such as Shiraz where berries remain hydraulically connected with the plant, backflow and BS are more likely. In contrast, Chardonnay presents a similar pattern of loss in cell vitality, but berry water content is maintained due to the low hydraulic connection between the berry and the plant at that stage. The high hydraulic resistance in the xylem of Chardonnay berries may prevent water backflow at that stage (Scharwies 2013). A third example presented by Tilbrook and Tyerman (2008) is Thompson Seedless, that remains hydraulically connected to the plant and yet supports the long xylem tension because it maintains mesocarp cell vitality at harvest and beyond this point.

Based upon this mechanistic hypothesis, environmental conditions that enhance MCD and at the same time contribute to the net water loss will more likely exacerbate BS in Shiraz, but may have minimum effects on Chardonnay (Chapter 3). For example, higher rates of berry transpiration and water backflow through the xylem may be anticipated for Shiraz when high evaporative combines with water deficit (Chapter 4). The maintenance of relatively high stomata conductance of Shiraz under soil water deficit and high evaporative demand implies larger fluctuation in plant water potential (Lovisolo et al. 2010, Prieto et al. 2010, Schultz 2003, Soar et al. 2006) that are transmitted from the leaf to the berry through the xylem (Keller et al. 2006, Tilbrook and Tyerman 2009).

## **1.6 Effects of Elevated Temperature and Water Deficit on Grapevine Composition**

The effects of temperature on grape berry composition and wine attributes have been recognised historically, to the extent that cultivars are often classified in terms of their thermal requirements. The prevalent thermal regime is therefore critical to characterising both wines and wine producing regions worldwide (Hall et al. 2009, Iland et al. 2011a, Jones et al. 2005, Keller 2010b). Likewise with temperature defining wine style, management practices such as regulated deficit irrigation (RDI) are widely used to modulate grapevine quality. RDI is extensively adopted in Mediterranean-like climates where seasonal cycles of soil drying determine temporal patterns of water deficit, and is being introduced nowadays in traditional European countries where irrigation has been a legal constraint for quality wine production. Trade-offs between water deficit and quality are not straightforward but moderate stress that reduces yield has been associated with quality grapes. Nevertheless, in the context of warming

(Section 1.2), irrigation strategies are needed with a dual aim of ensuring quality and counteracting undesirable thermal effects (Sadras and Schultz 2012e).

With emphasis on selected traits, this section revises thermal and water deficit effects on grape composition with emphasis on standard indices of berry maturity (sugar, total and titratable acidity and pH) and quality traits (phenolics); Box 1 outlines relevant features of these traits including measurement methods.

### **1.6.1 Thermal Effects**

Thermal effects on grape composition have mainly been gained indirectly from comparisons of thermally contrasting locations and seasons, or from studies that manipulated temperature in controlled environments (greenhouses or growing chambers) that do not necessarily replicate field conditions. With the increasing interest in climate change, season-to-season comparisons have expanded into formal time series analyses and new experiments have involved manipulation of temperature in field conditions. Drawbacks and limitations of these methods have been partially tackled in the literature (de Langre 2008, Marion et al. 1997, Passioura 2002, 2006, Sadras and Moran 2013, Sadras and Soar 2009, Soar et al. 2009, Tarara et al. 2000, Wolkovich et al. 2012) and are fully addressed in Chapter 2. Chapter 5 covers the effect of temperature in berry TA, pH and phenolics in grapes and wines. This section deals with information about temperature effects on berry sugar concentration.

Comparatively with TA and anthocyanins, berry sugar concentration for a given cultivar is a relatively stable trait under different environmental conditions (Dai et al. 2011). Nevertheless, there is a large discrepancy between studies assessing thermal effects on this trait. Studies where vines were warmed during the entire ripening period in greenhouses or growing chambers reported no differences (Mori et al. 2005b), increase (Buttrose et al. 1971) or reduction in sugar accumulation (Kliewer 1977b). When high temperature was applied during short spells in controlled conditions, results varied with the developmental stage; Matsui et al. (1986) reported that high temperature before veraison reduced sugar accumulation in both Thompson Seedless and Napa Gamay. Similar results were found when high temperature was applied at veraison and mid-ripening in Semillon (Greer et al. 2010) or before harvest in Chardonnay and Chenin Blanc (Sepulveda et al. 1986). On the contrary, Yamane and Shibayama (2006b) and Yamane et al. (2006a) reported no differences in Aki Queen (*V. labrusca* L. × *V. vinifera* L.) in response to timing of temperature treatment after veraison. Results are also conflicting between studies that assessed thermal effects in field-growing vines

enclosing the whole plant (Soar et al. 2009), using *ad hoc* devices to warm plant parts such as buds or canes (Bowen et al. 2004a, b, Radler 1965) or using open systems (Cohen et al. 2012b, Sadras et al. 2012c, Sadras and Soar 2009, Spayd et al. 2002). Studies with closed chambers found that TSS was increased (Bowen et al. 2004b) or reduced (Radler 1965) when temperature was increased during the entire cycle, or was unaffected when warming comprised short spells (Soar et al. 2009). TSS accumulation was hastened in studies that increased day and night time temperatures (Sadras and Moran 2012c) or damped daily temperature fluctuation (Cohen et al. 2012b) using open systems. These large discrepancies between studies can be associated with one or more factors including (i) differences in timing, duration and intensity of heat treatments; (ii) interactions with other factors (chiefly water supply); (iii) artefacts from controlled environments (Sadras and Soar 2009, Soar et al. 2009, Tarara et al. 2000); and (iv) deficiencies in experimental design (Poorter et al. 2012).

### **1.6.2 Water Effects**

Notwithstanding the significant body of literature dealing with the effect of water deficit on grape composition of red wine cultivars, results are controversial and inconclusive (Chaves et al. 2010, Sadras and Schultz 2012e). Differences in timing, severity and duration of water stress, cultivars and the interaction genotype  $\times$  environment, are frequent explanations for the discrepancies among studies. Unlike trade-offs between water deficit and grape composition that have been widely studied, information regarding the effects of plant water status on wine and sensory composition is scarce and limited mainly to red wine cultivars (Sadras and Schultz 2012e). Chapter 5 covers the effect of water deficit on berry TA, pH and phenolics in grapes and wines. This section focuses on berry sugar concentration.

Two mechanisms have been recognised in grapevines that promote sugar accumulation by moderate water deficit; an uptake of hexoses activated by the higher concentration of abscisic acid in the berry, or an indirect effect mediated by the increasing strength of berries as sinks of carbohydrates when secondary growth is inhibited by water deficit (Chaves et al. 2010). Reviews addressing the effect of water supply on sugar accumulation reported a wide range of responses that are far from conclusive; sugar concentration has been found to increase, decrease or remain stable in response to water deficit (Chaves et al. 2010, Dai et al. 2011). Putative causes accounting for this large discrepancy between studies include: (i) differences in source-to-sink ratio, e.g. over- or under-cropped vines, (ii) sampling strategy, e.g. sampling at the same date or at targeted

maturity and (iii) differences in timing, intensity and duration of water deficit (Chaves et al. 2010, Dai et al. 2011).

### **1.6.3 Combined Temperature and Water Effects**

The effect of water deficit on grape and wine composition has been widely investigated (Bindon et al. 2011, Casassa et al. 2013b, Chapman et al. 2005, Edwards et al. 2013, Intrigliolo et al. 2012, Ojeda et al. 2002, Roby et al. 2004a). Likewise the effects of temperature have attracted attention (Cohen et al. 2010, Downey et al. 2006, Jackson et al. 1993), but indirect methods comparing seasons, regions or controlled environments preclude establishing definitive cause-and-effect relationships relevant to realistic field conditions (Chapter 2). Interactions between thermal and water regimes are largely unknown. Indeed, interactions are the main cause of complexity and even the most basic interactions between two factors are largely unpredictable from knowledge of individual effects (Bandurska et al. 2013, Mooney et al. 1991, Spayd et al. 2002, Wootton 2002). Therefore, to provide the industry with indications of shifts in wine profiles that would require technological innovations in the vineyard and winery to maintain wine quality and style, field studies are necessary that anticipate drier and hotter conditions.

### **1.7 Summary and Aims of Research**

Significant effort has been invested to elucidate the effects of elevated temperature on grape composition; nevertheless, information has been gained mainly indirectly. Thermal effects have been inferred from natural gradients of temperature, from comparisons between contrasting regions and vintages or from time series, or by using direct methods that involve the experimental manipulation of temperature in closed systems (glasshouses and growing chambers) or in the field. Indirect methods are useful but bound to be inconclusive as climate factors are confounded. Direct methods are biased by experimental artefacts of enclosure or manipulation of temperature in the field. An assessment of methods used to elucidate thermal effects on grapevine composition is needed to emphasise drawbacks and limitations.

Thermal effects on grape composition have historically attracted attention for the characterization of producing regions; a new focus of research comprises the study of warming on berry ripening in a context of climate change. Warmer conditions have been associated with the abnormal ripening of grapes; the sugar concentrating effect of BS in Shiraz, for example, has been exacerbated in parallel with warming. The modulation of BS by environmental conditions has been anticipated and the link



between MCD and BS has attracted attention, but no studies have been carried out to assess the effect of elevated temperature on MCD and BS.

Shortage of water for irrigation and reduced rainfall compound the threat of increasing temperature for production in current warm areas. Both water deficit and temperature effects on grape and wine attributes have been widely investigated; however, interactions between thermal and water regimes in field growing vines are largely unknown. Therefore, it is imperative to quantify berry and wine responses to the combined effect of water deficit and temperature in realistic vineyard conditions. Studies that anticipate changing climates may provide the industry with indications to secure yield and regional wine styles.

The aims of the research presented in this thesis were to:

- (i) critically assess methods to investigate thermal effect in viticulture;
- (ii) determine cultivar (Shiraz, Chardonnay) differences between MCD and BS under two thermal regimes;
- (iii) evaluate the single and combined effects of temperature and water deficit on the dynamics of MCD and BS in Shiraz;
- (iv) determine relationships between grape and wine sensory traits and changes in berry biophysical traits as driven by warming and water deficit in Shiraz.

### **1.8 Linking Statement**

This dissertation is divided in six chapters, including two research chapters that have been published in peer-reviewed journals and two chapters that have been recently submitted for publication. The manuscripts embodied in each chapter have been structured according to the journal specifications, including abstract, introduction, material and methods, results and discussion; therefore, some information may appear duplicated in the general chapters (1 and 6) and the research chapters (2 to 5) of this thesis.

Chapter 1 provides a literature review covering topics that provide the theoretical framework for this research. A summary of the research gaps identified in the literature and general aims of research are presented at the end of Chapter 1.

Chapter 2 provides the state-of-the-art of methodological approaches used to investigate thermal effects on grape composition. Methods are critically assessed with emphasis on their main drawbacks and limitations to infer conclusions about temperature. Discrepancies and agreements between research methods are highlighted through this chapter for selected berry traits (total soluble solids, total and titratable acidity and anthocyanins).

Chapter 3 explores the effect of elevated temperature on the dynamics of berry MCD and BS for Shiraz and Chardonnay. Previous studies suggest that BS is a cultivar dependent-trait that correlates with MCD and which is putatively modulated by environmental conditions. Therefore, the effect of two temperature regimes on the onset and rates of progression of MCD and BS were followed up during ripening. A bilinear model was used to objectively discriminate the onset and rates of MCD. Trajectories of MCD and BS were assessed on thermal and chronological scales to account for experimental artefacts.

Chapter 4 complements the study presented in Chapter 3 by examining the individual and interactive effects of temperature and water on the same biophysical traits of Shiraz berries. MCD has also been associated with the development of berry flavours and aromas late in ripening; however, these associations have arisen indirectly by comparisons between grapes sourced from low and high grape quality. Therefore, by assessing 20 sensory traits from skin, pulp, seed and whole-berry, the sensory balance of berries was evaluated using temperature and water as drivers of cell death.

Chapter 5 builds on the previous two chapters by examining the individual and interactive effects of temperature and water deficit on essential grape and wine attributes. Links between Shiraz berry and wine composition (total soluble solids, acidity, pH and phenolics including anthocyanins and tannin), and wine sensory traits were investigated in this chapter.

Chapter 6 presents a summary of general conclusions of these studies and suggests future research directions.

**Box 1:** Standard indices of berry maturity and berry quality traits

Sugar concentration in the berry plays a critical role, initially, as a precursor for synthesis of organic acids, phenolics and aroma compounds, and, later, determining alcohol concentration in wines (Dai et al. 2011). Between 90 to 99% of sugar in berries is present as hexoses, i.e. glucose and fructose, with sucrose as the remaining fraction (Keller 2010b). The concentration of sugars in grapes is usually expressed as total soluble solids in °Brix or °Baume, and less commonly as SG (specific gravity) and °Oe (Oechsle). In a mature berry, sugar constitutes ~ 90% of its soluble solids whereas the remaining fraction is made up of organic acids (Keller 2010b). Two organic acids in grapes, tartaric and malic, account for 90% or more of the total acid content (Iland et al. 2011a). The acidity in grapes depends on the equilibrium between free organic acids and their potassium and, to a lesser extent, sodium salts; commonly used determinations include titration against an alkaline solution (titratable acidity or TA) or by determination of organic acid anions in the solution (total acidity) by methods such as high-performance liquid chromatography (HPLC), spectrophotometric or enzymatic analysis (Iland et al. 2011a). High TA is associated with low pH; nevertheless, this relation is not straightforward and depends on the concentration of the malic and tartaric forms in the solution and their combination with  $K^+$  and  $Na^+$  (Iland et al. 2011a). Acidity plays an important role in defining organoleptic properties in grapes and wines and contributes to the stabilization and preservation of wines during ageing (Rankine 2004). There is a great variability in sugar and organic acid composition within the *Vitis* genus; nevertheless, concentrations and profiles of sugars and organic acids are mainly determined by the interaction between genotype, environment and management practices (Dai et al. 2011).

Although quantitatively less important than sugar and acid in grapes, phenolics are critical to wine colour, mouthfeel and taste (Downey et al. 2006). Phenolics in grapes are grouped into non-flavonoid (i.e. hydroxybenzoic acids, hydroxycinnamic acids and stilbenes) and flavonoid compounds (i.e. flavones, flavonols, flavanones, flavan-3-ols and anthocyanins) (Teixeira et al. 2013). With the exception of hydroxycinnamic acids, the remaining phenolics from the non-flavonoids group are in low concentration in grapes and wines (Teixeira et al. 2013). Flavonoid compounds, on the contrary, are the main fraction of phenolics

## Box 1 (Continued)

found in grapes and red wines. Phenolic compounds share the benzene ring structure and the biosynthetic pathway from the aromatic amino acid phenylalanine (Cohen and Kennedy 2010); the chemical structure of phenolics compounds and their distribution between berry tissues and timing of accumulation have been recently reviewed (Cohen and Kennedy 2010, Teixeira et al. 2013). Similarly, analytical methods for the quantification of phenolics in grapes have been compared (Harbertson et al. 2006, Herderich et al. 2005, Seddon et al. 2008, Thorngate 2006); briefly, they include an initial extraction of phenolics from berry solids (skin or seeds) with solvents (Downey et al. 2007, Gishen et al. 2005, Kallithraka et al. 1995), e.g. ethanol, acetone and methanol in aqueous solution, and a subsequent determination by spectrophotometry, spectroscopy or chromatography.

In common with other berry metabolites, the composition and content of phenolic compounds within a cultivar is closely related to the environment (Downey et al. 2006, Teixeira et al. 2013). Nevertheless, the synthesis of phenolics is mainly associated with the activation of stress avoidance mechanisms (Cohen and Kennedy 2010). Water, light (quantity and composition), temperature and plant pathogens are well studied stressors affecting phenolics in grapes and wines (Cohen and Kennedy 2010, Downey et al. 2006, Teixeira et al. 2013).

## **Chapter 2**

# **Critical Appraisal of Methods to Investigate Temperature Effects on Grapevine Berry Composition**

*Accepted article - Australian Journal of Grape and Wine Research*

## Statement of Authorship

Bonada, M. and Sadras, V.O. (2014) Critical appraisal of methods to investigate temperature effects on grapevine berry composition. *Australian Journal of Grape and Wine Research* [Accepted]

### Author contributions:

**Marcos Bonada** conducted the review of literature, drafted and constructed the manuscript.

----- Date -----  
Signature

**Victor O. Sadras** provided guidance constructing the manuscript and contributed with its edition.

## **Abstract**

Grapevine is an economically important crop and a model species of long-lived perennials widely used in bioclimatic studies. Ambient temperature modulates berry composition, hence the significant research effort in establishing links between temperature, berry composition and wine attributes.

Our current understanding of the effect of temperature on berries and wines has been largely gained, however, from indirect methods or direct methods in controlled conditions. Indirect methods include comparisons of thermally contrasting locations and vintages; this approach is useful but is bound to be inconclusive as the effect of temperature is often confounded with other weather and climate factors (solar radiation, vapour pressure deficit and rainfall), management practices and soils. Direct experimental methods comparing fruit grown at a range of temperature are required to prove cause-and-effect, but attempts to modify the thermal regime of the plant often generate secondary effects. Experimental artefacts in controlled environments often include small soil volume, lack of wind and altered radiation regimes, with direct implications for plant physiology and berry composition. Experiments involving controlled temperature in vineyards aim at a higher degree of realism, but are constrained by cost, issues of scale in space and time, and are not necessarily free from artefacts.

Indirect and direct methods are of course non-mutually exclusive, but complementary. This review critically assesses the methods used to elucidate the effects of temperature on grape berry composition. It emphasises the limitations of studies where confounded effects are overlooked. With the focus on selected berry traits (total soluble solids, total and titratable acidity and anthocyanins), we analyse the dominant effects of temperature and highlight discrepancies and agreements between indirect and direct research methods.

**Key-words:** acidity, anthocyanins, climate change, radiation, region, sugars, time-series.

## Introduction

The effects of temperature on grape berry composition and wine attributes have been recognised historically, to the extent that cultivars are often classified in terms of their thermal requirements and the prevalent thermal regime is critical to characterise both wines and wine producing regions worldwide (Hall and Jones 2009, Iland et al. 2011a, Jones et al. 2005, Keller 2010b). Using the key words ‘temperature’ and ‘grapevine’, the Web of Science (Thomson Reuters 2013) returned 2753 papers. The first studies in these series were published at the beginning of the last century; however, the number of publications increased exponentially since 2000 (Figure 1). In parallel with the growth on the number of scientific journals and publications per author, as a major component for metrics of research performance (Cheek et al. 2006), global warming has been a likely contributor to the increasing interest in temperature effects in viticulture during the last decades (de Orduña 2010).

There are several aspects of warming relevant for grape and wine production. On the one hand, there is an increase in background average temperature that affects vines during the whole growing cycle, notwithstanding seasonal and daily asymmetries. Seasonal asymmetries indicate that warming during winter may be less marked than warming during summer in the course of berry ripening (Webb et al. 2013). Daily asymmetries involve stronger increase in minimum as compared with maximum temperature (Easterling et al. 1997). On the other hand, increased incidence of intensity and duration of heat waves (Intergovernmental Panel on Climate Change 2007) is also relevant for production of quality fruit and wine (Webb et al. 2010). Despite the close association between these diverse aspects of temperature, isolation of their effects is important to understand and manipulate the system against warming; likewise, it is also important separating the effect of associated variables such as temperature and radiation as some practices, e.g. irrigation to exploit evaporative cooling during ripening (Soar et al. 2009) would affect temperature but not radiation.

Historically, research has focused on the effects of temperature on vine physiology, and on grape and wine composition. A common focus of many of these studies is the effect of temperature on total soluble solids (TSS), pH, acidity and colour as traits associated with wine quality (Jackson and Lombard 1993). Research expanded to the effects of temperature on flavonoids (anthocyanins, tannins and flavonols) as these compounds are associated with colour, mouthfeel and taste of grapes and wines (Cohen and Kennedy 2010, Downey et al. 2006) and human health (Renaud et al. 1992). A more recent focus is the effect of elevated temperatures that could lead to over-ripening of



grapes and higher alcohol levels (Sadras and Moran 2012c), with marketing and health implications (Hoffmeister et al. 1999, Saliba et al. 2013).

Many reviews have dealt with environmental effects on grape and wine composition, where temperature is one of several factors (de Orduña 2010, Jackson and Lombard 1993). Other reviews had a primary focus on a particular group of compounds such as flavonoids (Cohen and Kennedy 2010, Downey et al. 2006). In this review, our aim is to critically assess the methods used to elucidate the effects of temperature, and to highlight the limitations of studies where confounded effects are overlooked (Büntgen et al. 2010, Ellwood et al. 2012, Freed et al. 2010, Keenan 2007, Sheehy et al. 2006, Wolkovich et al. 2012). From this view point, many of our conclusions are relevant for other crop species, e.g. apple (Caprio et al. 1999). This review analyses the effects of temperature on grapevines, and highlights discrepancies and agreements between different research methods with emphasis on selected traits including TSS, total and titratable acidity (TA) and anthocyanins.

### **Overview: direct vs. indirect methods**

Table 1 outlines the methods used to investigate temperature effects in viticulture, and the potential for confounded effects and artefacts. We divided methods in two main categories: indirect and direct. Indirect methods rely on natural gradients from variations in space or time; results are inferred from comparisons between thermally contrasting regions or vintages or from time series. Direct methods involve the experimental manipulation of temperature in glasshouses and growing chambers or in field conditions.

Indirect methods rely on statistical tools to relate temperature and plant, berry or wine characters. However, even when sophisticated statistics are used to untangle closely related factors such as temperature and water availability (Webb et al. 2012), confounded factors in the observational data are unavoidable (Büntgen and Schweingruber 2010, Ellwood et al. 2012, Freed and Cann 2010, Keenan 2007, Rutishauser et al. 2012, Sheehy et al. 2006, White 2013, Wolkovich et al. 2012). For example, time-series analysis combined with modelling concluded that early grapevine ripening was partially associated with soil drying in warming environments (Webb et al. 2012) but this conclusion was apparently inconsistent with long-term trends in annual rainfall (White 2013). Temperature effects on plant development assessed with time-series agree qualitatively but normally disagree quantitatively with experimental

observations in both natural ecosystems (Wolkovich et al. 2012) and vineyards (Sadras et al. 2013).

Indirect methods are useful to elucidate tendencies in contrasting environments, but results are often inconclusive, sometimes misinterpreted and may overlook emergent phenomena that arise from the collective behaviour of smaller scale processes (Levin 1992). These indirect methods are akin to epidemiological studies in medicine; they are extremely useful but need to be interpreted with caution, and used as the basis for direct studies in “clinical” trials with proper experimental design and controls.

Unequivocal results require direct methods in experiments where vines are exposed to different thermal regimes with minimal secondary effects. To prove cause-and-effect between environmental factors and plant processes, sufficiently small scales (in both space and time) are required (Hari 2008). However, direct methods are not free from confounded effects and artefacts. In the glasshouse and growth chamber, pot size and soil structure are major sources of experimental noise, particularly for a long-lived perennial plant with extensive root system like the grapevine (Passioura 2002, 2006). Unrealistic patterns of daily temperature and radiation, abnormal light quality (e.g. low U.V.) and lack of wind (de Langre 2008) are major obstacles to find definite answers in these experimental settings. Where heating systems have been deployed in the field to seek more realism, secondary effects on vine microclimate and experimental artefacts must be addressed explicitly (Sadras et al. 2012a, Sadras and Soar 2009, Soar et al. 2009, Spayd et al. 2002). Small scale heating systems commonly used in ecological field studies, for example, typically confound elevated temperature and soil dryness (Marion et al. 1997).

Indirect and direct methods are of course non-mutually exclusive, but complementary (Dunne et al. 2004). Where the two methods have been compared (Wolkovich et al. 2012), mismatches have been detected that can be interpreted with emphasis on the power and drawbacks of each method (Rutishauser et al. 2012) (Table 1). A comprehensive meta-analysis concluded that experimental methods under-estimate high temperature effects on plant development (Wolkovich et al. 2012). In contrast, carefully designed experiments in vineyards concluded that time-series over-estimated high temperature effects on vine development, and proposed that comparisons between indirect and direct approaches need to be evaluated on a case-by-case basis (Sadras and Moran 2013).

Irrespective of the research approach, it is important to note that the reliability of measurement varies with methods and berry traits, and a range of units of expression combine to make comparisons between studies difficult (Box 1).

### **Indirect methods**

Indirect methods include comparisons of thermally contrasting environments using variation in time, space or both (Table 2). This approach requires sound data bases, statistical skill and, in common to all other approaches, good understanding of viticulture and oenology; hence it is relatively inexpensive in comparison to experimental methods but it is bound to be inconclusive. Simulation models are commonly used to investigate environmental effects on grain crops (van Ittersum et al. 2003) but this indirect approach is rare in viticulture (Bindi et al. 1996, Santos et al. 2011, Webb et al. 2007). Here we present a critical analysis of indirect methods based on comparisons in time and space (Table 1).

### **Comparisons in time**

Variation in time spans from seasonal to long-term scales; decadal fluctuations have been identified for important climate factors such as temperature, rainfall and radiation (Jones et al. 1992, Jones et al. 1999, Kruger 1999, Neal et al. 2002, Viles et al. 2003, Wild et al. 2005). Here we consider vintage-to-vintage comparisons and long-term climate trends.

*Vintage comparisons:* In short-term comparisons, variation in fruit traits has been largely attributed to changes in temperature and rain (Cacho et al. 1992, Lorrain et al. 2011, Mateus et al. 2002, Miquel Ubalde et al. 2010, Ortega-Regules et al. 2006). Rarely, the interaction with other weather elements that vary alongside with temperature and available resources, is analysed; a hotter season, for example, is often a season with higher solar radiation, lower rainfall and higher vapour pressure deficit, VPD (Sadras et al. 2012d). Often, plant growth, source-sink ratios and water status, are poorly characterised; and these factors directly influence berry traits (Dai et al. 2010, Intrigliolo et al. 2011, Ollé et al. 2011, Sadras et al. 2008, Stoll et al. 2010).

Implicit in vintage-to-vintage comparisons are the effects of extreme events and the carryover effect of temperature from the previous season (Sadras and Moran 2013). Temperature during bud formation, for example, influences its fruitfulness and bunch size the following season (Dunn et al. 2000). The ripening stage at which berry traits are assessed determines another source of variation, which is rarely considered. In general,

berry traits are assessed at “commercial” harvest, which depends on wine style and is variable between vintages and locations (Lorrain et al. 2011).

Studies generally correlate compositional changes with gross temporal average temperatures, e.g. mean growing-season temperature; while valuable on climatological studies they inadequately describe biological responses to temperature and may overlook phenological windows where accumulation of berry compounds is more sensitive to temperature. Fruit soluble solids accumulation, for example, is more sensitive to temperature during the onset of accumulation, being less responsive to temperature during most of the season (Dai et al. 2011). On the contrary, malate accumulation responds to temperature during most of the season; however, malate concentration and acidity at harvest seem to be more related to temperature after veraison (Sweetman et al. 2009). The pattern of anthocyanins accumulation during ripening is represented by the balance between synthesis (Mori et al. 2005b, Yamane et al. 2006a) and degradation (Mori et al. 2007); however, the work of Yamane et al. (2006a) conducted under constant temperatures in growth chambers, showed that accumulation of anthocyanins in the skin of Aki Queen berries was more sensitive to temperature from one to three weeks after veraison. Experimental settings constrain the results of this study and limit its application to direct field conditions; nevertheless, these results highlight the effect that transient short-spell high temperature may have on anthocyanins accumulation at harvest, and, like for the other berry traits, questioned the functional relevance of studies where indirect associations are made between gross temporal average temperatures and berry composition.

*Long-term climate trends:* A comparison between thermally contrasting vintages is possibly the most common method to infer temperature effects on grape composition. With the increasing interest in climate change, vintage-to-vintage comparisons have expanded into formal time series analyses. Time series studies have covered different time and space scales; from long series of time in one specific region (Duchene and Schneider 2005, Grifoni et al. 2006, Jones and Davis 2000, Urhausen et al. 2011, Vrsic and Vodovnik 2012) to several contrasting regions in shorter time series (Cozzolino et al. 2010, Petrie and Sadras 2008, Sadras et al. 2007b, Soar et al. 2008).

Empirical associations arising from these studies should not be interpreted in terms of cause and effect. On the one hand, the increment in background temperature is associated with other climate elements, e.g. vapour pressure deficit, reference evapotranspiration, and CO<sub>2</sub> atmospheric concentration; warming is also associated with extremes events, e.g. heat waves and drought (Intergovernmental Panel on Climate

Change 2007). Higher quality vintages, for example, have been correlated with warmer and dryer years in some studies (Grifoni et al. 2006, Jones and Davis 2000). On the other hand, technological improvements in viticulture and oenology have historically contributed to shifts in grape and wine composition (Jones et al. 2005, Sadras et al. 2007b). New management practices overlapping with warming in time series (Bock et al. 2011, Jones and Davis 2000, Petrie and Sadras 2008, Urhausen et al. 2011, Vrsic and Vodovnik 2012) may mask direct associations between temperature and grape composition. Regulated deficit irrigation, adjustment of crop load and industry temporal trends in sugar maturity, e.g. extended ripening or "hang time", are practices more frequently demanded by winemakers seeking to increase the concentrations of certain quality indicators like anthocyanins (Keller 2010a, Petrie et al. 2006). Furthermore, evidence from these studies, while valuable, is constrained to the period of study. The statistical nature of fitting functions to multi-seasonal data determines that the sign and the magnitude of the slopes of regression analyses are highly influenced by extremes values, particularly in short time series (Sadras et al. 2007b), or by sub-periods with different trends in long term time studies (Jones and Davis 2000, Urhausen et al. 2011). Correlation analyses sometimes returns associations between climate factors and berry traits that are statistically strong but do not make biological sense; for example, temperature at flowering is unlikely to affect acidity (Jones and Davis 2000) or sugar content (Bock et al. 2011) at harvest.

Comparisons across cultivars, phenological stages and environments in time series studies require a common measure of temperature sensitivity, defined as the change in days per °C (Wolkovich et al. 2012). Unrelated time series in Australia and Italy returned temperature sensitivities for grapevine maturity between 7 and 9 d °C<sup>-1</sup> (Petrie and Sadras 2008, Sadras et al. 2011, Tomasi et al. 2011). The calculation of temperature sensitivity in these studies, however, assumes that all the variation in maturity is accounted for by the variation in temperature. This assumption is unlikely as showed in the refined analysis of Webb et al. (2012), thus leading to over-estimation of warming effects on grapevine maturity (Sadras and Moran 2013). Temperature sensitivity is therefore a sensible currency for comparisons, but this limitation must be made explicit.

Owing to the scarcity of data on other traits, most time series studies have focused on the effect of temperature on TSS and acidity (total and titratable). In parallel to warming, there is a general agreement between studies toward the increment in the relation TSS:acidity at harvest, associated with a reduction in acidity (Jones and Davis

2000), increment in TSS (Bock et al. 2011, Cozzolino et al. 2010, Duchene and Schneider 2005, Petrie and Sadras 2008) or both (Urhausen et al. 2011, Vrsic and Vodovnik 2012). However, field studies show that the response of juice TA to elevated temperature is more complex than suggested by these analyses. For example, direct methods showed that TA of Shiraz in the Barossa Valley, Australia, was unresponsive to an increment in mean-maximum growing temperature of 1.4 to 1.6 °C (Sadras et al. 2013) whereas time series analysis showed a drop on TA of  $\sim 1.52 \text{ g L}^{-1} \text{ }^{\circ}\text{C}^{-1}$  putatively associated with warming between 1950 and 1999 (Jones and Davis 2000).

### **Comparisons in space**

Spatial variation is a major influence on fruit composition with consequences for wine attributes. The scales span from the microclimate, as affected by canopy and bunch exposure and row orientation, to the meso- and macro-climatic variation across topography and regions (Bramley et al. 2011). In all cases, temperature varies alongside other sources of variation, and like all indirect methods, caution is needed in drawing conclusions on thermal effects.

*Bunch exposure and row orientation:* Experiments have indirectly investigated temperature effects by sampling canopy sections with different irradiance (Crippen et al. 1986a, b), by shading either using adjacent shoots (Haselgrove et al. 2000, Morrison et al. 1990), or light-exclusion materials (Chorti et al. 2010, Ristic et al. 2007, Rojas-Lara et al. 1989); leaf thinning has also been used to manipulate the thermal and radiation regimes of bunches (Chorti et al. 2010). This imperfect set of treatments, however, does not allow separating the effects of temperature and radiation. We present three examples of studies using these methods to highlight limitations of this approach.

Studies that assessed temperature of bunches growing at different solar radiation exposure found that the peak of irradiance and maximum ambient temperature coincides with the time of highest absolute fruit temperatures (Bergqvist et al. 2001, Spayd et al. 2002, Tarara et al. 2008). Depending on the hemisphere and row orientation, this occurs after the solar noon in the west-facing fruit in north-south orientated rows and north- and south-facing bunches in east-west orientated rows, for the southern and northern hemisphere respectively. Overall, these studies returned similar results; the level to which fruit solar-radiation exposure is beneficial for composition, mainly anthocyanins, is strongly dependent on the extent to which berry temperature is elevated in response to exposure. Effects on TSS and acidity are less conclusive, however.

In a study that involved east-west orientated rows of Cabernet Sauvignon and Grenache on the northern hemisphere, berry temperature increased linearly with incident photosynthetically active radiation (PAR) (Bergqvist et al. 2001). However, south-facing berries were  $\sim 4$  °C hotter at the same intensity of PAR than their counterparts on the north side. Berry anthocyanins concentration increased linearly in north-facing bunches in relation to PAR but declined in south-facing bunches after a peak at  $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$  at mid-day. Only north-facing bunches of Cabernet Sauvignon had greater TA and lower TSS than south-exposed bunches at a given mid-day PAR. The observed changes in composition were attributed to the interaction between radiation (intensity and proportion of diffuse radiation) and temperature.

A comparative study on the composition of Shiraz berries sampled with different solar radiation exposure in east-west oriented rows, in the southern hemisphere, returned differences between bunches in the centre and on the north-facing side of the canopy (Haselgrove et al. 2000). At the same TSS ( $\sim 23\text{-}24$  °Brix), berries from the shaded part of the canopy (centre) had higher concentration and content of total anthocyanins compared with their counterparts on the north-facing position. As proposed by the authors, the higher temperature in the north-facing bunches (up to 10 °C), as driven by higher radiation ( $\sim$  double), may be the key factor affecting anthocyanins concentration.

In north-south orientated rows in the northern hemisphere (Spayd et al. 2002), Merlot berries on the west-facing bunches had less anthocyanins than their counterparts on the east side (Spayd et al. 2002). The increase in skin anthocyanins found in this study when west-facing bunches were chilled to the temperature of shaded fruit, demonstrated the effect of temperature behind this change. As the authors suggested, synthesis of anthocyanins appears to exhibit some light dependence but excessively high berry temperatures may delay their formation, reduce their concentrations, and/or lead to an imbalance between the sugar:acids and anthocyanins:sugar in the fruit (Spayd et al. 2002).

Thus, whereas the interaction between temperature and radiation is an important driver of berry attributes such as anthocyanins (Spayd et al. 2002), these effects are confounded in indirect studies based on bunch exposure and row orientation.

*Topography:* Beyond the influence of row orientation and bunch exposure, vineyard elevation is an important factor determining canopy and fruit energy load. Studies that assessed temperature effects at different elevations have focused on grape sensory

profile (Falcao et al. 2007), proanthocyanidins composition (Mateus et al. 2001) and only few have reported effects in anthocyanins, acidity and TSS (Falcao et al. 2010, Koundouras et al. 2006, Mateus et al. 2002, Oliveira et al. 2008). The lapse rate at which temperature decrease with elevation in a natural situation is about 0.6 °C per 100 m (Jones 1992); which is a likely factor driving grape composition at different elevations (Mateus et al. 2002). However, temperature is not the only environmental factor affected by elevation that may alter vine metabolism. As total air pressure decrease with elevation, the partial pressure of its components gases (N<sub>2</sub>, O<sub>2</sub> and CO<sub>2</sub>) decreases in proportion (Jones 1992). Solar radiation becomes more intense and the proportion of diffuse radiation decreases in parallel with the diminishing atmospheric turbidity with elevation (Paul et al. 2003) (Jones 1992), leading to more risk of excessive energy load for directly illuminated leaves and bunches (Gladstones 2011) and higher photosynthesis (Roderick et al. 2003, Urban et al. 2007, Wohlfahrt et al. 2008). With elevation, ultraviolet radiation (UV-B ) increases, with direct consequences for plant and fruit metabolism (Ballare 2003, Ensminger 1993); high UV-B radiation, for example, changed anthocyanin profile in Malbec berries (Berli et al. 2011) and induced the expression of the anthocyanin biosynthetic genes in apples under low temperature (Ubi et al. 2006). Furthermore, implicit in comparisons at different elevations are the effects on grape composition associated with the topsoil of the different terraces that may influence plant nutrition, water availability or soil temperature. For example, limited plant water availability, as driven by natural soil water-holding capacity and higher evaporative demand at different elevations, was associated with compositional changes in Agiorgitiko grapes and wines (Koundouras et al. 2006). Slope orientation and inclination also contribute to the mesoclimatic conditions of the vineyard, and add further complexity to the interaction between solar radiation and temperature on grape composition in spatial studies (Failla et al. 2004).

*Sites and regions:* Indirect studies that compare thermally contrasting regions or sites arrive to similar conclusions; grapes growing in warmer conditions have lower TA and anthocyanins concentration (Barnuud et al. 2013, Cozzolino et al. 2010, Nicholas et al. 2011). A difference in temperature, VPD or rainfall between regions rather than radiation is most likely to be the main determinant in these compositional changes; solar radiation may be limiting only in regions with heavy cloud-cover for extended periods. Radiation supply usually exceeds maximum requirements; grapevine leaves, for example, become light-saturated in the range from about 200 to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  incident PAR, in leaves at the bottom and the top of the canopy respectively (Iland et al. 2011a, p. 96), and anthocyanins concentration peaks at  $\sim 51\text{-}100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR at



mid-day (Bergqvist et al. 2001). Temperature effects, however, are confounded with other factors in region-to-region studies. Besides aspects inherent to the site that are highly correlated with temperature, such as elevation (see above), latitude and location (coastal vs. inland), management practices and production objectives differ between regions and wine styles. For example, fruit from a cool environment such as Adelaide Hills in Australia, mainly aimed at premium wines, is subject to different vineyard management than fruit from a hot region such as Riverland, mainly aimed at high volume production (Dry et al. 2004). Implicit in region-to-region comparisons is the historical adaptation of cultivars to a particular site by acclimation and, management practices, which may modify its response to the weather and hence wine quality or style (Soar et al. 2008). Furthermore, the influence of soils in grape composition makes it very difficult to separate the effects of soil and climate in site comparisons (Koundouras et al. 2006).

### **Direct methods**

Direct methods need to be critically assessed against the criteria summarised in Table 1. Some artefacts such as pot size and soil conditions have been analysed extensively (Passioura 2002, 2006). Here we critically analyse artefacts from the methods used to manipulate temperature from a wide bibliography base (Table 3).

### **Glasshouse and growing chambers**

Table 3 summarises studies assessing temperature effects on berry composition in controlled environments. Most of these experiments were conducted with potted plants or artificial-soil systems constraining the duration to one growing season. Thermal treatments have been mainly forced to the period pre-veraison to harvest, some assessed differential effects of day and night temperature, and some sought to determine the most favourable stage and optimal temperature range for the accumulation of specific compounds. Some limitations from these approaches have been emphasised (Sadras and Soar 2009, Soar et al. 2009, Tarara et al. 2000); these include: (i) difficulties to up-scale results from plant or lower level of organisation to the crop level (Azuma et al. 2012, Koshita et al. 2007, Yamane et al. 2006a), or from hours of treatments to complete seasons (Soar et al. 2009); (ii) unrealistic growing conditions that do not match current and projected warming (Yamane et al. 2006a) or do not reproduce daily and seasonal cycles of temperature, CO<sub>2</sub>, VPD and light (Buttrose et al. 1973, Buttrose et al. 1971, Yamane et al. 2006a). Main artefacts associated with the experimental design (Poorter et al. 2012), include: (i) hardly sufficient replication or independency between

experimental units (Mori et al. 2007, Mori et al. 2005a, Yamane et al. 2006a) that compromise reproducibility and statistical power; or artefacts from controlled environments, such as: (ii) lack of wind that reduces thigmomorphogenic effect (Jones 1992), evaporative demand and convective heat loss from the berry (Smart et al. 1976); (iii) abrupt changes on cycles of temperature and light or extreme temperatures, e.g. 40/20 °C (day/night) during 8 to 12 days ; (iv) altered radiation (intensity and composition) and (iv) enrichment of CO<sub>2</sub> concentration and its consequences, for instance, on stomata conductance; (v) altered tissue temperature, which affect plant growth and metabolism (De Boeck et al. 2012) . These artefacts and limitations of controlled environments need to be considered in the interpretation of data.

## **Field methods**

*Closed systems:* Closed systems have been used in ecological and agricultural research (Burkart et al. 2007, Kellomaki et al. 2000, Perez-Priego et al. 2010). High resolution studies with portable growing chambers in viticulture have focused on the carbon turnover in the continuum atmosphere-plant-soil (Pérez Peña et al. 2004, Petrie et al. 2009). Focusing on thermal effects, chambers have been used to increase temperature enclosing the whole plant (Ezzahouani 2003, Galat Giorgi et al. 2013, Soar et al. 2009) and *ad hoc* devices have been used to warm plant parts such as buds or canes (Bowen et al. 2004a, b, Petrie et al. 2005, Radler 1965). Closed field chambers allow for vines in actual soil but share artefacts of enclosure with greenhouses and growing chambers. Often, the cover material can reduce radiation intensity and change spectral composition; polyethylene cover, for example, filters out UV-B radiation and increases the proportion of diffuse radiation, which may alter both phenolics synthesis (Berli et al. 2011, Spayd et al. 2002) and carbon assimilation (Berli et al. 2013) in comparison with control plants growing in natural conditions. Air speed is reduced in the chamber altering boundary layer and plant energy balance; the poor heat dissipation under calmer conditions may cause a substantial warming of plant tissues (De Boeck et al. 2012). This effect may be exacerbated in water-stressed vines where the evaporative-cooling system of leaves is shut down with stomata closure (De Boeck et al. 2012). Owing the close relationship between temperature and humidity, the VPD is altered. Collectively, low VPD and reduced atmospheric turbulence in the chamber may alter gas exchange and CO<sub>2</sub> assimilation, thus confounding thermal effects (Bowen et al. 2004a, b). The abnormal pattern of soil drying and warming in closed systems that comprise the root-zone (Ezzahouani 2003, Galat Giorgi et al. 2013, Soar et al. 2009) may alter the normal vegetative and reproductive development of vines (Galat Giorgi et al. 2013, Marion et

al. 1997, Rogiers et al. 2014). Warmer root-zone temperature early in development increased root-carbohydrate mobilization and hastened plant development; the source-sink ratio of treated vines was altered, resulting in larger berries with higher TSS, anthocyanin content and lower TA at harvest (Rogiers et al. 2014).

*Open-top chambers and open systems:* Open-top chambers (Sadras et al. 2012a, Sadras and Soar 2009) and chamber-free (Tarara et al. 2000) methods to investigate temperature effects on grapevine berry composition have been designed to reduce the secondary effects typical of closed systems. However, these methods tackled different aspects of warming and integrated different physiological responses; while the chamber-free system of Tarara (2000) manipulates bunch temperature, open-top chambers affect whole-canopy temperature.

Desirable attributes and drawbacks of chamber-free and open-top chambers to manipulate temperature in realistic field conditions have been discussed (Aronson et al. 2009, Beier et al. 2004, De Boeck et al. 2012, Sadras et al. 2012a). Well-designed open-top chambers aim at: (1) tracking daily and seasonal cycles of temperature; (2) maintenance of relative humidity, hence allowing for increments in VPD; (3) minimum biologically secondary effects, e.g. radiation and temperature interaction; (4) a large-scale system that allows for proper experimental design and sampling, including buffers between treatments. Open-top chambers can be potentially expanded to manipulate both temperature and CO<sub>2</sub> using free air CO<sub>2</sub> enrichment (FACE) technology. Potential secondary effects of open-top chambers include soil drying and disturbance of crop boundary layer (Marion et al. 1997, Sadras et al. 2012a). To directly probe for artefacts using open-top chambers, a two-step analysis has been proposed (Sadras and Moran 2013). First, the trajectory of the trait of interest is plotted in a chronological scale for control and heated treatments (e.g. TSS vs date); statistical differences in the time-trajectory between treatments can be primarily attributed to temperature treatments but artefacts cannot be excluded. Second, the trajectory of the trait of interest is plotted on a thermal-time scale (e.g. TSS vs degree days) for both treatments; a statistical test showing no difference between treatments is strong evidence that experimental artefacts are unlikely, as accounting for temperature in the thermal-time scale removes all the differences between treatments (Bonada et al. 2013a, Bonada et al. 2013b, Sadras and Moran 2013). Artefacts would be evident as residual significant differences between heated and control treatments on a thermal-time scale. Indirect methods support the reduction in juice TA (see long-term climate trend section) and increase in pH (Iland et al. 2011a, Keller 2010b) as general responses to elevated

temperature, but direct manipulation of temperature using open-top chambers in the field showed a more complex interaction between temperature, season and cultivar on these traits, thus illustrating again the caution necessary in the analysis of indirect evidence (Sadras et al. 2013).

To unequivocally separate the effect of temperature and radiation, Tarara et al. (2000) developed a chamber-free field method for heating or cooling grape bunches in the vineyard. Studies in Merlot showed a strong temperature x radiation interaction (Spayd et al. 2002). Sun-exposed bunches had always higher total anthocyanins concentration than their shaded counterparts; however, cooling increased total anthocyanins in sun exposed bunches and heating reduced the content in shaded bunches. This illustrates the limitations in studies relying on sun exposure/thinning as a source of thermal differences.

Infra-red heaters above the canopy and heat-resistance cables buried or in the soil surface form the basis of chamber-free methods to manipulate temperature of natural ecosystems (Grime et al. 2000, Kimball et al. 2009), and their advantages and disadvantages have been fully discussed (Aronson and McNulty 2009, De Boeck et al. 2012). These approaches, however, have not been used in vineyards.

## **Conclusion**

The effect of temperature on berry composition is biologically interesting and has practical implications for wine attributes. Historically, indirect methods using comparisons in space and time have been used to infer thermal effects on berry and wine properties. With global warming affecting many wine regions of the world, this indirect approach has been extended as to include time series analyses. Other indirect methods include comparisons of fruit grown under different solar radiation exposure, as affected by latitude, elevation, row orientation or experimental manipulation affecting both radiation and temperature. Owing to confounded factors, indirect methods are bound to be inconclusive; however valuable, conclusions from this approach need to be considered carefully. Direct assessments in controlled conditions are also valuable, but explicit consideration of artefacts and sources of variation that do not relate to field conditions must be made explicit. Field experiments seek a higher degree of realism, but are not free from artefacts either. Research under more realistic field conditions using chamber-free and open-top chambers has confirmed some well-established patterns from indirect studies or studies in controlled environments, but has also challenged some text-book patterns. Large scale settings during long-term experiments account for

acclimation and are important checks for time series analyses in climate change studies. Modelling remains an under-developed approach to investigate berry composition in response to temperature.

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**Table 1:** Methods to assess temperature effects in viticulture and their potential for confounded effects and artefacts. Each experimental method needs to be critically assessed against the recommended sources of variation; a poor assessment of these hidden factors (shaded area) may lead to misinterpretations of results.

Source of variation	Indirect					Greenhouse & growth chambers	Direct			
	Comparisons in time		Comparisons in space				Field			
	Vintage-to-vintage	Long term climate trends	Bunch exposure & row orientation	Topography	Sites & regions		Closed systems	Open-top chambers	Open systems	
								Chamber-free	Infra-red heaters ††††	
<b>Scale effects</b>										
Extreme values (atypical years)										
Sub-periods with different trend										
<b>Climate elements</b>										
Rainfall										
Radiation										
Quantity †		¶¶¶¶								
Quality ‡										
Diffuse §										
VPD ¶										
Wind										
CO <sub>2</sub> ††		¶¶¶¶								
<b>Vineyard management</b>										
Yield										
Water availability										
Technological improvements										
Production objectives										
<b>Site</b>										
Elevation	‡‡‡	‡‡‡								
Location ††	‡‡‡	‡‡‡								
<b>Experimental settings</b>										
Soil structure		‡‡‡								
Heating system										
Carry-over effect §§										
Source-sink ratio										
Pot size										
Soil drying pattern										
Warming pattern ¶¶¶										
Replicability										
Duration										
Timing of assessment †††										
Timing of treatment										

† Defined by the intensity of solar radiation ( $\mu\text{mol}/\text{m}^2 \text{ sec}$ ) in the photosynthetically active range (PAR 400-700nm).  
‡ Defined by changes in spectral composition of light; of particular importance for plant photomorphogenesis are changes in the red (R; 660nm) to far-red (FR; 730nm) ratio, and changes in UV (10-400nm) and infrared (700-3000nm) proportion.  
§ Defined by the proportion of isotropic radiation (flux density independent of angle). The photon flux density of diffuse radiation is relatively constant within 400-900nm.  
¶ Vapour pressure deficit.  
†† Defined by the variation in the concentration of carbon dioxide (CO<sub>2</sub>) by natural and anthropogenic causes (important in time series studies) or as a consequence of the enclosure.  
‡‡ Defined by sun-exposure, slope and continentality.  
§§ Defined by the effect of temperature in the reproductive cycle (inflorescence primordial initiation and development) in the season preceding flowering and development of fruit.  
¶¶¶ Defined by the daily and seasonal cycles of temperature.  
††† Defined by the ripening stage at which berry traits are assessed.  
‡‡‡ If time series include variations in space, sources of variation from the site may be important.  
¶¶¶¶ If time series are long, global dimming (Stanhill and Cohen, 2001) and atmospheric CO<sub>2</sub> enrichment (IPCC 2007) may be important.  
†††† Infra-red heaters have not been used in viticulture but we include them here for completeness.

**Table 2:** Responses of grape berry composition to temperature in studies that used indirect assessments based on comparisons in space and time.

Reference	Cultivar	Environmental factor-sources of variation	Range of temperature	Berry trait	Timing of assessment	Response
Lorrain et al. (2011)	Merlot Cabernet Sauvignon	Vintage (2006-09)	Difference of 1.8 °C (May-Sep) between 2007 and 2009 (contrasting seasons)	Anthocyanin	Maturity	Lower concentration in 2009 (warmer, higher radiation and wetter at flowering)
Miquel Ubalde et al. (2010)	Cabernet Sauvignon	Vintage (2003-05)/ site (two)	Difference from 1 to 2.2 °C in mean seasonal temp.	Anthocyanin Acidity TSS	During ripening	Lower in 2003 (warmer and wetter) Higher in 2004 (cooler) Higher in 2005 (drier)
Cacho et al. (1992)	Tempranillo Moristel Grenache	Vintage (1987-88)	Mean seasonal temp. was between 1.2 to 2.5 °C higher in 1987	Anthocyanin	During ripening	Lower in 1987 (warmer and drier)
Mateus et al. (2002)	Touriga Nacional Touriga Francesa	Vintage (1997-99) Elevation/Site (Δelev. 150 - 200m a.s.l.)	Differences of 3 °C (Mean seasonal temp.) between 1997 and 1998 (contrasting seasons) and ~ 5 °C between sites	Anthocyanin	From veraison to harvest	Lower in 1997 (cooler and wetter) Enhanced at higher elevation (cooler)
Jones and Davis (2000)	Merlot Cabernet Sauvignon	Time series (1952-97)	Increment in mean seasonal temp. was ~ 1.76 °C in 49 years (0.036 °C year <sup>-1</sup> )	TSS Acidity	Harvest	No trend during the last 26 years Decreased for both cultivars during the last 26 years
Duchene and Schneider (2005)	Riesling	Time series (1972-2003)	Increment in mean annual temp. was 2 °C over 30 years (0.06 °C year <sup>-1</sup> )	Alcohol (TSS)	Harvest	Positive association between warming and alcohol (0.08% vol year <sup>-1</sup> )
Petrie and Sadras (2008)	Chardonnay Cabernet Sauvignon Shiraz	Time series (1993-2006) Regions (18)	Increment in mean monthly ranged from negligible up to 0.19 °C year <sup>-1</sup>	TSS	Harvest (designated maturity 21.8 °Brix)	Stable with time in Chardonnay. Increased up to ~ 0.3 °Brix year <sup>-1</sup> for Cabernet Sauvignon and Shiraz
Cozzolino et al. (2010)	Cabernet Sauvignon Shiraz Merlot Grenache	Time series (1999-2008)/ Regions (14)	Difference of 0.77 °C between the coldest and the warmest seasons	Anthocyanin	Harvest (between 18 to 25.2 °Brix)	Positive association between temperature and anthocyanins concentration Lower anthocyanins concentration in warmer regions
Bock et al. (2011)	Müller-Thurgau Riesling Silvaner	Time series (1949-2010)	-	TSS Acidity	Harvest (designated maturity 60 °Oe)	Increased by 0.24 °Oe year <sup>-1</sup> over the period 1960-2010 No significant trend from 1960 onward
Urhausen et al. (2011)	Riesling	Time series (1951-2005)	Increment in mean seasonal temp. was ~ 1.37 °C in last 25 years (0.055 °C year <sup>-1</sup> ) and ~ 1.57 °C during the whole period (0.029 °C/year)	TSS Acidity	Harvest	Increased by 0.3 ± 0.2 °Oe year <sup>-1</sup> over the period 1965-2005 Decreased by 0.09 ± 0.069 g L <sup>-1</sup> year over the period 1965-2005
Vrsic and Vodovnik (2012)	Sauvignon Blanc Riesling Welschriesling Bouvier	Time series (1950-2009)	Increment in mean seasonal temp. was ~ 1.77 °C in last 30 years (0.061 °C year <sup>-1</sup> ) and ~ 2.12 °C during the whole period (0.036 °C year <sup>-1</sup> )	TSS Acidity	At harvest (between 76 and 84 °Oe)	Increased by 0.01 to 0.3 °Oe year <sup>-1</sup> over the period 1980-2009 Decreased by 0.09 to 0.15 g L <sup>-1</sup> year <sup>-1</sup> over the period 1980-2009
Bergqvist et al. (2001)	Cabernet Sauvignon Grenache	Temperature/Radiation (south vs north bunches)	Ambient + 3 °C in the south facing bunches at mid-day	Anthocyanin Acidity TSS	-	Reduced in south-facing bunches Reduced in south-facing bunches (hotter) Reduced in north-facing bunches (cooler)

**Table 2:** continued

Reference	Cultivar	Environmental factor-sources of variation	Range of temperature	Berry trait	Timing of assessment	Response
<i>Haselgrove et al. (2002)</i>	Shiraz	Temperature/Radiation (north vs centre bunches)	Temp of exposed berries was 2.6 °C > than shaded	Anthocyanins TSS	Mature grapes (based on °Brix)	Reduced in north-facing bunches (hotter) Temp./light altered anthocyanin profile Unaffected by exposure
<i>Koundouras et al. (2006)</i>	Agiorgitiko	Sites/Elevation (elev.) (from ~ 300 to 700m a.s.l.) Vintage (1997-98)	1998 was warmer than 1997 (20.6 °C vs 19.1 °C in mean seasonal temp) Mean daily temp was ~ 3 °C lower at higher elevation	Anthocyanin TSS and pH	From veraison to harvest	Higher in 1998 than in 1997 (1998 was warmer and drier) Mid elevation site, with the highest water deficit, had the highest anthocyanin content Higher elevation site had the lowest sugar content and the highest total acidity (higher water availability)
<i>Falcao et al. (2010)</i>	Cabernet Sauvignon	Elevation (from ~ 800 to 1400m a.s.l.)/Sites (5)/ Vintage (2005-06)	Mean seasonal temp ranged from 15.4 (higher elev.) to 20.2 °C (lower elev.)	TSS Acidity pH Anthocyanin	Harvest (between 20 to 24 °Brix)	Variable between seasons and elev./sites No trend in season 2005, higher at higher elev./sites in 2006 Variable between seasons and elev./sites Variable between seasons and elevation/sites. Lowest values at the low elevation
<i>Oliveira and Correia (2008)</i>	Touriga Nacional	Elevation (from ~ 100 to 300m a.s.l.)/Sites (18)/ Vintage (3)/Exposure (6)	-	Anthocyanin TSS	Harvest	Increase with elevation SW and NW exposures surpassed the N, S, NE, and SE exposures Reduced with elevation SW, S and SE exposures surpassed the other exposures
<i>Failla et al. (2004)</i>	Nebbiolo	Elevation (~300-700m a.s.l.)/ Orientation (SE-SW)/ Slope (15-35°)/ Vintage (1998-2000)	From 1100 to 1800 GDD between high and low elevations. Mean yearly temp in vineyard slopes was between 1 to 2 °C > than in the valley floor. Most vineyards between 2700 and 3200 MJm <sup>-2</sup> (PAR)	TSS, acidity and pH Anthocyanin	From veraison to harvest	Elevation explained more than 50% of the variance in these traits Explained mainly by: PAR x elevation, vine crop load and its interaction with PAR
<i>Nicholas et al. (2011)</i>	Pinot noir	Sites (8)/ Vintage (2005/07)	-	Anthocyanin	Two observation points: during ripening and at harvest	Positively correlated with temp between 16 and 22 °C from veraison to harvest Increased light interception was correlated with lower levels of anthocyanin Warm temp. from budburst to bloom increased [phenolic]; warmer temp. during the preceding fall and during ripening offset these effects Lower anthocyanin concentration in warmer sites



**Table 3:** Responses of grape berry composition to temperature in studies where thermal regimes were directly manipulated in glasshouses and growing chambers or field conditions.

Reference	Cultivar	Environmental factor-sources of variation	Range of temperature	Heating system	Timing of treatment	Berry trait	Response
<b>Greenhouse and growth chambers</b>							
<i>Buttrose et al. (1971)</i>	Cabernet Sauvignon	Temperature (day)	I) 20/15 °C day/night; whole period II) 30/15 °C day/night; whole period	Greenhouse	Before veraison to harvest	Anthocyanin TSS Acidity	Berry colour decreased in treatment II (~ 2 to 3 fold) Increased in II Reduced in II. High temp. reduced malate concentration Tartaric acid was unaffected
<i>Mori et al. (2007)</i>	Cabernet Sauvignon	Temperature (day)	I) 35/20 °C day/night II) 25/20 °C day/night	Phytotron	Before veraison to harvest	Anthocyanin	Decreased in treatment I (less than half of II)
<i>Mori et al. (2005)</i>	Darkridge <i>V. vinifera L. x V. labrusca L.</i>	Temperature (night)	I) 30/15 °C day/night II) 30/30 °C day/night	Phytotron	Before veraison to harvest	Anthocyanin TSS	Decreased in treatment II Unaffected
<i>Koshita et al. (2007)</i>	Aki Queen <i>V. labrusca L. x V. vinifera L.</i>	Temperature (night)	Low: control - 5 °C at night High: control + 5 °C at night Control in greenhouse	Greenhouse (with heaters)	Before veraison to harvest	Anthocyanin	High temp. inhibited accumulation
<i>Kliewer and Torres (1972)</i>	Cardinal Tokay Pinot noir Cabernet Sauvignon	Temperature (day and night)	Cool: 15 °C Warm: 25 °C Hot: 35 °C	Phytotron	From veraison to harvest	Anthocyanin	Cool day increased coloration in Cardinal, Pinot noir and Tokay Hot day and night reduced or completely inhibited coloration Cool night did not reverse the effect of hot day temp.
<i>Yamane and Shibayama (2006)</i>	Aki Queen <i>V. labrusca L. x V. vinifera L.</i>	Temperature (day and night)	Low: 23/18 °C day/night High: 33/28 °C day/night	Greenhouse	Four ripening stages: I) 4-11, II) 11-18, III) 18-25 and IV) 25-32 days after onset coloring	Anthocyanin Acidity TSS	Reduced by high temp. stages II and III Enhanced by low temp. stage II Temp. between 8-21 days after onset of coloring is critical for coloration Enhanced by low temp. in stage I Unaffected
<i>Yamane et al. (2006)</i>	Aki Queen <i>V. labrusca L. x V. vinifera L.</i>	Temperature (day and night)	Low: 20 ± 2 °C day and night High: 30 ± 2 °C day and night	Greenhouse	Four ripening stages: Before veraison (I) and after onset coloring (II-IV)	Anthocyanin Acidity TSS	Reduced by high temp. in stage III Enhanced by low temp. in stage III Enhanced by low temp. in stage IV Unaffected
<i>Matsui et al. (1986)</i>	Thompson seedless Napa Gamay	Temperature (day and night)	High: 40/22 °C day/night for 4 days Control in greenhouse	Phytotron	Before veraison (Stage I)	TSS	Reduced by high temp. Reduced by high temp.
<i>Azuma et al. (2012)</i>	Pione <i>Vitis x Labruscana</i>	Temperature/Radiation (day and night)	10 days at: I) 15 °C + light (UV+white), II) 15 °C + dark, III) 35 °C + light and IV) 35 °C + dark. Light: 80 µmol m <sup>-2</sup> s <sup>-1</sup>	In vitro	At onset veraison (9.2-10.8 °Brix)	Anthocyanin	High temp. (III, IV) or dark (II, IV) treatments suppressed accumulation Accumulation was dependent on both low temp. and light Temp. and light affected anthocyanin composition
<i>Kliewer (1977)</i>	Emperor	Temperature (day and night)	High: 37/32 °C day/night Control in field conditons (23.6/17.1 °C day/night)	Phytotron	From two weeks before veraison to harvest	Anthocyanin/TSS	High temp. inhibited accumulation

**Table 3:** continued

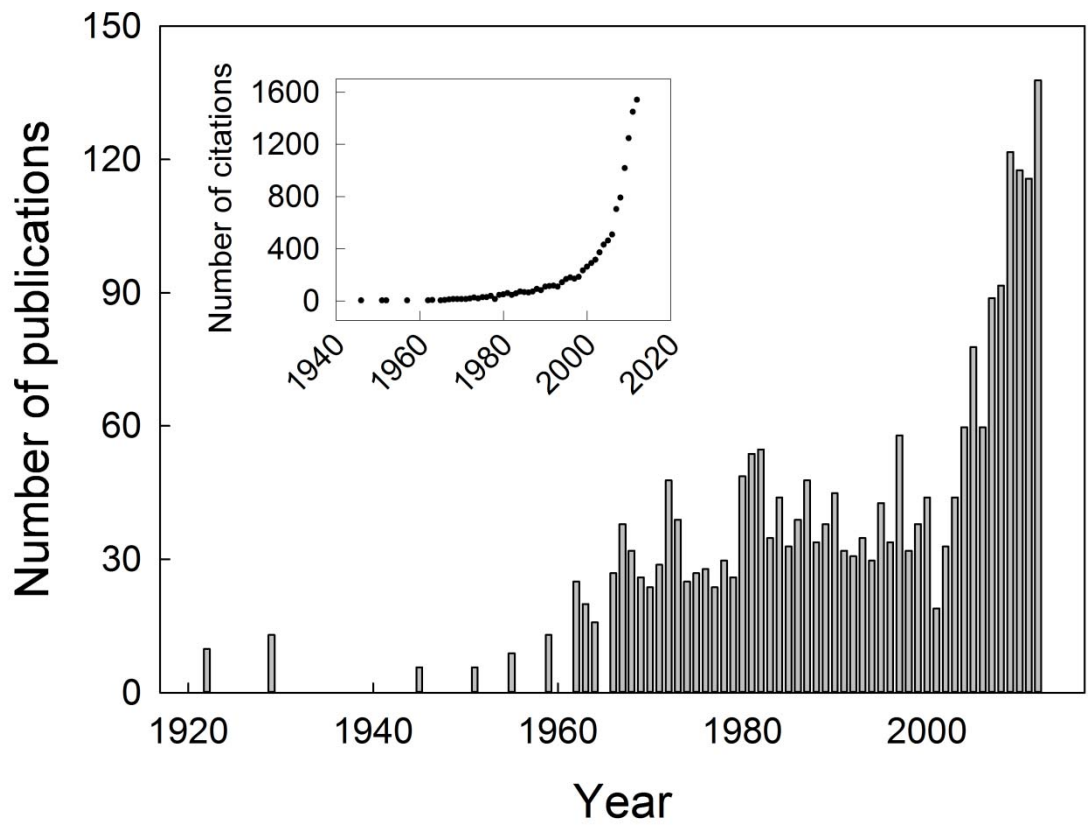
Reference	Cultivar	Environmental factor-sources of variation	Range of temperature	Heating system	Timing of treatment	Berry trait	Response
<i>Greer and Weston (2010)</i>	Semillon	Temperature (heat waves)	High: 40/25 °C day/night for 4 days Control in growth chamber (25/15 °C day/night)	Growth chamber	At flowering, fruit set, veraison and mid-ripening	TSS	Reduced when heated at veraison and mid-ripening
<i>Sepúlveda and Kliewer (1986)</i>	Chardonnay Chenin Blanc	Temperature (day and night)	High: 40/20 °C day/night Control in greenhouse (25/15 °C day/night) 0, 4, 8 and 12 days + recovery time (8 days)	Phytotron	Seven weeks post-veraison	TSS	Reduced by high temp. Heat stress increased glucose:fructose ratio
<b>Field experiments</b>							
<i>Radler (1965)</i>	Thompson seedless	Temperature (day and night)	Heated: 33 ± 2 °C day and night Control: ambient temp.	Bunch heaters (with black lamp)	From flowering to harvest	Acidity TSS	Reduced by high temp. Reduced by high temp.
<i>Bowen et al. (2004b)</i>	Merlot	Temperature (day)	Heated: ambient max temp. + 5-8 °C day Control: ambient temp.	Plastic sleeves (passive)	Before budburst to harvest	Acidity TSS	Reduced by high temp. High temp. increased TSS:TA (w:w) ratio Increase by high temp.
<i>Soar et al. (2009)</i>	Shiraz	Temperature (heat waves)	Heated: up to 40 °C day for 3 days (day) Control: ambient temp.	Closed chamber (combine active and passive)	At post-set, pre-veraison, veraison and pre-harvest	TSS	Unaffected
<i>Spayd et al. (2002)</i>	Merlot	Temperature/Radiation (east vs west, exposed vs shaded and heated vs cooled bunches)	Ambient/shaded + up to 13 °C in sun-exposed berries	Active, with blowers	From bunch closure to harvest	Anthocyanin  TSS Acidity	Higher in east-exposed bunches Increased by sun-exposure Increased in cooled sun-exposed bunches and reduced in heated shaded bunches Unaffected by exposure Reduced in west-exposed bunches
<i>Sadras and Soar (2009)</i>	Shiraz	Increase in background temperature	Ambient + ~4 °C	Open-top chamber (passive)	At budburst, after flowering, pea-size, verison and before harvest	TSS	Unaffected
<i>Cohen et al. (2008)</i>	Merlot	Temperature (diurnal temperature fluctuation)	I) ambient; II) control (blower); III) day-time +4 °C (heat) ; IV) night-time -4 °C (cool); V) damped ±4 °C ; VI) double-damped ±8 °C	Active, with blowers	From: I) fruit-set to veraison; II) veraison to harvest	Anthocyanin	Increased in damped treatments
<i>Cohen et al. (2012)</i>	Merlot	Temperature (diurnal temperature fluctuation)	I) ambient; II) control (blower); III) damped ±8 °C (cooling during the day and heating at night)	Active, with blowers	From: I) fruit-set to veraison; II) veraison to harvest	Anthocyanin  TSS	Variable (not different or reduced by damped temp.) Damping altered the proportion of anthocyanin derivatives Increased in damped treatments
<i>Sadras and Moran (2012)</i>	Shiraz	Increase in background temperature	Ambient + ~2 °C	Passive (day) and combine (day and night)	From budburst to harvest	Anthocyanins:TSS ratio	Temp. delayed anthocyanin accumulation

**Box 1.** The concentration of TSS is a reliable marker for ripening; digital refractometers are commonplace for both research and industry measurements. For comparisons, measurements of TSS need to be adjusted for temperature (Bayindirli 1993), but the reported data are not necessarily consistent in this correction. Concentration of TSS is generally reported in °Brix and °Baume or, less commonly, in SG (specific gravity) and °Oe (Oechsle), and these units can be converted for comparisons. There is a large discrepancy of methods used for anthocyanins determination (Harbertson and Spayd 2006), which combined with the different units used in the expression of the results, e.g. anthocyanins per gram of berry, per berry basis or per gram of skin, makes comparisons between studies extremely difficult, sometimes impossible. Organic acids are commonly determined by titration with a standard solution of sodium hydroxide; as if all the titratable ions were associated with one acid, results are expressed in tartaric acid at an equivalence point of pH 8.2, e.g. Australia and the U.S.A., or sulphuric acid at an equivalence point of pH 7, e.g. France. However, values expressed as g/L of sulphuric acid are lower than if they are expressed as their tartaric acid equivalent, conducting to misleading comparisons (Iland et al. 2011a, p. 127). Similarly, total acidity, as given by the additive concentration in g/L of tartrate and malate, is always higher than TA; the interchangeable use of total acidity and TA to refer to acid concentration in grapes and wines may result therefore in misleading interpretations (Boulton 1980a, b).

Implicit in thermal studies is the effect of temperature on berry size (Greer and Weston 2010). The complex nature of concentrations is widely recognised, but the widespread use of chronological scales for comparisons implies that ontogenetic drift effects are often overlooked (Sadras and McCarthy 2007a). Comparisons of size-independent traits, e.g. anthocyanin-to-TSS ratio, in thermally contrasting regimes are more reliable currency for comparisons (Sadras and Moran 2012c).

### **Caption to figure**

**Figure 1:** Yearly distribution of indexed publications containing the key words 'temperature' and 'grapevine' between 1909 and 2012. Inset shows the historical evolution in the number of citations of publications related to these key words. Data were obtained on November of 2012 from the Web of Science (Thomson Reuters 2013).



**Figure 1**



## **Chapter 3**

### **Effect of Elevated Temperature on the Onset and Rate of Mesocarp Cell Death in Berries of Shiraz and Chardonnay and its Relationship with Berry Shrivel**

*Published article - Australian Journal of Grape and Wine Research*

19, 87–94

## Statement of Authorship

Bonada, M., Sadras, V.O. and Fuentes, S. (2013) Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Australian Journal of Grape and Wine Research* 19, 87–94.

### Author contributions:

**Marcos Bonada** designed and conducted all research experiments, analysed the data and drafted and constructed the manuscript.

----- Date -----  
Signature

**Victor O. Sadras** contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.

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Signature

**Sigfredo Fuentes** contributed to research ideas, microscope-image analysis and editing the manuscript.



Bonada, M., Sadras, V.O. & Fuentes, S. (2013) Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Australian Journal of Grape and Wine Research*, v. 19(1), pp. 87-94

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## **Chapter 4**

### **Elevated Temperature and Water Stress Accelerate Mesocarp Cell Death and Shrivelling, and Decouple Sensory Traits in Shiraz Berries**

*Published article - Irrigation Science 31, 1317–1331*

## Statement of Authorship

Bonada, M., Sadras, V.O., Moran, M.A. and Fuentes, S. (2013) Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrigation Science* 31, 1317–1331.

### Author contributions:

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**Victor O. Sadras** contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.

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**Martin A. Moran** provided technical assistance and support of field experiments.

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**Sigfredo Fuentes** contributed to research ideas and editing the manuscript.

Bonada, M., Sadras, V.O. Moran, M. & Fuentes, S. (2013) Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrigation Science*, v. 31(6), pp. 1317-1331

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## **Chapter 5**

# **Impact of Elevated Temperature and Water Deficit on the Chemical and Sensory Profiles of Barossa Shiraz Grapes and Wines**

*Manuscript submitted on 23 January 2014 to Australian Journal of  
Grape and Wine Research*

## Statement of Authorship

Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A. and Sadras, V.O. (2014) Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Australian Journal of Grape and Wine Research* [Submitted manuscript]

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Signature

**David W. Jeffery** contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.

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Signature

**Paul R. Petrie** provided technical assistance and support of winemaking experiments, contributed to research ideas and editing the manuscript.

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**Martin A. Moran** provided technical assistance and support of field experiments.

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**Victor O. Sadras** contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.

## **Abstract**

*Background and Aims:* Interactions are the main cause of complexity in field experiments; however, no studies have combined water and temperature regimes in field-growing vines. Here we assessed grape and wine attributes from a field trial where these factors were directly manipulated.

*Methods and Results:* Shiraz vines grown in a 2<sup>2</sup> factorial experiment with two temperature (control and heated) and two water regimes (deficit and irrigated) during two seasons (2010/11 and 2011/12). Sensory and compositional data of grapes and wines were assessed on both seasons, whereas detailed phenolic profiles were determined by spectrophotometry in 2011/12. We found additive effects (i.e. lack of interaction) between temperature and water for berry weight and components, fruit composition (TA and pH), grape phenolics (12.5% ethanol-extracted and most of the 70% acetone-extracted), wine phenolics (chemical age 2) and wine sensory traits (floral aromas and berry flavours). Significant, previously unrecorded interactions between temperature and water were found for grape phenolics (70% acetone-extracted skin- and seed-tannins and total phenolics per berry), wine phenolics (colour density, total tannins and phenolics) and wine sensory traits (floral aromas, cooked fruit flavours and tannin structure).

*Conclusions:* The effect of water deficit leading to colourful, flavoursome and phenolic-rich wines may not be held under high temperature.

*Significance of the Study:* Our study anticipates the drier and hotter climate of the Barossa Valley, and provides industry with indications of shifts in wine profiles that would require technological innovations to maintain the identity of Barossa Shiraz, the flagship of the Australian wine industry.

**Keywords:** anthocyanins, berry, climate change, colour, phenolics, sensory



## **Introduction**

Matching parcels of grapes to wine of a target style and quality is commercially important but remains challenging due to the difficulty in developing rapid, reliable and inexpensive predictors and the complex relationship between analytical measures and perceived quality. In red wines, where grape-derived phenolic compounds are critical to colour, mouthfeel and taste, attempts to predict wine quality from grape composition are often based on the assessment of phenolic compounds and colour, using measures such as total phenolics, grape anthocyanins and polymeric flavan-3-ols (tannins) (Kennedy 2008). More recently, volatile aroma compounds liberated by acid hydrolysis of grape components have been gauged as indicators of potential wine quality as well (Ristic et al. 2010). Approaches which combine the analysis (targeted or not) of volatile and non-volatile grape constituents appear most suited to aiding the determination of style and quality, but knowledge of environmental and viticulture factors that modulate important grape components is necessary to grow grapes to achieve a desired wine quality outcome.

Grapevines are subjected to management techniques to modulate vine growth and improve fruit quality. Regulated deficit irrigation, for example, is a widely adopted practice to reduce vine vigour and yield, and improve bunch zone microclimate and grape and wine composition (Iland et al. 2011a). Trade-offs between yield and berry traits related to wine quality are not straightforward, but moderate water deficit that reduces yield is generally beneficial for production of quality grapes (Basile et al. 2011, Intrigliolo et al. 2012, Roby and Matthews 2004b). Compositional changes in grapes by transient water deficit are related to indirect effects, leading to a concentration of berry metabolites, and direct effects, through up-regulation of genes in the biosynthetic pathway of phenolic compounds (Castellarin et al. 2007, Deluc et al. 2009, Roby et al. 2004a). Links between deficit irrigation and berry quality traits need to be revised, however, in a scenario that anticipates warmer and drier growing conditions. Effective water management needs to accomplish the dual purpose of constraining water supply at certain phenological windows to favour grape quality traits, while ensuring plant water status to counteract deleterious effects of elevated temperature in water-stressed plants (Sadras and Schultz 2012e).

Numerous studies have assessed the effects of water deficit on berry composition (Intrigliolo et al. 2012, Ojeda et al. 2002, Roby et al. 2004a) and wine phenolic compounds (Bindon et al. 2011, Casassa et al. 2013b, Chapman et al. 2005, Edwards and Clingeleffer 2013). Similarly, many studies have dealt with temperature effects on

grape (reviewed by Cohen and Kennedy 2010, Downey et al. 2006, Jackson and Lombard 1993) and wine quality (Bock et al. 2011, Dalu et al. 2013, Grifoni et al. 2006, Rodó et al. 2000, Sadras et al. 2007b, Soar et al. 2008). However, these assessments have been indirect, i.e. based on comparisons of thermally contrasting seasons or sites, or from experiments in controlled conditions (glasshouse and growing chambers). These approaches are useful but are bound to be inconclusive as temperature effects are often confounded with other weather factors (solar radiation, vapour pressure deficit and rainfall), management practices and soils, or by experimental artefacts in controlled environments (small soil volume, lack of wind and altered radiation regimes), with direct implications for plant physiology and berry composition. To probe for cause-and-effect and to quantify the interaction between water deficit and high temperature, factorial field experiments are required (Sadras et al. 2012a, Spayd et al. 2002).

Water deficit and high temperature have been combined in controlled (Edwards et al. 2011, Goto-Yamamoto et al. 2009) or field conditions (Sadras et al. 2012b, Sadras and Moran 2012c, Sadras and Moran 2013, Sadras and Moran 2013), Increased temperature during the whole growing cycle affected grape composition and wine sensory attributes (Sadras et al. 2013). A difference of  $\sim 1.3$  °C in maximum temperature from anthesis to harvest decoupled anthocyanin and sugar accumulation in berries, potentially leading to high-alcohol and low-colour wines (Sadras and Moran 2012c). Increased background temperature also hastened berry development and decoupled berry sensory traits (Bonada et al. 2013b, Sadras et al. 2013); seed ripening, for example, was advanced in relation to other berry tissues. This asynchrony in development of seed vs other berry tissues, as driven by temperature, may change the tannin extractability from seeds and skin during alcoholic fermentation (del Llaudy et al. 2008) and the resultant phenolic composition and sensory attributes of wines (Ristic et al. 2010). Under the experimental conditions of Casassa et al (2013b), extraction of the major phenolics classes from seed and skin of fully ripe fruit into wine was unaffected by water deficit; however, the chemical effects of water deficit combined with high temperature are unknown.

In a context of a field experiment aimed at anticipating the effects of warmer and drier climates on vine phenology, physiology and yield (Sadras et al. 2012b, Sadras and Moran 2013, Sadras and Moran 2013), this work is the first attempt to assess the effects of water deficit combined with high temperature on the phenolic composition of Shiraz grapes and wines. Previous reports from this experiment focused on the effect of temperature and water on total anthocyanins and sugar accumulation in berries during

the first experimental season 2010/11 (Sadras and Moran 2012c). Here we present a deeper analysis of phenolic compounds in season 2011/12 and include sensory and compositional data of grapes and wines from both seasons. In doing so, we address the following objectives: to assess the possible interaction between deficit irrigation and high temperature on berry and wine composition (total soluble solids, acidity, pH and phenolics including anthocyanin and tannin), and wine sensory traits; to explore the effect of temperature and water regime on the extraction of the major phenolics classes from seed and skin in fully ripe fruit and their relative contributions to the final wine phenolic profile. The effects of irrigation and temperature on vegetative growth and yield components measured in this trial during both experimental seasons have been reported elsewhere (Sadras and Moran 2013).

## ***Methods***

### **Field experiments**

The experimental settings, including plant and site, experimental design and vineyard management, have been described in previous papers (Bonada et al. 2013b, Sadras and Moran 2012c). Briefly, treatments were laid-out in a split plot design with three replicates; temperature was assigned to main plots and water to sub-plots. Interference between treatments was minimised by buffer zones between replicates within the row (~13m) and between rows (one guard row at each side). Heated plants were compared with control vines at ambient temperature. To increase temperature under realistic field conditions open-top chambers were used. Full construction details, performance and limitations are in Sadras et al. (2012a). Water regimes were imposed in two consecutive growing seasons (2010/11 and 2011/12) based on calendar applications (Bonada et al. 2013b). During the season 2010/11, well-watered plants were watered seven times from 24 January to 7 March, while water deficit vines were irrigated only twice, on 21 and 28 February. In the drier season 2011/12, irrigation scheduling started earlier and control plants received 13 irrigations from 26 November to 24 February, in comparison with their water deficit counterparts that were irrigated only twice, on 24 January and 17 February.

The approach used for characterizing thermal and water regimes in this experiment has been reported elsewhere (Sadras et al. 2012b, Sadras and Moran 2012c). During both seasons pre-dawn water potential was measured as previously reported in Sadras and Moran (2013). In 2011-12, stem water potential was also measured at midday, as reported in Bonada et al. (2013b).

## **Winemaking**

In 2010/11, heated and control temperature treatments were differentially harvested on 22 and 25 March respectively, whereas in 2011/12 all treatments were harvested on 27 February. Approximately 46 kg of fruit from each field replicate was transported to the Penfolds winery facility at Magill, South Australia. Field replicate identity was maintained during the vinification process to avoid confounded field and fermentation effects (Sadras et al. 2013) affording a total of 12 wines. The fruit was crushed and destemmed using a small electric stainless steel crusher (Beta 35, Zambelli, Camisano Vicentino, Italy) and pressed using a hand racked press. Must was inoculated with selected dry yeast (*Saccharomyces cerevisiae* Lalvin EC-1118, Lallemand Inc., Montreal, Canada) and the following day with malolactic bacteria (*Oenococcus oeni* Lalvin VP 41, Lallemand Inc.) following the manufacturer's instructions. Fermentation was carried out on skins in 75 L plastic bins at ambient temperature ( $\sim 22^{\circ}\text{C}$ ), with the ferment being plunged once a day. After 7-days, when the wines were approximately 2  $^{\circ}$ Brix, the wine was pressed and racked into 10 L demijohns. After sugar ( $< 3$  g/L combined glucose and fructose) and malolactic fermentation ( $< 0.1$  g/L malic acid) was complete, wine was treated with potassium metabisulfite (to maintain  $\sim 20$  mg/L free  $\text{SO}_2$ ) and tartaric acid to reach a target of  $\sim 7$  g/L. The wine was settled and cold stabilised at  $4^{\circ}\text{C}$  and then racked clean from the lees and bottled (375 ml bottles)  $\sim 75$  days after crushing in 2011 and after 126 days in 2012. Bottles were sparged with dry ice prior to filling and sealed with screw-cap closures. The wine was stored in a temperature controlled room ( $\sim 17^{\circ}\text{C}$ ) until the time of assessments.

## **Fruit and wine basic analyses**

From each field replicate, 100 berries were randomly chosen from the seven central plants; berries were separated from the clusters by cutting with scissors through the pedicel as close as possible to their point of attachment and placed into polyethylene bags cooled with ice in the field. A sub-set of 80 berries was selected at random for TSS, pH and TA determinations on the same day of sampling, as previously described (Sadras and Moran 2012c). The remaining 20 berries were stored at  $-18^{\circ}\text{C}$  for phenolics analysis.

Standard juice analyses were undertaken immediately after crushing following the guidelines of Iland et al. (2000); these included total soluble solids (TSS) (digital refractometer; HI 96801, HANNA, Woonsocket, RI, USA), titratable acidity (TA) and pH (autotitrator; Crison Instruments, Barcelona, Spain). Residual sugar (as glucose plus

fructose) and malic acid were analysed using a Gallery Automated Photometric Analyser (Thermo Scientific, Massachusetts, USA).

Ethanol concentrations were measured using an AlcoLyzer Wine M (Anton Paar, Graz, Austria). The aspiration method was used for the determination of free and total SO<sub>2</sub> and the cash still method was used for volatile acidity (Iland et al. 2004).

### **Extraction of phenolics from grape skin and seed**

Each 20-berry sample (see above) was divided at random into two sets of 10 berries. After weighing the sub-samples, seeds and skin were collected from frozen berries and extracted separately. Pulp was carefully scraped from the skin and skin from the first 10-berry sub-sample was pooled, weighed and transferred into a 15 mL glass vial containing 10 mL of 70% acetone in water (v/v) (Kallithraka et al. 1995). Vials were sparged with nitrogen before being sealed and incubated at 23 °C in the dark for 24 h on an orbital shaker (200 rpm). Whole seeds from the first sub-sample were counted, weighed and put into separate 15 mL glass vials and extracted with 70% acetone as described for skin samples. In the same manner, seeds and skin from the second 10-berry sub-sample were separately weighed/counted and extracted with 10 mL of hydroalcoholic solution containing 12.5% (v/v) ethanol and 2 g/L potassium hydrogen tartrate (pH 3.6) (Bautista-Ortín et al. 2012). Prior to incubation, sodium azide (0.02% w/v) was added to inhibit microbial activity. The 15 mL vials were sparged with nitrogen before being sealed and incubated at 23 °C in the dark for 72 h on an orbital shaker (200 rpm). All extracts were decanted from the solids after incubation and their volumes were recorded. Acetone extracts were concentrated under vacuum at 20 mbar using a rotatory evaporator at 38 °C, the aqueous residue was transferred to a volumetric flask and adjusted to 10 mL with 50% (v/v) ethanol prior to being frozen at -20 °C until required. Ethanol extracts were stored without further manipulation at -20 °C until required.

### **Spectrophotometric analysis**

Absorbances were recorded with a Thermo Scientific Multiskan® Spectrum (Thermo Fisher Scientific Inc., USA) using UV semi-micro 1.5 mL disposable cuvettes (Brand, Stennick Scientific, Melrose Park, SA, Australia). To check for spectrophotometer performance, linearity and accuracy were tested prior to the formal analysis (Iland et al. 2004). All the parameters were measured in triplicate for grape extracts or duplicate for wines obtained from freshly opened bottles; two bottles of each field replicate were analysed 338 days after bottling.

Anthocyanins and total phenolics from skin and seeds were determined according to the method described by Iland et al. (2004) with some minor modifications. A 0.25 mL sub-sample of extract was diluted with 5 mL of 1 M HCl and incubated at room temperature (21-23 °C) in the dark for 3 h. Samples were centrifuged for 5 min at 4000 rpm using an Eppendorf 5810 R centrifuge (Eppendorf, Hamburg, Germany) and absorbance was measured at 280 and 520 nm with a 10 mm path length. Anthocyanin content was expressed as mg of malvidin-3-glucoside equivalents per berry and per gram of berry fresh mass from the absorbance at 520 nm (equation 1). The content of total phenols per berry and per gram of berry was calculated from the sum of total phenolics from seeds and skin based on absorbance measurements at 280 nm (equation 2).

$$\text{Anthocyanins (mg/berry)} = \frac{\text{Abs (520nm)}}{500} \times \text{DF} \times \text{EV} \times \frac{\text{WB}}{\text{WT}} \quad \text{eq. 1}$$

$$\text{Total phenols (au/berry)} = \text{Abs (280nm)} \times \text{DF} \times \text{EV} \times \frac{\text{WB}}{\text{WT}} \times 0.001 \quad \text{eq. 2}$$

where DF is the dilution factor of the extract in 1 M HCl, EV is the extracted volume after maceration with 12.5% ethanol or 70% acetone, WB is the weight of 10 berries, and WT is the weight of berry tissue (seeds or skin) from 10 berries. The value of 500 is based on a previous report (Somers et al. 1974) that estimated the extinction coefficient of malvidin-3-glucose in g/100 mL of solution. Anthocyanin and total phenolics per gram of berry were calculated from the mean of berry weight (g).

Wine anthocyanin equilibria and phenolic composition were determined using the modified Somers colour assay (Mercurio et al. 2007). Condensed tannins in wine and grape extracts (seeds or skin) were quantified by the methyl cellulose precipitable (MCP) tannin assay (Sarneckis et al. 2006) using the protocol of Mercurio and Smith (2006). Control samples prepared as part of the MCP tannin assay were used for quantifying total phenolics at 280 nm in the wine samples.

The epicatechin equivalent concentration was determined as previously described (Mercurio and Smith 2006) with minor modifications. Briefly, duplicate 1000 mg/L stock solutions of (–)-epicatechin (Sigma-Aldrich, Castle Hill, NSW, Australia) in 10% aqueous ethanol (v/v) were used to prepare a duplicate series of calibration solutions containing 10, 40, 70, 100, 130, 160, 190 and 220 mg/L of epicatechin. The total tannin concentration of each sample, expressed as epicatechin equivalents (mg/L) using the 8-

point epicatechin standard curve ( $r^2 = 0.998$ ) after correcting for dilution, was determined by UV–vis spectrophotometry.

The chromatic CIELab coordinates  $L^*$  (lightness),  $a^*$  (green/red component), and  $b^*$  (blue/yellow component) were calculated using Spectral Colour software, version 1.5 (GBC Scientific Equipment Ltd, Australia). Measurements were undertaken at room temperature (21–23 °C) using a Cintra 40 spectrophotometer (GBC Scientific Equipment Ltd, Australia). Wine samples were centrifuged for 5 min at 4000 rpm prior to analysis. Wines were scanned from 380–780 nm at 0.43 nm intervals in a 1 mm quartz cuvette (Starna, Australia) at the daylight illuminant D65 with a 10 degree observer angle, using Milli-Q water as a blank. A holmium filter (Azzota Corporation, USA) was used as a standard to check the spectrophotometer performance (Vandenberg 1961). To explore overall chromatic differences between treatments in the Euclidean space,  $L^*$ ,  $a^*$ , and  $b^*$  were further considered to calculate the CIELab colour difference ( $\Delta E^*$ ), the saturation or chroma ( $C^*$ ) and the hue angle ( $H^*$ ) (Pérez-Magariño et al. 2003).

### **Sensory assessment**

Wine sensory assessment was undertaken by a tasting panel of 15 (vintage 2010/11) and 20 (vintage 2011/12) professional winemakers from Treasury Wine Estate. Prior to the formal tasting in 2011, winemakers defined the most contrasting attributes between treatments; by consensus four attributes were chosen (berry flavours, floral aromas, cooked fruit flavours and tannin structure). Tannin structure was defined by the combination of ‘drying’ (feeling of lack of lubrication or desiccation in the mouth) and ‘dynamic’ (sensation involving some form of mouth movement) (Gawel et al. 2000). Seeking consistency, the same attributes were assessed at the time of the formal assessment on 9 December (2011) and 31 January (2013). The ranking test procedure was used to assess sensory intensity of each single attribute across treatments (Lawless et al. 2010). Sample wines of each treatment were presented in a random order to each panellist who was asked to rank each attribute in order from least intense (1) to most intense (4). Wines (~ 25 mL) were presented at room temperature (23 °C) in coded in 210 mL ISO standard tasting glasses covered by plastic Petri dishes. In both years, wine testing included three replicates and all samples were tested in a single 2 h session.

### **Statistical analysis**

The effects of temperature, water and their interaction on berry and wine composition were determined by two-way analysis of the variance (ANOVA). Both non-parametric (Friedman test) and parametric (ANOVA) methods were used to analyse the ranking

data for each sensory attribute (Juanola et al. 2004). To test for significance of treatment effects, Friedman's statistic (F) was calculated and compared with the  $\chi^2$  distribution for three degrees of freedom at a 5% level of significance (Lawless and Heymann 2010). Assuming that the ranks were quantitatively related to the intensity of the attribute, the analysis of the variance was done testing for the effect of temperature, water and the temperature  $\times$  water interaction for each growing season. Data analysis was performed using StatView version 5.0 (SAS Institute Inc., Cary, NC, USA). Interaction line plots were used to illustrate the effect of temperature and water regimes on grape and wine traits (DeWitt et al. 2004, Mazer et al. 1991, Valladares et al. 2007). Principal component analysis (PCA) was performed with no rotation on the table of observations/variables including the replicates, using the statistical package XLSTAT-Pro 2013, version 4.08 (Addinsoft SARL, Paris, France). Correlation analysis between berry, wine and sensory data was run with XLSTAT-Pro 2013 software.

## **Results**

### **Growing conditions**

The 2011/12 season was warmer and drier than 2010/11. During the growing period September to March, differences in mean minimum temperatures were negligible between seasons ( $< 0.02$  °C) but the mean maximum temperature was  $\sim 1.5$  °C higher in 2011/12. The accumulated rainfall during season 2010/11 (September to March) was 509 mm, which contrasts with the 257 mm for the same period during 2011/12.

Performance of the heating system, including potential artefacts, and thermal regimes during the experiments has been fully described (Sadras et al. 2012b, Sadras and Moran 2012c). Across two growing seasons and the intervening winter, from September 2010 to March 2012, mean daily temperature at canopy level in the heated treatments was  $1.31$  °C (SD = 0.63) higher than in controls (Sadras and Moran 2013).

Temperature treatments did not affect pre-dawn leaf water potential; the data pooled across temperature regimes showed significant differences in pre-dawn leaf water potential between irrigated and deficit treatments in both growing seasons as detailed in Sadras and Moran (2013). Briefly, differences started to develop shortly before veraison and lasted for 3 weeks in 2010/2011 and extended to shortly before harvest in 2011/12. Measurements of midday stem water potential in 2011/12 showed that control treatments were maintained at or above  $-1.0$  MPa from pea size to harvest (Bonada et al. 2013b). Moderate to severe stress ( $< -1.2$  MPa) developed in the water deficit



treatments from veraison to harvest. Heated vines had lower stem water potential (more negative) compared to vines at ambient temperature ( $p = 0.02$ ).

### **Berry size and components**

There were significant, additive effects (i.e. no significant interaction) of thermal and water regimes on berry weight, and skin-to-berry and pulp-to-berry ratios (Table 1). Mean berry weight was reduced by both elevated temperature (~ 5%) and water deficit (~ 7%), for data pooled across temperature and water, respectively. The proportion of skin per berry increased and the proportion of pulp per berry decreased with elevated temperature and water deficit (Table 1). The skin-to-pulp ratio increased (~ 13%) with both high temperature ( $p = 0.0116$ ) and water deficit ( $p = 0.0074$ ), which was primarily associated with a reduction in the mass of pulp per berry ( $p < 0.0113$ ) (data not shown).

### **Berry composition**

To minimize compositional differences between treatments associated with maturity level, we aimed at harvesting grapes at a common TSS between treatments within the season; this was achieved in both years (Figure 1ab). There was a significant seasonal effect ( $p < 0.0001$ ) whereby TSS was higher in 2011/12 (~ 26.6 °Brix) than in 2010/11 (~ 23.2 °Brix).

TA and pH responded to both temperature ( $p < 0.0001$ ) and temperature  $\times$  season interaction ( $p < 0.01$ ). To interpret this interaction, data were split by season (Figure 1). High temperature reduced TA in both seasons, however, this effect was 3.5-fold more pronounced in 2010/11 (from 5.98 to 4.73 g/L) compared to 2011/12 (from 6.45 to 6.09 g/L) and only statistically significant in 2010/11 (Figure 1cd). Similarly, high temperature increased juice pH, with a 5-fold more pronounced effect in 2010/11 (from 3.51 to 3.87) than in 2011/12 (from 3.67 to 3.74) (Figure 1ef).

The phenolic composition of grape tissue was determined in terms of total anthocyanins, tannins and phenolics using two extraction methods for separated skin and seed (Table 2). Aqueous acetone (70% v/v), a common and high-yielding solvent for polyphenol extraction (Cheng et al. 2012), was compared with a wine-like extraction medium containing 12.5% (v/v) ethanol (Bautista-Ortín et al. 2012). The relative contribution of skin and seed tannins to the total berry composition was also explored. Phenolic compounds in this study were expressed in terms of berry fresh weight (content) and per gram of fresh berry weight (concentration) to provide an indication as

to whether changes in phenolic compounds occurred as a result of changes in biosynthesis or were simply related to berry size or component proportions.

The extraction of skin anthocyanins with 70% acetone was ~1.6-fold higher than the extraction with 12.5% ethanol (based on means from Table 2 pooled across three replicates and four treatments). Regardless of the extractant, only the effect of temperature on berry anthocyanin was significant (Table 2); for both per berry and gram of berry weight, heated berries had between 14 to 24% less anthocyanin than their counterparts at ambient temperature.

Total tannin extracted with 70% acetone was ~ 3-fold higher than that extracted with 12.5 % ethanol and decreased significantly in heated treatments by up to 20% (Table 2). These differences in total tannin for heated treatments were mainly explained by a distinctive response of skin and seed tannins to temperature; while skin tannins were unresponsive to temperature, seed tannins were 15 to 37% lower in heated treatments compared to controls for either extraction solvent, when expressed both as content or concentration. Water regime significantly affected the concentration of seed and skin tannins on a mg/g of seed or skin basis for acetone extracts, as did the interaction of water and temperature. In contrast, differences were constrained to the effect of temperature on seed tannin concentration (mg/g of seed) when 12.5 % ethanol was the extractant (Table 2).

Elevated temperature increased the proportion of skin-derived tannin and reduced the proportion of tannin from seed; the overall effect of temperature on lower extractability of tannins from seed therefore resulted in an increase in the skin-to-seed tannin ratio in heated treatments compared with controls. There was also an evident effect of extractant on the skin- to-seed tannin ratio; data from Table 2 pooled across temperature and water treatments showed that means for the 12.5% ethanol solution were 1.4-fold higher than with the 70% acetone solution ( $p < 0.001$ ). The relative lower contribution of seed tannin when ethanol was used as extracting agent is the most likely reason behind this effect, due to the higher percentage of organic component and greater tannin extraction from mature seeds with acetone (Bautista-Ortín et al. 2012).

The effect of temperature on anthocyanins and tannins translated into total phenolics, which were lower in heated treatments. With the exception of total phenolics per berry (au/g fresh berry weight), which was affected by the interaction between temperature and water when 70% acetone was used as extractant, only the effect of temperature was

significant for total phenolics (Table 2). Values were ~ 1.9-fold higher when 70% acetone was used as extractant, and total phenolics were between ~ 8 to 23% lower in heated treatments compared with controls on both per gram of berry and per berry basis.

### **Wine composition**

Wines prepared in 2012 were analysed for basic composition after ~ 11 months of bottle ageing (Supplementary Table 1). Treatments had no effects on wine attributes, except for TA that was ~ 6% lower in wines from heated treatments. The difference in TA, however, may be attributed to the acid adjustment employed as part of the vinification process rather than a real temperature effect. The latter can be also supported by the lack of effects on berry TA by temperature in 2011/12 (Figure 2d).

To compensate for any small differences in SO<sub>2</sub> and pH of the wines, which may affect colour density and other spectrophotometric colour expression parameters, wines were analysed using the modified Somers colour assay (Mercurio et al. 2007). In this method pH, SO<sub>2</sub> and alcohol content of wines are standardised in a one-step adjustment with a model wine buffer solution prior to the analysis. After ~ 11 months of bottle ageing, the concentration of total anthocyanins in wines was unaffected by treatments (Table 3). In contrast, total tannins and total phenolics responded significantly to the interaction between water and temperature; while water deficit treatments had higher concentrations of tannins and total phenolics than their irrigated counterparts at the control temperature, differences were undetectable under high temperature. Wine colour density (with and without SO<sub>2</sub> correction) and SO<sub>2</sub>-resistant pigments (i.e. non-bleachable coloured compounds) showed a similar response; they were unresponsive to temperature in well-irrigated treatments, but colour density was reduced by up to 26% and SO<sub>2</sub>-resistant pigments by ~ 30% when water deficit was combined with elevated temperature. Chemical age 2, which represents the extent to which polymeric pigments produced during ageing account for total wine colour at pH < 1 (Somers et al. 1977), differed significantly among water treatments. Chemical age 2 was an average of ~ 22% higher in deficit compared to irrigated treatments, indicating a higher proportion of polymeric pigments containing the flavylum ion in water deficit wines. Other measurements of wine colour (hue, degree of ionization of anthocyanins and chemical age 1) were not significantly affected by either water, temperature or their interaction (Table 3).

Chromatic differences between wines in their natural state, without SO<sub>2</sub> or pH adjustment, were further characterised by the CIELab parameters (Figure 2). Both water

and temperature significantly affected wine lightness ( $L^*$ ) and saturation ( $C^*$ ). The  $L^*$  was higher in irrigated and heated treatments, indicating lighter wines (Figure 2a). The overall contribution of red ( $a^* > 1$ ), blue ( $b^* < 0$ ), and yellow ( $b^* > 1$ ) components define  $C^*$ ; higher  $C^*$  corresponds to more vivid colours (Gil-Muñoz et al. 1997). The  $C^*$  was higher for control-deficit (in particular) and control-irrigated treatments, indicating more colourful wines (Figure 2b). Control-deficit wines had significantly lower  $L^*$  (~ 12%) and higher  $C^*$  (~ 27%) than their irrigated and heated counterparts; mainly by the higher contribution of red colours (Figure 2c) rather than a difference in yellow and blue tones between wines (Figure 2d). There were no significant effects of water, temperature and their interaction on the hue angle between treatments (data not shown).

The CIELab colour difference  $\Delta E^*$  predicts chromatic differences discernible by the human eye between any given pair of wines. Under the same CIELab standard conditions ( $10^\circ$  standard observer and illuminant D65) the literature indicates potentially detectable visual differences between a given pair of wines when  $\Delta E^*$  is greater than one (Kwiatkowski et al. 2007, Skouroumounis et al. 2005), approximately three (Ayala et al. 1997, Martínez et al. 2001) or five CIELab units (Pérez-Magariño and González-Sanjosé 2003). We defined the strictest  $\Delta E^* = 5$  threshold to confidently attribute colour differences between wines from this indirect spectral method. Based on this criterion, differences in colour between control-irrigated and heated treatments were perceptually indistinguishable, regardless of the water regime (Table 4). Only wines from the control-deficit treatment resulted in a discernibly higher colour compared to the other wines.

### **Wine sensory**

Sensory scores were analysed using both parametric and non-parametric statistics. Both methods returned similar results; non-parametric analysis, however, precluded discriminating single effects. Table 5 and Figure 3 summarise the single and combined effects of temperature and water on wine sensory attributes assessed during both vintages. A differential response of wine sensory attributes to temperature and water treatments between vintages is partially reflected in the significant temperature  $\times$  vintage effect ( $p = 0.0032$ ).

In 2010/11, treatments affected two attributes (Table 5); high temperature increased wine floral aromas for both irrigated and water deficit treatments (Figure 3c) and increased cooked fruit flavours in water deficit but not in irrigated treatments (Figure

3e). In 2011/12, the four assessed attributes were affected by temperature, water and/or by their interaction (Table 5). Regardless of temperature, water deficit enhanced berry flavours (Figure 3b). There was an interaction between temperature and water in floral aromas whereby this attribute was enhanced by high temperature under water deficit but was reduced with irrigation (Figure 3d). Similar effects of temperature and water were found for cooked fruit flavours and tannin structure attributes (Figure 3fh); high temperature slightly increased these attributes in irrigated treatments but strongly reduced these characters under water deficit.

### **Relationship between water and temperature with berry, wine and sensory traits**

The compositional data (including replicates) of significantly different traits between treatments were subjected to principal component analysis to further explore the influence of both temperature and water treatments on the composition of grapes and wines (Figure 4). The first four principal components with eigenvalues greater than one were originally retained, accounting for ~ 95% of the observed variation in grape and wine composition between treatments. The fourth component explained less than ~ 5% of the observed variability; therefore it was excluded from the analysis to simplify the interpretation.

Component one explained ~ 53% of the variation and was a function of the temperature treatment; the remaining 37% was explained by component two and three, which were related to the water treatment. These differences suggest a comparatively higher impact of temperature over water on grape and wine composition. Irrespective of the water treatment, high temperature grouped on the negative region of component one, and was strongly correlated ( $r \geq 0.84$ ) with wine lightness. The PCA component loadings on the positive side strongly associated ( $r \geq 0.85$ ) control temperature treatments with grape total phenolics (concentration), wine colour density, SO<sub>2</sub>-resistance pigments, total tannin concentration, total phenolics, colour saturation and red components. Grape anthocyanin (content and concentration), total tannin and phenolics (content), and wine SO<sub>2</sub>-resistance pigments, also correlated with component one but to a lesser extent ( $0.76 \leq r \leq 0.80$ ).

Component two, which explained ~ 25% of the variation, segregated treatments as a function of irrigation, and it was mainly driven by the proportion of seed- and skin-derived tannin ( $r = -0.89$  and  $0.89$ , respectively) and the skin-to-seed tannin ratio ( $r = -0.84$ ). Regardless of temperature treatments, water deficit clustered at the lower end of component two, indicating a higher proportion of skin-derived tannin and overall higher

skin-to-seed tannin ratio. On the contrary, control-irrigated treatments located at higher end of component two indicating a higher proportion of seed-derived tannin. Heated-irrigated treatments located at the median region of component two. Similar to component two, component three, which explained 12% of the variation, segregated water deficit treatments from the irrigated counterparts by their higher chemical age 2 ( $r = 0.84$ ).

The principal components showed a clear separation of treatments within the compositional and sensory space. Control-deficit treatments were defined by higher concentrations of phenolics in grapes and wines and more intensely coloured wines. Grapes were richer in anthocyanins, skin-derived tannin and total tannin and phenolics. Wines made from these treatments were characterised by more intense red tones ( $a^*$ ), and higher colour saturation, colour density (with and without  $SO_2$  correction),  $SO_2$ -resistance pigments, total phenolics, total tannins, and a higher proportion of polymeric pigments (chemical age 2). Also, three out of four wine sensory-traits, i.e. cooked fruit flavours, berry flavours and tannin structure, were associated with control-deficit wines. Control-irrigated treatments were broadly similar to their control-deficit counterparts, but had a lower concentration of grape phenolics, being mainly characterised by the higher proportion of seed-derived tannin, and overall lower values for wine phenolic and colour measures. In opposition, and regardless of water regime, heated treatments clustered together and were characterised by the lower phenolic concentrations of both grapes and wines and by the lower chromatic properties (higher  $L^*$  and lower  $a^*$  and saturation) and sensorial composition of the wines.

## **Discussion**

The effect of water deficit on grape and wine composition has been widely investigated. Likewise the effects of temperature have attracted attention, but indirect methods comparing seasons or regions or controlled environments preclude establishing definitive cause-and-effect relationships relevant to realistic field conditions. Interactions between thermal and water regimes are largely unknown; interactions are indeed the main cause of complexity and even the most basic interactions between two factors are largely unpredictable from knowledge of individual effects (Mooney et al. 1991, Wootton 2002). Our study demonstrated for the first time the combined effects of elevated temperature and water deficit on grape and wine composition that may be comparable with realized and predicted climate change (Giorgi and Lionello 2008, Webb et al. 2005). This study reproduced increments in background temperature

equivalent with environments where temperature and water deficit increase in parallel during the growing season (Brunetti et al. 2000, Ramos et al. 2008).

### **Berry composition**

Both water deficit and high temperature reduced berry weight and increased the skin-to-pulp ratio, and these effects were additive. The effect of water deficit reducing pericarp volume and berry weight due to restricted cell expansion has been well documented (Bindon et al. 2011, Ojeda et al. 2002, Ojeda et al. 2001, Roby and Matthews 2004b). However, the understanding on the effect of temperature in berry growth is constrained to studies that manipulated temperature in controlled environments that do not necessarily replicate field conditions and/or applied heat during short spells at different phenological stages that do not capture the effects of warming explored in our study (Greer and Weston 2010, Kliewer 1977a, Matsui et al. 1986, Soar et al. 2009). As indicated for the analysis of berry tissues, the reduction in berry weight at harvest was due to a reduction in the weight of pulp (Table 1). Studies in growing chambers have shown that depending on the timing and the intensity of temperature treatments, smaller berries can result from a reduction in the number of pericarp cells (Kliewer 1977a) or in pericarp cell expansion (Hale et al. 1974).

Environmental conditions in 2011/12 shortened the growing season by ~ 3 to 4 weeks in comparison to 2010/11; nonetheless, TSS and TA were higher in 2011/12 by ~ 3.3 °Brix and ~ 0.92 g/L respectively (Figure 1a-d). The influence of temperature and water on berry composition was examined at a common TSS within the year. Water regimes had no substantial effects on berry TA and pH during the 2-yr study (Figure 1c-f), which is coincident with studies that indicate a degree of unresponsiveness on these traits to irrigation (Ginestar et al. 1998, Junquera et al. 2012, Reynolds et al. 2005, Sivilotti et al. 2005). However, studies are not conclusive in this regard; other authors have reported an increase in berry TA under increased water availability (Esteban et al. 1999) or a reduction in TA under early season water deficit (Intrigliolo et al. 2012, Matthews et al. 1988). Responses of juice TA and pH to elevated temperature do not agree either, between direct studies in field growing conditions and indirect comparisons from contrasting sites or time series studies. Indirect evidence suggests a reduction in TA (Barnaud et al. 2013, Jones and Davis 2000, Urhausen et al. 2011, Vrsic and Vodovnik 2012) and increase in pH with temperature (Iland et al. 2011a, Keller 2010b); in contrast, direct manipulation of temperature using top-open chambers in the field has shown a more complex interaction between temperature, season and cultivar (Sadras et al. 2013) or between temperature and light (Spayd et al. 2002) in these traits. For

example, Sadras et al. (2013) found that TA and pH for Shiraz were unresponsive to an increment in mean-maximum growing temperature of 1.4 to 1.6 °C while Barnuud et al. (2013) reported no trend for pH but a drop on TA of 0.51 g/L °C along a 700-km transect of climate gradient. The interaction between temperature and season found here compares with the results of Sadras et al. (2013) and highlights the need for a closer look at the effects of temperature on the seasonal dynamics of malate, tartrate, K<sup>+</sup> and Na<sup>+</sup> accumulation to further understand the responses of TA and pH. For example, malate accumulation in grapevines seems to have a distinctive response to temperature during berry development; while it is favoured by warm temperature pre-veraison, peaks at 20 – 25 °C and drops sharply at ~ 38 °C (Lakso et al. 1975), malate concentration and acidity at harvest seem to be more related to temperature post-veraison (Sweetman et al. 2009). This highlights the effect that short periods of high temperature may have on acid accumulation at harvest and questions the functional relevance of studies where indirect associations are made between gross temporal average temperatures, e.g. mean growing-season, and berry composition.

No interactions were found between water and temperature on the phenolic composition of berries (Table 2); therefore, the effects of water and temperature were addressed separately. Water stress was effective at reducing berry size and increasing the skin-to-pulp ratio; however, this effect was not reflected in a quantitative improvement of berry composition. No differences were found in anthocyanin content or concentration in berries under different water regimes in the current study. This contrasts with studies that show an increase in skin anthocyanin under water deficit either by a berry-size concentrating effect or by up-regulation of the biosynthetic pathway (Castellarin et al. 2007, Deluc et al. 2009, Roby et al. 2004a). Nevertheless, the relationship between water deficit and berry quality traits is not universal; the curvilinear response of anthocyanins to post-veraison water deficit found in potted Tempranillo vines, for example, showed a narrower range of water deficit that increases anthocyanin accumulation (Girona et al. 2009). Similar for Monastrell, midday stem water potential < 1.4 MPa post-veraison decreased the accumulation of flavonoids in berries, resulting in no differences in total anthocyanins between control and water deficit treatments (Romero et al. 2010). Some studies have related the accumulation of berry metabolites with the limited carbon assimilation under severe water deficit (Basile et al. 2011, Girona et al. 2009, Romero et al. 2010). The severe water stress (- 1.2 to -1.5 MPa) imposed during the time of anthocyanin biosynthesis in the current study may have induced a decrease in the net of carbon assimilation. Using biomass accumulation as an



indicator of carbon assimilation, water deficit reduced pruning weight by ~ 16% and yield by 46% whereas it did not affect starch concentration in roots and trunks (Sadras and Moran 2013); this is consistent with the proposed carbon-limitation effect on phenolic biosynthesis under severe water stress. The content and concentration of total tannins, total phenolics and skin- and seed-derived tannins were unaffected by water regimes. This is in agreement with studies that show a reduced response of tannins to irrigation treatments (Bindon et al. 2011, Bucchetti et al. 2011, Ollé et al. 2011). In our work, the onset of water deficit was likely too late in terms of berry development to affect tannin biosynthesis (Downey et al. 2003).

Many studies have dealt with the effects of temperature on phenolics (reviewed by Cohen and Kennedy 2010) but results are constrained by experimental settings and artefacts (Sadras and Soar 2009, Soar et al. 2009, Tarara et al. 2000) and are therefore not comparable with direct assessment in field conditions (Sadras and Moran 2013). We found a general reduction in the flavonoid concentration of berries under high temperature (Table 2). The reduction in berry colour with high temperature in our experiment is consistent with the observed reduction in anthocyanin concentration in response to elevated temperature reported by Tarara et al. (2008); however, results are not commensurable. The experiments tackled different aspects of warming and integrated different physiological responses; while Tarara et al. (2008) manipulated bunch temperature between veraison and harvest, our experiment comprised the integrative effect of temperature at the whole canopy level during two growing seasons and the intervening dormant period (Sadras et al. 2012b, Sadras and Moran 2013, Sadras and Moran 2013). Warming the whole canopy during the entire growing cycle, Sadras and Moran (2012c) found that differences in anthocyanin accumulation between control and heated treatments during the season were related to a shift in the onset rather than the rate of accumulation. With the snapshot at harvest, our data suggest a similar effect of temperature on anthocyanins; however, temperature-driven changes in the net accumulation of anthocyanins resulting from differences between synthesis (Mori et al. 2005b, Yamane et al. 2006a) and degradation (Mori et al. 2007) cannot be discarded. The reduction in total tannin by temperature contrasts with findings from other studies, which suggest a less consistent relationship between temperature and tannin accumulation (Cohen et al. 2012a, Cohen et al. 2012b, Goto-Yamamoto et al. 2009); however, temperature treatments do not compare between studies.

The higher skin-to-seed tannin ratio found under high temperature compared to controls, resulted from a relative higher contribution of tannins from skin and lower

contribution from seed (Table 2). Some explanations could be put forward to account for this observation, with an effect of temperature that affects tannin extractability from seed or skin by indirectly (i) modifying the number of seeds or skin weight per berry, (ii) uncoupling berry seed and skin development, or directly by (iii) affecting accumulation of tannin in seed or skin during development. No differences were found between temperature treatments on seed number or skin weight that support the first interpretation; however, effects of temperature hastening seed development in relation to the rest of the berry has been reported (Bonada et al. 2013b, Sadras et al. 2013). As seed development advances, tannins are progressively bound to cell wall components (reviewed by Hanlin et al. 2010) and are less extractable by low yielding extractants such as aqueous ethanol (Bautista-Ortín et al. 2012). This explains the differential concentration of seed- and skin-derived tannins in model wines made from grapes at different maturity (del Llaudy et al. 2008), and is consistent with the higher skin-to-seed tannin ratio found under high temperature in this study. However, this indirect effect on extractability may mask the direct effect of temperature on tannin accumulation, as extractability relates to both the ripening stage (del Llaudy et al. 2008) and the extractant used (Bautista-Ortín et al. 2012). To test whether changes in concentration of tannin resulted from changes in accumulation or were simply related to extractability, we used a high-yielding solvent (70% aqueous acetone) for polyphenol extraction. Not surprisingly, total tannin (seed + skin) extracted with 12.5 % ethanol represented ~ 32% of the total tannins present in the berry (i.e. assumed to be those extracted with acetone). Furthermore, some interactions emerged between water and temperature on seed and skin tannin (per gram of seed or skin) and on total phenolics (per berry) (Table 2) when acetone was used as extractant. This suggests that, even when they were not reflected with the aqueous ethanol extractions (which most likely did not emulate extraction conditions present during winemaking) water and temperature generated compositional differences in berries which may account for differences in wine between treatments.

### **Wine composition and its relation with berry traits**

Water and temperature driven differences in berry composition were only partially reflected in wine composition, as expected (Bindon et al. 2008, Bindon et al. 2011). Water effects were negligible on grape composition; however, spectral analyses of wines ~ 11 months after bottling revealed a number of significant interactions between water and temperature on composition that resulted in favour of control temperature-water deficit treatment (Table 3). As shown in the PCA analysis (Figure 4), control-deficit treatments were positively associated with anthocyanin, total tannin and total

phenolics in grapes, and colour density, polymeric pigments and total tannin in wines. Despite no significant differences in grape composition this higher flavonoid concentration in wines of control-deficit treatments suggests a concentration effect during winemaking driven by the higher skin-to-pulp ratio. Nevertheless, changes in the composition of anthocyanin and tannin in grapes by water deficit (Chaves et al. 2010) or during fermentation, e.g. from pyranoanthocyanin formation, that accounted for wine colour formation and stabilization cannot be discarded (Bindon et al. 2008, Bindon et al. 2011). That includes the effect of water deficit on other compounds not assessed in this study such as flavonols, which are highly dependent on berry microclimate (Spayd et al. 2002). Flavonols primarily mediate the formation of more stable compounds during the early stages of wine ageing (Boulton 2001) and contribute to the chromatic characteristics of finished wines (Schwarz et al. 2005). Owing to the reduced canopy size in water stressed vines (Dayer et al. 2013), it is possible that the responses found here could have arisen from a greater sun exposure of bunches rather than a direct effect of water deficit. In addition, the correlation between colour density and non-bleachable pigments suggests an association between anthocyanin and tannin toward the formation of stable polymeric pigments (Cheynier et al. 2006), which was also reflected in the relatively high chemical age 2 of control-deficit wines.

In accord with the enhanced flavonoid composition, control-deficit wines were chromatically and sensorially more intense than the others (Figure 4). The higher relative contribution of yellow ( $b^* > 1$ ) vs blue tones ( $b^* < 0$ ) found in all the treatments is an indicative characteristic of aged red wines (Casassa et al. 2012). Nevertheless, the lower  $L^*$  and higher  $C^*$ , coupled with the higher  $a^*$ , revealed that control-deficit wines were comparatively darker, more colourful and with more intense red tones. These overall chromatic attributes make control-deficit wines distinguishable from the remaining wines when appreciated by the human eye ( $\Delta E^* > 5$ ) (Table 4). Furthermore, these wines had more intense berry and cooked fruit flavours and greater tannin structure, in comparison with wines from the other treatments. Positive significant correlations were found between wine berry flavours and berry skin-tannin and anthocyanin, and with wine colour density, total phenolics and polymeric pigments. That suggests a relationship between skin tannins, anthocyanins, coloured wine adducts and flavour compounds localized in the skin, supporting a previous report that highlighted the link between phenolic composition, flavour intensity and wine quality (Ristic et al. 2010). As pointed out in other studies, deficit irrigation has been associated with enhancement of fruity characters (Casassa et al. 2013a, Chapman et al. 2005), and water deficit may have favoured the concentration of fruity  $C_{13}$ -norisoprenoid odorants

in wines through the interconversion of their carotenoid precursors by altering bunch microclimate (Bindon et al. 2007). Consistent with the higher phenolic composition of control-deficit wines, sensory tests detected greater tannin structure. Similarly to the work of Ristic et al. (2010), astringency-related attributes in our work (as defined by the combination of drying and dynamic sensation), were positively associated with wine colour density, total phenolics and polymeric pigments, and with berry skin-tannin. Although overall wine quality was not assessed in our study, the correlation between tannin structure and berry skin-tannin implies an improvement in quality of control-deficit wines, since tannins derived from grape skin, for example, are smoother and less bitter than those equivalently sized tannins derived from seed (Kennedy 2008). Finally, cooked fruit flavours were positively associated with wine chemical age 2 and SO<sub>2</sub> resistant pigments but negatively associated with floral aromas, suggesting a contrasting effect of wine ageing on these attributes.

Compared to control-deficit, control-irrigated treatment was mainly associated with a higher proportion of seed-derived tannin in grapes and characterised by a lower colour and flavonoid concentration in wines (Figure 4). Seed mass, seed number or its relative contribution to the final berry weight were unaffected by water or temperature regimes. Therefore, presumably, the higher extractability of tannin from seeds was more related to seed ripening (Bautista-Ortín et al. 2012, del Llaudy et al. 2008). On the bases of these studies, a higher extractability is expected with the model wine solution from unripe seeds under conditions that delay seed maturity, such as irrigation (Bonada et al. 2013b). Despite the lack of detectable differences in grape composition, control-irrigated wine had a lower phenolic concentration in comparison to its irrigated counterpart. As judged by the skin-to- pulp ratio, the higher contribution of pulp to the final wine volume may have resulted in a dilution effect during winemaking (Salón et al. 2005). Nevertheless, the relative higher contribution of seed-derived tannin may have also affected colour equilibria in the wine. For example, poor concentrations of anthocyanins and skin tannin coupled with a high concentration of seed tannin in grapes has been associated with low colour density and polymeric pigments in wine (Ristic et al. 2010).

A more consistent response was found in heated treatments. Regardless of water regime, heating reduced flavonoids in grapes, which was translated into the corresponding wines (Figure 4). Wines from heated treatments had the lowest colour density, polymeric pigment and total tannin concentration, and, as judged by the

CIELab, these differences were translated into the chromatic attributes of wine. Elevated temperature increased the proportion of skin vs seed tannin (by reducing seed tannin extractability); a higher proportion of skin-derived tannin suggests a change in the mean degree of polymerization of wine tannins, which has been related to an improvement in wine colour density and sensory properties (Ristic et al. 2010). Nevertheless, the balance between berry flavonoid compounds seems to be as important as the overall concentration; wines examined after 12 months of ageing revealed a positive correlation between skin tannin concentration in grapes and the concentration of polymeric pigments, total tannin and colour density (Ristic et al. 2010). However, this response was driven by the relative availability of other compounds, such as anthocyanins, mediating the formation of coloured adducts. In the current work, heating increased the proportion of skin tannins, but reduced the concentration of total tannins and anthocyanins; therefore, it is likely that the greater availability of skin tannin per unit of anthocyanin did not result in an increase in the formation of polymeric pigments.

## **Conclusion**

The effect of water deficit on grape and wine composition has been widely investigated. Likewise the effects of temperature have attracted attention, but indirect methods comparing seasons, regions or controlled environments preclude establishing definitive cause-and-effect relationships relevant to realistic field conditions. Interactions between thermal and water regimes are largely unknown. Indeed, interactions are the main cause of complexity and even the most basic interactions between two factors are largely unpredictable from knowledge of individual effects (Wootton 2002). We thus combined two water regimes and two thermal regimes in realistic vineyard conditions to quantify berry and wine responses. We found additive effects (i.e. lack of interaction) for berry weight and components (skin-to-berry, pulp-to-berry and skin-to-pulp ratios), fruit composition (TA and pH), grape phenolics (12.5% ethanol-extracted and most of the 70% acetone-extracted phenolics), wine phenolics (chemical age 2) and wine sensory traits (floral aromas and berry flavours). Significant, previously unrecorded interactions were found for grape phenolics (70% acetone-extracted skin- and seed-tannins and total phenolics per berry), wine phenolics (colour density, total tannins and total phenolics) and wine sensory traits (floral aromas, cooked fruit flavours and tannin structure). Our study thus anticipates the drier, hotter climate of the Barossa Valley, and provides industry with indications of shifts in wine profiles that would require technological innovations in the vineyard and winery to maintain the identity of Barossa Shiraz, the flagship of the Australian wine industry.

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**Table 1:** Berry fresh weight, number of seeds per berry and the proportion of skin, seed and pulp at harvest for 2011/12 vintage. Values are mean  $\pm$  SE and  $p$  values are from the ANOVA, accounting for effects of temperature (T), water (W) and their interaction (T  $\times$  W).

Treatment	Berry weight (g) <sup>†</sup>	Number of seeds per berry <sup>†</sup>	Proportion of skin per berry (%) <sup>†</sup>	Proportion of seeds per berry (%) <sup>†</sup>	Proportion of pulp per berry (%) <sup>†‡</sup>
Control-Deficit	1.47 $\pm$ 0.015	2.5 $\pm$ 0.070	13.2 $\pm$ 0.16	4.8 $\pm$ 0.22	81.9 $\pm$ 0.25
Control-Irrigated	1.53 $\pm$ 0.044	2.3 $\pm$ 0.140	12.3 $\pm$ 0.28	4.3 $\pm$ 0.18	83.4 $\pm$ 0.34
Heated-Deficit	1.35 $\pm$ 0.006	2.6 $\pm$ 0.064	14.3 $\pm$ 0.15	4.9 $\pm$ 0.20	80.8 $\pm$ 0.20
Heated-Irrigated	1.50 $\pm$ 0.029	2.7 $\pm$ 0.176	13.2 $\pm$ 0.61	4.7 $\pm$ 0.32	82.2 $\pm$ 0.75
<b>Source of variation (<math>p</math>)§</b>					
Temperature (T)	<b>0.0368</b>	0.1080	<b>0.0123</b>	0.4405	<b>0.0166</b>
Water (W)	<b>0.0033</b>	0.6486	<b>0.0082</b>	0.1247	<b>0.0047</b>
(T $\times$ W)	0.1995	0.1189	0.8138	0.5421	0.8892

<sup>†</sup> Average of 3 replicates, each comprising 20 berries.

<sup>‡</sup> Pulp was determined by subtraction of fresh skin and seed weight from berry weight.

<sup>§</sup> Significant  $p$  values are shown in bold ( $p < 0.05$ ).

**Table 2:** Phenolic composition of grape solids (skin and seed) at harvest for 2011/12 vintage, using two extraction conditions (see Materials and Methods for details). Values are mean  $\pm$  SE and  $p$  values are from the ANOVA, accounting for temperature (T), water (W) and their interaction (T  $\times$  W).

Extract/parameters	Control-Deficit	Control-Irrigated	Heated-Deficit	Heated-Irrigated	$p$ $\forall$		
					(T)	(W)	(T $\times$ W)
<b>Acetone</b>							
Anthocyanin (mg/g) <sup>†</sup>	8.19 $\pm$ 0.668	8.45 $\pm$ 0.532	7.01 $\pm$ 0.142	6.60 $\pm$ 0.373	<b>0.0123</b>	0.8766	0.5021
Anthocyanin (mg/berry) <sup>†</sup>	12.03 $\pm$ 0.816	12.59 $\pm$ 1.284	9.89 $\pm$ 0.478	9.85 $\pm$ 0.695	<b>0.0230</b>	0.0912	0.7349
Total tannin (mg/g) <sup>‡</sup>	3.69 $\pm$ 0.117	3.74 $\pm$ 0.132	3.23 $\pm$ 0.133	3.27 $\pm$ 0.164	<b>0.0097</b>	0.7497	0.9766
Total tannin (mg/berry) <sup>‡</sup>	5.43 $\pm$ 0.218	5.53 $\pm$ 0.108	4.54 $\pm$ 0.081	4.85 $\pm$ 0.098	<b>0.0005</b>	0.1340	0.4620
Seed tannins (mg/g seed) <sup>‡</sup>	30.14 $\pm$ 0.768	36.97 $\pm$ 1.488	25.27 $\pm$ 0.620	24.83 $\pm$ 0.827	<b>&lt; 0.0001</b>	<b>0.0117</b>	<b>0.0061</b>
Skin tannin (mg/g skin) <sup>‡</sup>	15.94 $\pm$ 0.494	19.54 $\pm$ 0.690	14.39 $\pm$ 0.275	14.50 $\pm$ 0.399	<b>0.0002</b>	<b>0.0046</b>	<b>0.0063</b>
Seed tannin (mg/g) <sup>‡</sup>	1.60 $\pm$ 0.028	1.66 $\pm$ 0.042	1.23 $\pm$ 0.062	1.27 $\pm$ 0.028	<b>&lt; 0.0001</b>	0.3103	0.8009
Seed tannin (mg/berry) <sup>‡</sup>	2.36 $\pm$ 0.092	2.22 $\pm$ 0.162	1.72 $\pm$ 0.017	1.89 $\pm$ 0.095	<b>0.0018</b>	0.8993	0.1975
Skin tannin (mg/g) <sup>‡</sup>	2.09 $\pm$ 0.116	2.23 $\pm$ 0.143	2.00 $\pm$ 0.076	2.00 $\pm$ 0.191	0.2877	0.6114	0.2442
Skin tannin (mg/berry) <sup>‡</sup>	3.07 $\pm$ 0.175	3.31 $\pm$ 0.270	2.81 $\pm$ 0.078	2.97 $\pm$ 0.193	0.1557	0.3339	0.8338
Proportion of seed-derived tannins (%)	43.46 $\pm$ 1.421	42.76 $\pm$ 1.967	38.04 $\pm$ 0.671	38.97 $\pm$ 2.697	<b>0.0371</b>	0.9529	0.6690
Proportion of skin-derived tannins (%)	56.54 $\pm$ 1.421	57.24 $\pm$ 1.967	61.96 $\pm$ 0.671	61.03 $\pm$ 2.697	<b>0.0371</b>	0.9529	0.6690
Skin tannin:seed tannin ratio <sup>§</sup>	1.30 $\pm$ 0.077	1.34 $\pm$ 0.103	1.63 $\pm$ 0.046	1.59 $\pm$ 0.188	<b>0.0404</b>	0.9875	0.7384
Total phenolics per berry (au/berry) <sup>¶</sup>	13.39 $\pm$ 0.230	15.29 $\pm$ 0.653	11.80 $\pm$ 0.472	11.80 $\pm$ 0.472	<b>0.0028</b>	0.0954	0.1836
Total phenolics per berry (au/g) <sup>¶</sup>	9.10 $\pm$ 0.211	10.30 $\pm$ 0.036	8.36 $\pm$ 0.042	8.07 $\pm$ 0.342	<b>&lt; 0.0001</b>	0.0559	<b>0.0063</b>
<b>Ethanol</b>							
Anthocyanin (mg/g) <sup>†</sup>	5.28 $\pm$ 0.556	5.06 $\pm$ 0.225	4.19 $\pm$ 0.067	4.08 $\pm$ 0.349	<b>0.0175</b>	0.6512	0.8815
Anthocyanin (mg/berry) <sup>†</sup>	7.71 $\pm$ 0.864	7.96 $\pm$ 0.274	6.13 $\pm$ 0.467	6.01 $\pm$ 0.337	<b>0.0111</b>	0.9025	0.7384
Total tannin (mg/g) <sup>‡</sup>	1.22 $\pm$ 0.112	1.12 $\pm$ 0.063	0.98 $\pm$ 0.043	1.05 $\pm$ 0.035	0.0579	0.8167	0.2500
Total tannin (mg/berry) <sup>‡</sup>	1.78 $\pm$ 0.176	1.75 $\pm$ 0.047	1.49 $\pm$ 0.092	1.55 $\pm$ 0.091	<b>0.0395</b>	0.6668	0.5030
Seed tannins (mg/g seed) <sup>‡</sup>	9.36 $\pm$ 0.388	9.39 $\pm$ 0.577	7.10 $\pm$ 0.067	7.15 $\pm$ 0.383	<b>0.0016</b>	0.9325	0.9969
Skin tannin (mg/g skin) <sup>‡</sup>	6.03 $\pm$ 0.714	5.67 $\pm$ 0.396	5.24 $\pm$ 0.090	5.41 $\pm$ 0.403	0.2860	0.8307	0.5814
Seed tannin (mg/g) <sup>‡</sup>	0.41 $\pm$ 0.024	0.43 $\pm$ 0.022	0.26 $\pm$ 0.011	0.32 $\pm$ 0.045	<b>0.0043</b>	0.2902	0.5695
Seed tannin (mg/berry) <sup>‡</sup>	0.60 $\pm$ 0.037	0.68 $\pm$ 0.037	0.45 $\pm$ 0.017	0.47 $\pm$ 0.077	<b>0.0117</b>	0.3703	0.6392
Skin tannin (mg/g) <sup>‡</sup>	0.81 $\pm$ 0.100	0.69 $\pm$ 0.072	0.72 $\pm$ 0.031	0.73 $\pm$ 0.010	0.7511	0.4097	0.3242
Skin tannin (mg/berry) <sup>‡</sup>	1.18 $\pm$ 0.154	1.07 $\pm$ 0.084	1.05 $\pm$ 0.030	1.08 $\pm$ 0.021	0.5112	0.7095	0.4524
Proportion of seed-derived tannins (%)	34.07 $\pm$ 2.722	38.77 $\pm$ 3.176	28.90 $\pm$ 0.551	29.86 $\pm$ 3.437	<b>0.0322</b>	0.3298	0.5107
Proportion of skin-derived tannins (%)	65.93 $\pm$ 2.722	61.23 $\pm$ 3.176	71.10 $\pm$ 0.551	70.14 $\pm$ 0.344	<b>0.0322</b>	0.3298	0.5107
Skin tannin:seed tannin ratio <sup>§</sup>	1.97 $\pm$ 0.220	1.61 $\pm$ 0.218	2.46 $\pm$ 0.065	2.45 $\pm$ 0.449	<b>0.0420</b>	0.5284	0.5441
Total phenolics per berry (au/berry) <sup>¶</sup>	7.68 $\pm$ 0.428	7.92 $\pm$ 0.061	6.12 $\pm$ 0.765	6.10 $\pm$ 0.155	<b>0.0053</b>	0.8101	0.7917
Total phenolics per berry (au/g) <sup>¶</sup>	5.27 $\pm$ 0.264	5.04 $\pm$ 0.164	4.15 $\pm$ 0.227	4.13 $\pm$ 0.221	<b>0.0018</b>	0.3200	0.6401

<sup>†</sup> Anthocyanin determined colorimetrically at 520 nm (Ilанд et al. 2004), expressed as malvidin-3-glucoside units.

<sup>‡</sup> Tannin determined by the MCP tannin assay (Mercurio and Smith 2006), expressed as mg of epicatechin units per gram of seed/skin, per gram of fresh mass or per berry.

<sup>§</sup> Determined as the ratio between skin tannin:seed tannin in mg/g.

<sup>¶</sup> Determined from absorbance at 280 nm (Ilанд et al. 2004), expressed in absorbance units (au) per berry or per gram of berry fresh mass.

$\forall$  Significant  $p$  values are shown in bold ( $p < 0.05$ ).



**Table 3:** Anthocyanin equilibria and phenolic composition of Shiraz wines after ~ 11 months bottle ageing. Values are mean  $\pm$  SE and  $p$  values are from the ANOVA, accounting for temperature (T), water (W) and their interaction (T  $\times$  W) during the 2011/12 vintage.

Parameters	Control-Deficit	Control-Irrigated	Heated-Deficit	Heated-Irrigated	$p$ §		
					(T)	(W)	(T $\times$ W)
Total anthocyanins (mg/L)†	363.49 $\pm$ 36.689	325.99 $\pm$ 19.358	310.40 $\pm$ 4.752	310.08 $\pm$ 34.360	0.2166	0.4924	0.4996
Total tannins (mg/L)‡	1009.94 $\pm$ 63.616	681.68 $\pm$ 50.842	710.18 $\pm$ 61.562	658.58 $\pm$ 12.112	<b>0.0051</b>	<b>0.0014</b>	<b>0.0140</b>
Total phenolics (au, 10mm)†	45.84 $\pm$ 1.417	37.16 $\pm$ 0.915	39.31 $\pm$ 0.471	37.28 $\pm$ 0.878	<b>0.035</b>	<b>0.0012</b>	<b>0.0294</b>
Colour density (au)†	9.92 $\pm$ 0.311	7.02 $\pm$ 0.018	7.32 $\pm$ 0.452	6.78 $\pm$ 0.165	<b>0.0001</b>	<b>&lt; 0.0001</b>	<b>0.0008</b>
Colour density SO <sub>2</sub> corrected (au)†	11.07 $\pm$ 0.644	8.22 $\pm$ 0.358	8.47 $\pm$ 0.299	7.94 $\pm$ 0.400	<b>0.0042</b>	<b>0.0011</b>	<b>0.0169</b>
SO <sub>2</sub> resistant pigments (au)†	2.60 $\pm$ 0.096	1.73 $\pm$ 0.063	1.83 $\pm$ 0.120	1.66 $\pm$ 0.027	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.0005</b>
Chemical age 2 (au)†	0.12 $\pm$ 0.006	0.09 $\pm$ 0.005	0.10 $\pm$ 0.006	0.09 $\pm$ 0.008	0.2137	<b>0.0201</b>	0.0771
Hue †	0.70 $\pm$ 0.004	0.70 $\pm$ 0.006	0.73 $\pm$ 0.016	0.70 $\pm$ 0.007	0.1172	0.1299	0.1093
Chemical age 1 (au)†	0.39 $\pm$ 0.011	0.35 $\pm$ 0.012	0.36 $\pm$ 0.012	0.35 $\pm$ 0.019	0.2787	0.0604	0.2630
Degree of ionisation of anthocyanins (%)†	18.38 $\pm$ 1.182	14.83 $\pm$ 0.465	15.54 $\pm$ 1.240	15.78 $\pm$ 1.473	0.4202	0.1663	0.1154

au = absorbance units.

† Determined by the modified Somers assay (Mercurio et al. 2007).

‡ Tannin determined by the MCP tannin assay (Mercurio and Smith 2006), expressed as mg of epicatechin units per liter.

§ Significant  $p$  values are shown in bold ( $p < 0.05$ ).

**Table 4:** Double entry table showing the CIELab colour difference ( $\Delta E^*$ ) in Shiraz wines from a factorial ( $2^2$ ) temperature by water experiment in the Barossa Valley. Values are mean of three replicates in CIELab units. Numbers in bold indicate a  $\Delta E^* > 5$ , being the perceptible visual difference for a panellist evaluating the wine under CIELab standard conditions ( $10^\circ$  standard observer and illuminant D65).

Treatment		Control		Heated	
Temperature	Water	Irrigated	Deficit	Irrigated	Deficit
Control	Irrigated		<b>11.12</b>	1.61	1.02
	Deficit			<b>12.69</b>	<b>11.22</b>
Heated	Irrigated				1.88
	Deficit				

**Table 5:** Effect of temperature, water regime and their interaction on Shiraz wines sensory traits during two vintages in the Barossa Valley. *p* values are from the ANOVA and Friedman's test (df = 3).

Vintage	Descriptor	<i>p</i> (ANOVA)			Friedman	
		Water	Temperature	Interaction	F	<i>p</i>
2010/11	Berry flavours	0.0953	0.1247	0.3157	4.78	0.1889
	Floral aromas	0.6877	<b>0.0331</b> ‡	0.2287	4.89	0.1813
	Cooked fruit flavours	0.7384	0.6402	<b>0.0286</b>	3.93	0.2695
	Tannin structure†	0.1820	0.5038	0.0623	4.58	0.2052
2011/12	Berry flavours	<b>0.0102</b>	0.0654	0.3206	9.13	<b>0.0276</b>
	Floral aromas	0.5955	0.6796	<b>0.0002</b>	10.62	<b>0.0140</b>
	Cooked fruit flavours	<b>0.0094</b>	<b>0.0181</b>	<b>0.0132</b>	13.66	<b>0.0032</b>
	Tannin structure	0.0607	<b>0.0186</b>	<b>0.0149</b>	11.75	<b>0.0083</b>

† Defined by the combination of 'drying' (feeling of lack of lubrication or desiccation in the mouth) and 'dynamic' (sensation involving some form of mouth movement) (Gawel et al. 2000).

‡ Significant *p* values are shown in bold (*p* < 0.05).

**Supplementary Table 1:** Basic chemical analysis of Shiraz wines after ~ 11 months bottle ageing. Data are mean  $\pm$  SE and  $p$  values are from the ANOVA, accounting for temperature (T), water (W) and their interaction (T  $\times$  W) during the 2011/12 vintage.

<b>Treatment</b>	<b>Titrateable acidity (g/L)</b>	<b>pH</b>	<b>Ethanol (% v/v)</b>	<b>Acetic acid (g/L)</b>	<b>Free SO<sub>2</sub> (mg/L)</b>
Control-Deficit	7.34 $\pm$ 0.056	3.77 $\pm$ 0.040	14.76 $\pm$ 0.167	0.55 $\pm$ 0.034	26.49 $\pm$ 5.800
Control-Irrigated	7.39 $\pm$ 0.201	3.74 $\pm$ 0.034	14.41 $\pm$ 0.068	0.55 $\pm$ 0.023	24.18 $\pm$ 1.968
Heated-Deficit	6.81 $\pm$ 0.115	3.79 $\pm$ 0.048	14.68 $\pm$ 0.490	0.54 $\pm$ 0.026	27.56 $\pm$ 4.315
Heated-Irrigated	7.00 $\pm$ 0.142	3.75 $\pm$ 0.054	14.40 $\pm$ 0.326	0.54 $\pm$ 0.031	29.16 $\pm$ 6.126
<b>Source of variation (<math>p</math>)†</b>					
Temperature (T)	<b>0.0107</b>	0.8288	0.8958	0.7857	0.7138
Water (W)	0.4166	0.4778	0.3366	0.9186	0.9654
(T $\times$ W)	0.6271	0.9425	0.9082	0.9728	0.8120

† Significant  $p$  values are shown in bold ( $p < 0.05$ ).

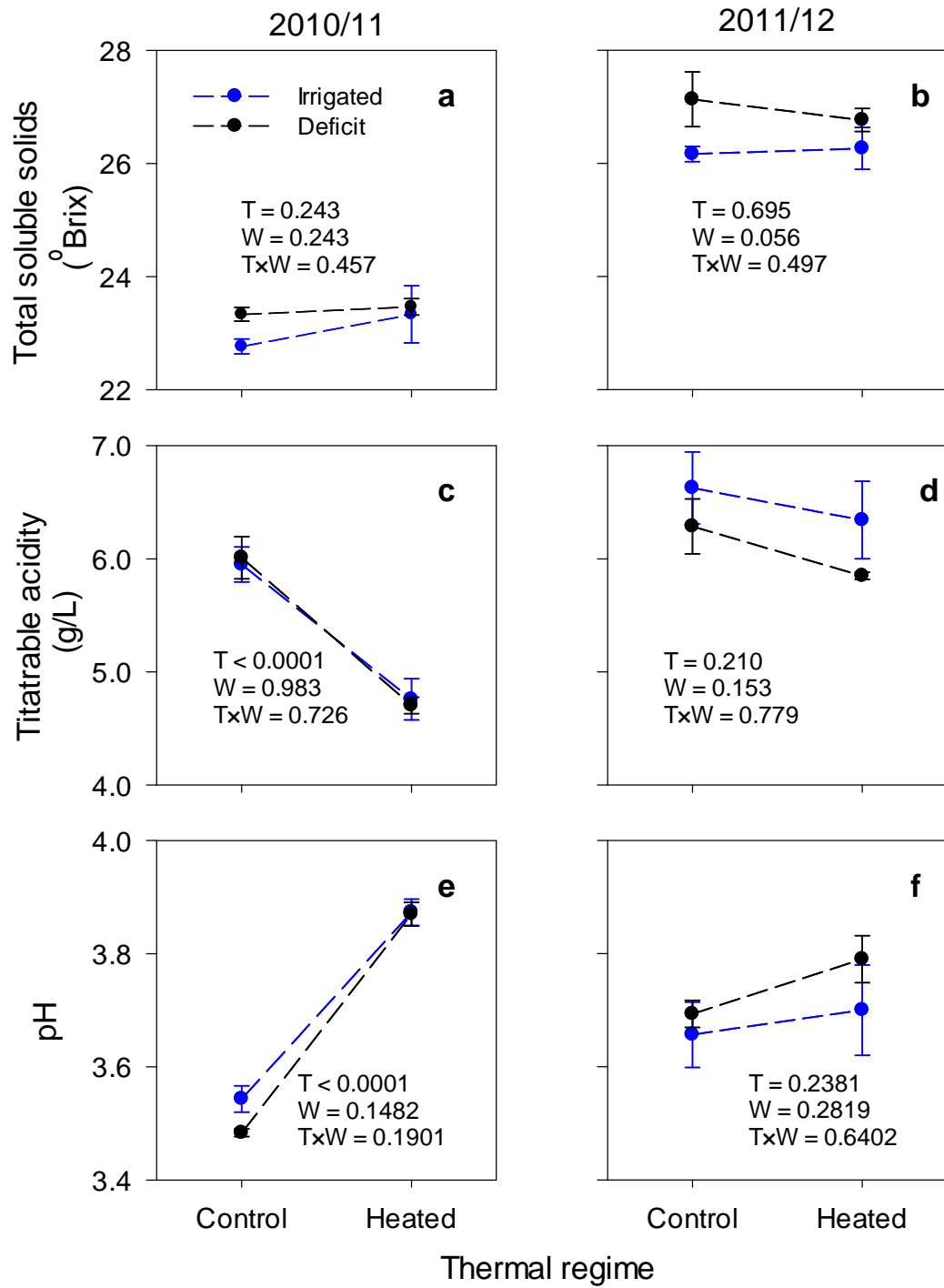
## Caption to figures

**Figure 1:** (a, b) Total soluble solids (TSS), (c, d) titratable acidity (TA) and (e, f) pH of grape juice at harvest for Shiraz berries grown in a  $2^2$  factorial experiment combining two temperature (control and heated) and two water regimes (deficit and irrigated). Data are from two consecutive seasons in the Barossa Valley. Values are mean  $\pm$  SE from three repetitions. Insets in figures represent  $p$  values from the ANOVA accounting for single and combined water and temperature effects.

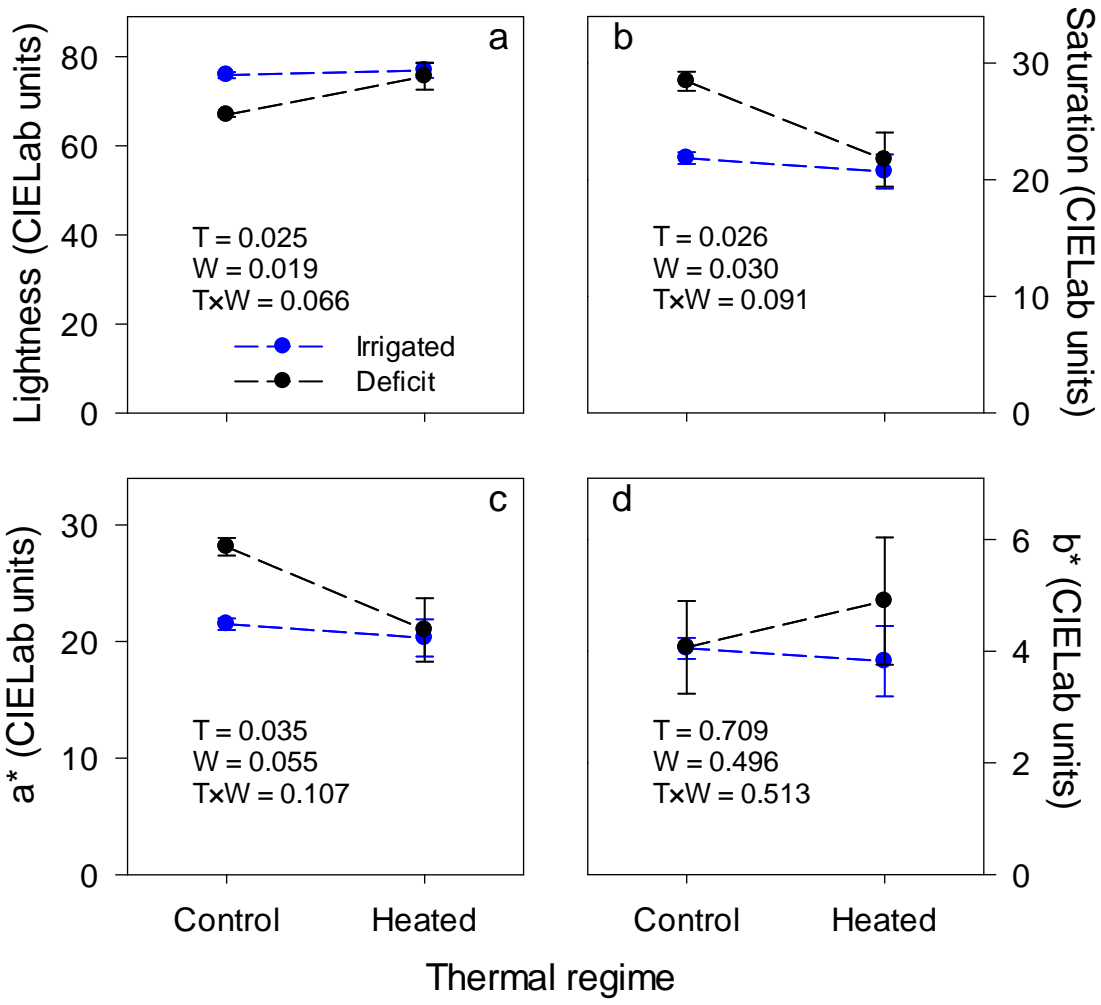
**Figure 2:** Effects of temperature (T) water (W) and their interaction ( $T \times W$ ) on the CIELab colour parameters of Shiraz wines after  $\sim$  11 months bottle ageing: (a) lightness, (b) saturation, (c)  $a^*$  (red/green chromaticity) and (d)  $b^*$  (yellow/blue chromaticity). Values are mean  $\pm$  SE from three repetitions. Insets in figures represent  $p$  values from the ANOVA accounting for single and combined water and temperature effects. Data are from 2011/12.

**Figure 3:** Sensory attributes of wines from Shiraz grapes grown under two temperature (control and heated) and two water regimes (deficit and irrigated). Values are mean  $\pm$  SE for two consecutive seasons in the Barossa Valley.

**Figure 4:** Principal component analysis of significant Shiraz grape (black font) and wine (red font) compositional and sensory (blue font) data during the 2011/12 vintage. Data of grapes used in the analysis correspond to phenolics extracted with hydroethanolic solution (12.5% v/v). The factorial combination of treatments is identified by temperature (C, control; H, heated) and water regime (D, deficit; I, irrigated).



**Figure 1**



**Figure 2**

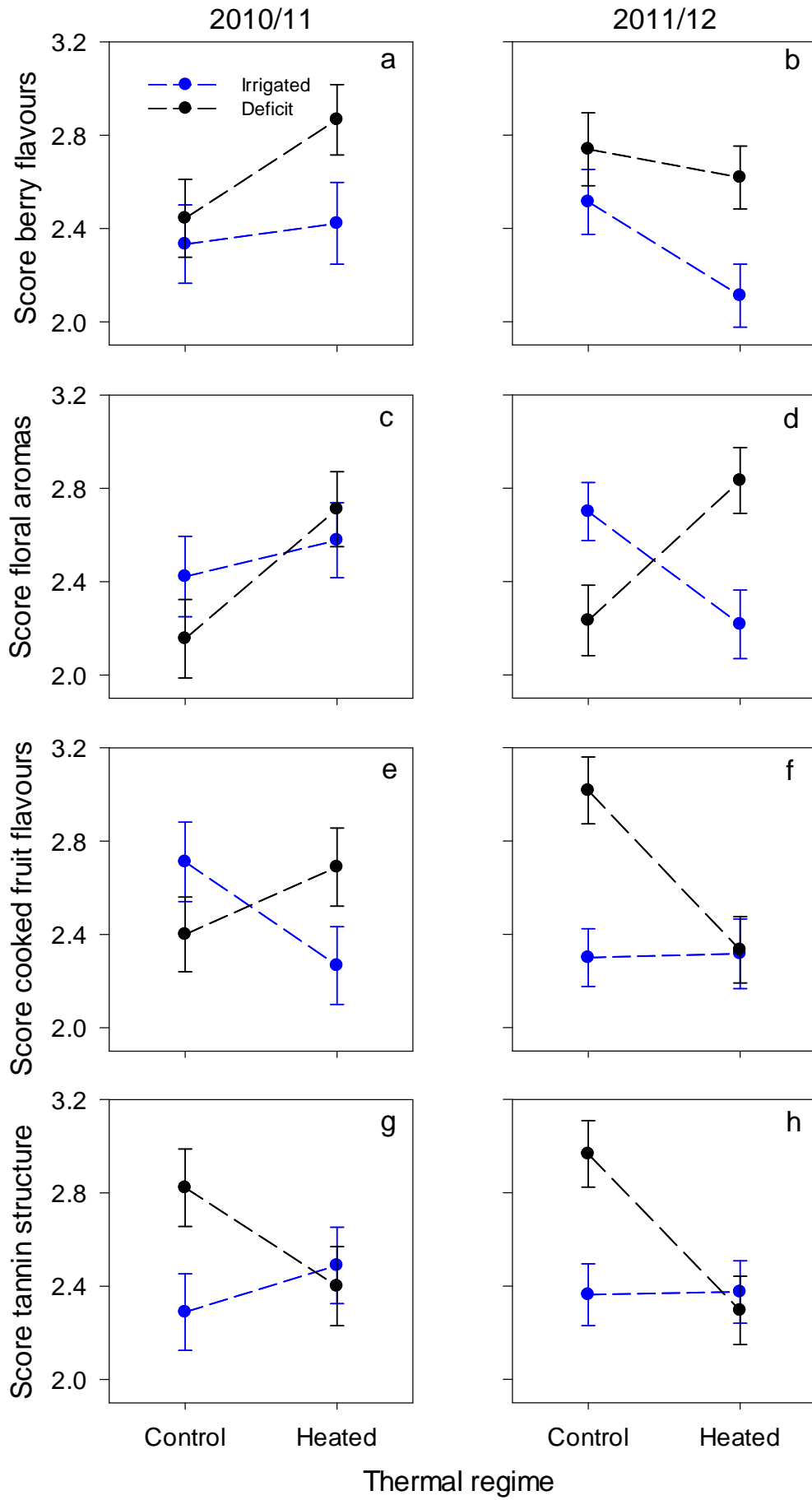
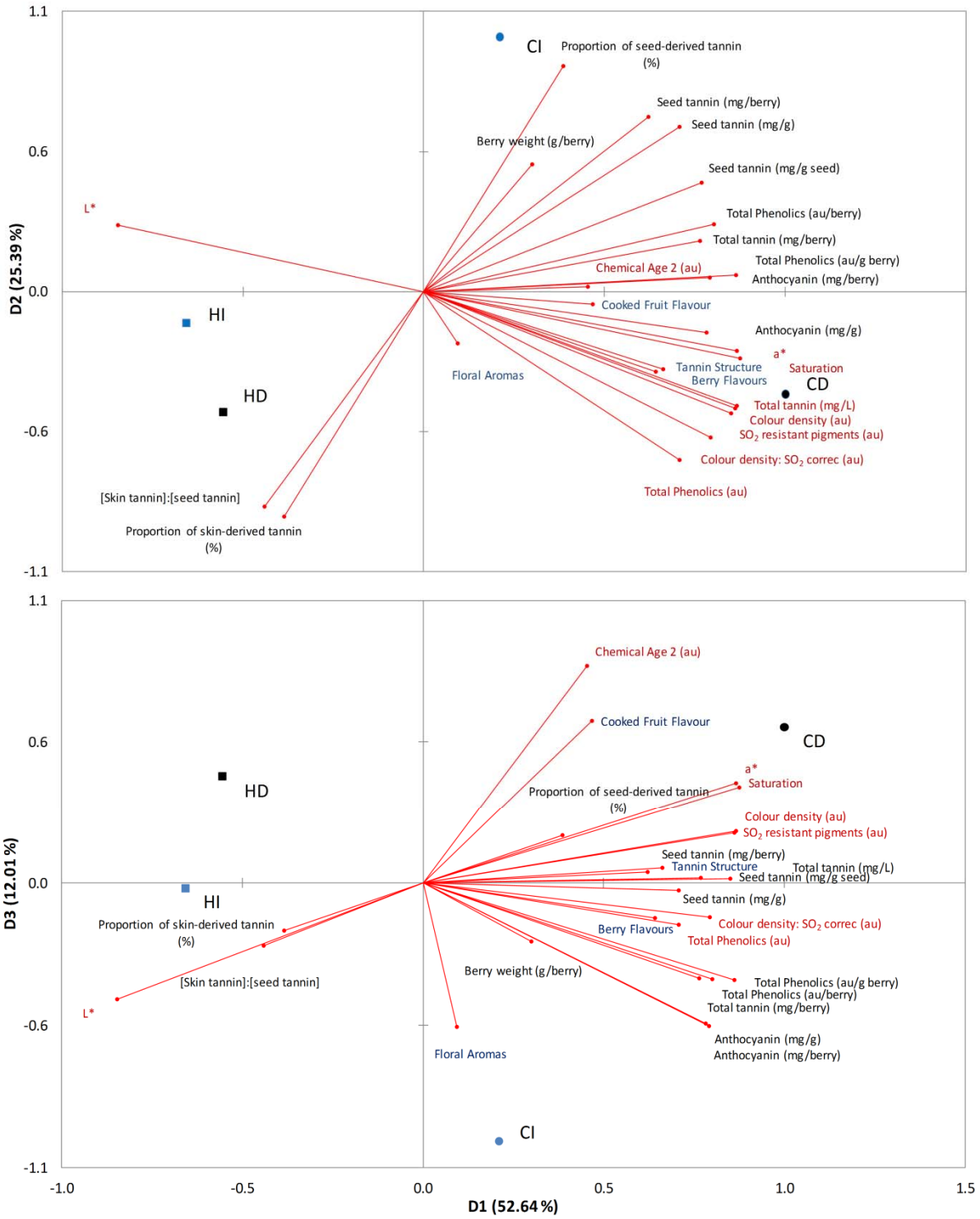


Figure 3





**Figure 4**

## **Chapter 6**

### **Conclusions and Future Research**

## Conclusions and Future Research

Against the backdrop of projected warmer and drier futures in grape growing regions of Australia, this thesis achieved four goals related to the objectives outlined in Chapter 1. First, it critically assessed methods used to investigate thermal effects in viticulture. Drawbacks were identified for indirect methods, which rely on natural gradients of temperature from variations in space or time, and for direct methods, where temperature is experimentally manipulated (Chapter 2). Indirect and direct methods are of course non-mutually exclusive, but complementary; research under more realistic field conditions using chamber-free and open-top chambers confirmed some well-established patterns from indirect studies or studies in controlled environments, but also challenged some text-book patterns. Second, the thesis demonstrated cultivar-specific patterns of MCD and BS in response to elevated temperature (Chapter 3). Chardonnay and Shiraz were selected for comparison based on contrasting berry physiology (Chapter 1) and their relevance as these cultivars jointly account for ~ 45% (710,000 tonnes) of the wine grapes produced in Australia (ABS 2012). Cultivar differences in MCD and BS under two thermal regimes were found for the first time. Third, the thesis evaluated the single and combined effects of temperature and water regime on the dynamics of MCD and BS in Shiraz (Chapter 4). Fourth, the thesis identified changes in berry biophysical traits, as driven by thermal and water regime, and their implications for Shiraz berry and wine chemical and sensorial composition (Chapters 4 and 5). This is the first study unequivocally addressing the interaction of water and temperature under realistic field conditions and its implications for berry physiology and wine attributes.

Historically, the effects of temperature on berries and wines have been studied with indirect methods or direct methods in controlled conditions. Indirect methods include comparisons of thermally contrasting locations and vintages; this approach is useful but is bound to be inconclusive as temperature effects are often confounded with other weather and climate factors (solar radiation, vapour pressure deficit, and rainfall), management practices and soils. With global warming affecting many wine regions worldwide, vintage-to-vintage comparisons have extended as to include time series analyses. Other indirect methods include comparisons of fruit grown under different solar radiation exposure, as affected by latitude, elevation, row orientation or experimental manipulation affecting both radiation and temperature. Direct experimental methods comparing fruit grown at different temperatures are required to prove cause-and-effect, but attempts to modify the thermal regime of the plant often generate secondary effects. Experimental artefacts in controlled environments often include small soil volume, lack of wind and altered radiation regimes, with direct

implications for plant physiology and berry composition. Experiments involving controlled temperature in vineyards aim at a higher degree of realism, but are constrained by time- and space-scale issues and cost, and are not necessarily free from artefacts.

Previous studies on cell death have been mostly qualitative (Krasnow et al. 2008, Tilbrook and Tyerman 2008). Here for the first time, mathematical models with biologically meaningful parameters were used to advance on the quantitative nature of cell death (Chapter 3). A bilinear model fitted to the progression of living tissue was used to objectively define three relevant parameters: the onset of rapid cell death, and the rates of cell death before and after the onset. Comparisons of the dynamics of MCD were based on both chronological and thermal scales to unequivocally separate the effects of temperature (Sadras and Moran 2013). Elevated temperatures and water deficit showed a significant effect on berry biophysical traits; both increased the proportion of MCD and BS at harvest (Chapters 3 and 4). MCD response to temperature was primarily explained by the advance in onset, while the effect of water deficit was associated with the increment in the rate of MCD after the onset.

The cultivar-dependent association between MCD and BS is a well-accepted concept (Fuentes et al. 2010, Tilbrook and Tyerman 2008); the differential response between Chardonnay and Shiraz found in this study reaffirms this idea (Chapter 3). Elevated temperature accelerated MCD and BS in Shiraz, and increased MCD but had not impact on BS in Chardonnay, which demonstrates that MCD is necessary but not sufficient to explain BS. However, if the link between MCD and BS is functional, as suspected for Shiraz (Tilbrook and Tyerman 2009), management practices that uncouple these processes during berry ripening may help to reduce the severity of BS in conditions that increase MCD, such as warmer and drier environments. The delay in the onset of net water loss observed in irrigated treatments compared to water deficit under high temperature supports this hypothesis (Chapter 4), and provides some evidence in the use of irrigation management as a palliative practice for BS. Even when rapid MCD has started, lower fluctuations may be expected in the xylem tension by transpiring leaves, and lower backflow and BS, if a favourable plant water status is maintained in a period of high atmospheric water demand. A new line of research is expected that disentangles the underlying nature of the relation between MCD and BS, and that reveals the signalling pathway involved in the onset of MCD in grapevines. The role of lipoxygenase (LOX) enzymes triggering the deterioration of membranes during

senescence of normally developing organs or as response to stress (Bhattacharjee 2005, Maccarrone et al. 2001) suggests some possible research directions. Gene expression studies of LOX candidate genes that encode this enzymatic pathway may add conclusive evidence in this unexplored area. Studies may expand to other signalling molecules such as jasmonates or reactive oxygen species widely studied in model species due to their role in programmed cell death (Mittler et al. 2004, Zhang et al. 2008).

Regulated deficit irrigation is widely adopted practice to modulate grape composition; however, the results presented here (Chapters 4 and 5) indicate that the effects of water deficit leading to colourful, flavoursome and phenolic-rich grapes and wines may not hold under high temperature. Sensory traits typical of ripening berries were associated with higher MCD (Chapter 4), which validates the practice of letting grapes hang on the plant beyond targeted sugar content, waiting for the development of flavours and aromas. However, warming and water deficit hastened ripening and altered the balance of berry traits in pulp, skin, seeds and whole-berry. This finding conclusively demonstrated that the positive association between berry aromas and flavours and MCD may not be held when temperature drives cell death in water stressed berries. Significant, previously unrecorded interactions were found between temperature and water for sensory and compositional data of wines that further supported this conclusion; water deficit only increased phenolics in wines under control temperature (Chapter 5). Overall, these findings highlight the dominant effect of temperature driving grape and wine composition, and provide industry with indications of shifts in wine profiles that would require technological innovations in the vineyard and winery to maintain the identity of Barossa Shiraz, the flagship of the Australian wine industry.

Adaptation and mitigation strategies in a context of risk management are needed that restore the balance between quality traits. While long-term strategies may require development of new cultivars and land allocation changes, short-term palliative measures mostly comprise changes in crop management practices (Fraga et al. 2013). Effective practices should be pointed to reduce the sugar concentrating effect of BS leading to high alcohol wines, and to restore the anthocyanin:sugar ratio (Sadras and Moran 2012c) and the equilibrium between sensory attributes, disrupted by elevated temperature (Chapter 4). Three approaches are identified that may help to counteract thermal effects: (i) reducing energy load in the canopy, directly, by shading nets, sprinklers or sunscreens, or, indirectly, by varying row orientation and/or trellis systems; (ii) irrigation management that exploits the evaporative cooling capacity of the canopy during ripening and that maintain vine water status during periods of high

evaporative demand; or (iii) shifting vine development by chemical applications, e.g. auxins, (Böttcher et al. 2011) or pruning practices that move ripening to more favourable cooler conditions (Favero et al. 2011, Friend et al. 2007). On a case-by-case study, trade-offs may need to be evaluated on further factorial field experiments combining thermal regimes with management practices.

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