Heat wave mitigation strategies for wine grape production and measures of the impact of heat on berry ripening and wine composition

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Abstract

Loss of berry mass, commonly referred to as berry shrivel, can occur in some cultivars late in ripening. This phenomena is particularly common in Shiraz where it has been linked to a decrease in berry cell vitality (*cell death*) at high sugar concentrations. Moreover, both cell death and loss of mass can be triggered by high temperatures and water stress during berry ripening. Since the mass lost is predominantly water, sugars are concentrated resulting in an increase in the level of alcohol in wine. This increase in alcohol content has been especially evident in red wines in Australia during the last three decades. Global warming evidence and forecasted more intense and longer heatwaves requires that mitigation strategies be investigated that can reduce the high temperature impacts on berry development and in particular loss of berry mass and cell death.

The aims of this research were: (i) develop a more practical and objective technique to detect berry cell death progression based on impedance spectroscopy; (ii) understand grapevine physiology and the ripening process in shaded conditions and to assess various shading options to mitigate heatwaves; (iii) test the use of micro-sprinkler cooling to mitigate heatwaves; (iv) assess if wrapping arms for cordon establishment compromises the vasculature of the cordon, which would represent a progressive stress potentially compounding the effects of heatwave events.

Impedance spectroscopy measurements revealed that berry cell death could be described as a function of berry electrical impedance. The electrical impedance over a range of stimulus frequencies was measured through berry development on Shiraz berries from two locations. From veraison to the onset of cell death, berry impedance was proportional to sugar concentration measured as total soluble solids. Thereafter, impedance decreased in proportion to the level of berry cell death. Changes in berry

cell death were objectively determined by impedance spectroscopy, two models were developed with a high determination coefficient (r^2 >0.83).

Various shade configurations on Shiraz vines at two sites and 3 seasons using a 60% attenuation indicated that an overhead shade system was the most effective. Overhead shade applied from veraison to harvest resulted in maximum temperatures within the canopy being reduced by about 2 °C compared to control vines when temperatures were above 40 °C. This resulted in a 6% decrease in thermal time accumulated and a reduction of around 15 % in the maximum daily VPD. Despite this small difference, shaded vines had a more functional canopy indicated by higher leaf chlorophyll content and photosynthetic rate at saturating light. Lower ψ indicated a different water balance compared to control vines. Berries from shaded vines accumulated sugars at a lower rate, had higher water content and higher total acidity than controls. Berry mass loss under shade was delayed, occurred at a lower rate and berry cell vitality in ripened berries was higher in overhead shade compared to control. The wines corroborated the above trend showing a decrease in the alcohol level at two harvest times, but no difference in anthocyanin, tannins or polyphenols.

A microspray system was installed inside Cabernet Sauvignon canopies and adjusted to activate cyclically for 20 seconds every 10 minutes during berry ripening if the day time temperature was forecast to be higher than 40° C. The mist was able to decrease the air maximum temperature by ~3° C and leaf temperatures were reduced from 4° C in dry leaves to 14° C in wetted areas surrounding the microspray units. Fresh berry mass increased in misted vines, but berry sugar concentration was not altered resulting in higher sugar per berry. The efficiency of cooling was examined relative to the theoretical maximum using certain assumptions, and a suggested index of water

use efficiency calculated that could be compared between studies, and to assist grape growers to achieve optimum cooling for minimal water use.

The hypothesis that wrapping arms on to a cordon wire represents a continuous and progressive stress for the arm vasculature, thus affecting the normal flow of water and nutrients, was tested in a commercial Shiraz block at Barossa. Although, these are preliminary results from just one season, they confirm the hypothesis. Pruning mass was 20 % lower in wrapped vines compared to non-wrapped vines. The arm transversal area decreased more in wrapped vines from the proximal part of the cordon near the trunk to the distal portion. Berry assessment at harvest suggested that non-wrapped vines were exposed to less stress. Since this stress is potentially related to restriction of cordon vasculature, it might compromise the ability of these wrapped vines to deal with heatwaves and water stress. This becomes especially important from a global warming perspective and matches the significant increment in dead arm incidence across Australia.

In summary, in this thesis a robust model to objectively predict berry cell death in Shiraz is presented. This provides a new tool for berry quality assessment. Overhead shade applied from veriason to harvest was effective in heat protection, leading to berries with less sugar, more water and more cell vitality. The vine physiology revealed vines under less stress. Microspray cooling inside the canopy was efficient in protecting the vines from heatwaves using a small volume of water. The wrapping treatment represents an important contribution to the wine industry. It provides a physiological basis to amend this traditional management, which in a global warming context might be best avoided.

Thesis declaration

This work contains no material which has been accepted for award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The Author acknowledges that copyright of the published works contained within this thesis (as listed below) resides with the copyright holders of those works. I also give permission for the digital version of my thesis to be made available on the Web, via the University's digital research repository, the library catalogue, The Australian Digital Thesis Program (ADTP) and also through wed search engines, unless permission has been granted by the University to restrict access for a period of time.

Professors Steve Tyerman and Cassandra Collins provided expert and technical advice when required, and editorial advice on drafts of papers and thesis.

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Related Publications Arising During Candidature

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Caravia L, Collins C, Shepherd J and Tyerman SD (2015) Wrapping arms for cordon establishment could be a stressful practice for grapevine. Wine and Viticulture Journal, Vol. 30, No. 6, Nov/Dec 2015: 46-50.

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Dedication

To my father Luciano who could not see this achievement, but he has been an inspiration for me to go beyond my limitations. To my grandma Betty and my mother Sonia who have inspired me with their life and for their unconditional love.

Chapter 1

Introduction and Literature Review

1.1 Overview

The effects of global warming have been very obvious to the wine industry with earlier ripening dates, increased sugar concentration in grape berries, and increased incidence of berry shrinkage (shrivel) and loss of cell vitality in the berry being associated with warming. The shrinkage decreases the yield as a consequence of water loss, concentrating sugar and contributing to increased alcohol in red wine. This study expected to develop an easier evaluation of cell vitality in berries to assist viticultural decisions. It also sought to understand and evaluate causes of berry shrivel and to explore techniques to mitigate heat stress on the level of this phenomenon and berry cell death. During the study, observations in vineyards indicated that wrapping arms for cordon establishment may compound heat and water stress. Therefore a trial was established to test the hypothesis that wrapping arms on a cordon wire may cause water stress responses, devigoration of distal shoots, and reduced carbohydrate storage.

1.2 Introduction

The effect of climate change on the Australian wine Industry has become more evident in recent decades and there have been consistent changes in vine performance and grape quality. Most significantly, predictions about future weather indicate a warmer trend around the world. Furthermore, Australian Bureau of Meteorology and the Commonwealth Scientific and Industrial Research Organisation (BOM and CSIRO, 2014) estimated that by 2030 there will be a rise in the average temperature in Australia between 0.6 to 1.5° C regardless of further greenhouse gas emissions. From this perspective, the physiology and phenology of grapevines, which is closely

related to temperature, may change, leading to potential changes in wine style (Webb et al., 2012).

There has been an increase in studies concerning berry shrivel since McCarthy (1997) provided the first concrete data on this phenomenon. Although, shrivel results in a decrease in yield, it has been seen that late shrivelled berries have reached normal and desirable composition for wine making, since substantial changes in berry composition have not been observed in shrivelled berries, other than an increase in sugar concentration (Fang et al., 2011). These authors suggest that, some level of shrinkage could be beneficial to wine quality in terms of increasing concentration of flavour compounds, and improving phenolic profile. However, the main direct effect of this phenomenon is a high concentration of sugar, which would be an important contributing factor to the high level of alcohol trends observed in red wines (Petrie and Sadras, 2008). Causes of berry mass loss (shrivel) seem to be linked to both berry (Tilbrook and Tyerman, 2009, Scharwies, 2013) and vine (Bonada et al., 2013a, Bonada et al., 2013b) water balance under a high evaporative demand and to the phenomenon of cell death in the mesocarp of the berry (Krasnow et al., 2008, Krasnow et al., 2009, Clarke et al., 2010, Fuentes et al., 2010, Bondada and Keller, 2012, Bonada et al., 2013a, Bonada et al., 2013b). Confirmation of these links would have important effects in developing mitigation strategies.

Another aspect related to climate warming is related to vine physiology. During field work the author had the chance to realise some basic differences in vineyard management in Australia compared to Chile. Wrapping canes on to a wire to establish a cordon appeared to be an antagonistic management strategy given that this could restrict water flow to the distal shoots on an established cordon. Such a water restriction if it occurs would add additional stress to vines under water and heat stress.

Therefore, an experimental program testing the hypothesis that wrapping canes on a wire could restrict vascular flow was incorporated during the last year of this study.

1.3 Literature review

1.3.1 Warmer weather conditions in Australia

The Australian annual mean temperature has risen in the last century by 0.9 ° C, being a marked trend since 1950 (BOM and CSIRO, 2014). Analogously, the warmer weather trend would appear to be verified by records showing an increase in the minimum and average temperature in Australia during the last century by 0.96 and 0.76 °C respectively (Sadras et al., 2010). Similarly, Sadras and Petrie (2011) confirm this trend through the annual average temperature records during wine grape growing seasons between 1996 and 2008 in three climatic wine regions of South Australia (Coonawarra, Barossa and Riverland). A clear increase in the maximum temperature over the period could be seen in this study. As a result, vine performance has apparently been modified in terms of the length and the inception of some of the phenological stages.

Estimations of impact of climate warming are profuse worldwide and there is general agreement that wine regions in Australia will be seriously affected. The predictions of the BOM and CSIRO (2014), regarding the foreseen increase in the average temperature in Australia by 2070 ranges from 1.0 to 5.0 °C depending on the gas emission scenarios, both extreme predictions have their own range built into them according to the location. Moreover, most of the current wine regions in Australia would be affected, requiring that the wine regions shift to higher latitudes to maintain the climatic variables suitable for the currently grown cultivars (Hall and Jones, 2009, Webb et al., 2013). The Commonwealth Scientific and Industrial Research

Organisation, CSIRO (2007) report indicated that this increase is expected to be strong in southern Australia, with a marked increase in the number of days with temperatures over 35 °C. There is also expected to be an increase in the number of warm nights between 15 to 50 % by the end of the century. This would put the Australian wine industry in a dangerous situation with many regions unsuitable to properly grow wine grapes. In addition, wine quality is highly dependent on temperature.

There will be both changes in the range of wine cultivars suitable of being properly cultivated into the current wine regions and in the boundaries of the current wine regions as a consequence of an increase in the average temperature (Schultz, 2000, Webb et al., 2013). In addition, in the period 1971-2000 three of the 61 recognised wine region in Australia exceeded an average temperature during the growing period of 21°C, which is accepted as the highest optimum limit of temperature in which it is possible to obtain a quality wine (Hall and Jones, 2009). By 2070 it is expected that 21 of the in Australian wine regions will exceed this temperature. As a consequence, imminent damage to wine quality appears probable. In this regard, it has been suggested that potentially the increment in phenolic compound that deficit irrigation cause in Shiraz, could be absent if the ripening occurs at 1.5 °C above normal temperature, which could led to wines with different sensory properties (Bonada et al., 2015).

1.3.2 Changes in vine performance

Grapevine phenological stages are occurring earlier in the seasons as a result of warmer weather (Webb et al., 2007b). This would mean vines reach their thermal requirements sooner and growing seasons are therefore shorter (Duchêne et al., 2010,

Mira de Orduña, 2010). From this perspective, predictions regarding an increase in average annual temperature would lead to ripening in some areas occurring 30 days earlier, moving this process to a warmer period of the year (Webb et al., 2007a, Hall and Jones, 2009, Sadras and Petrie, 2011, Webb et al., 2012). That would place an added stress during berry ripening that could increase the occurrence of shrivelled berries. Consequently, the difficulty of maintaining the characteristics of the grapevine that lead to a distinctive wine style is the main impact of global climate change on the wine industry (Anderson et al., 2008, Webb et al., 2012).

Higher temperatures up to 35℃ tends to favour photosynthesis (Kriedemann, 1968) and thus sugar synthesis, resulting in an early accumulation of sugar (Sadras and Petrie, 2011). However, with more rapid ripening all the desirable flavour components of grapes are not synchronised. Although red wine grapes may reach an adequate level of sugar earlier, the harvest time may not be earlier if other components have not adequately developed (Sadras and Petrie, 2011). For example, vines that grew in a modified warm temperature presented a lower level of anthocynin at equal level of sugar compared to unheated vines (Sadras and Moran, 2012). The ripening condition in a grape berry involves an optimal balance of all the varied attributes (Coombe and McCarthy, 1997). Moreover, the resulting high level of sugar in grapes would be a consequence of a delay in the harvest, in order to obtain the peak in colour or aromatic maturity (Mira de Orduña, 2010, Sadras and Moran, 2012). Therefore, there is not a proportional advance of the harvest time due to a dissimilar development of the other attributes of the grapes. Analogously, there is an improvement in the aromatic profile in more mature grapes, although there is also a rise in sugar, which results in more alcoholic wine (AWRI, 2005).

In addition, as Keller (2010) comments, unharvested grapes past 25°Brix are mainly the result of water loss in the range of 10 to 20%**.** In Merlot the increment in sugar during berry mass loss seems to be equivalent to the mass lost (Keller et al., 2015). Essentially, the increase of sugar in grapes means a high level of alcohol content in the wine. To exemplify this point, alcohol content of Australian red wines tested by the Australian Wine Institute, clearly depicted an upward trend. The observed changes, range from an average alcohol level in wine of 12.4% in 1984 to an average of 14.4 % in 2008 (Godden and Muhlack, 2010). Interestingly, an increase in residual sugar (glucose + fructose) and total dry extract was also reported for this period.

1.3.3 Berry growth

The berry growth process has two remarkable and significant physiological milestones, which are the onset of ripening (veraison) and maximum mass. These milestones allow the berry growth process to be divided into three different phases, formation (from flowering to veraison), maturity (from veraison to maximum mass) and then from the point of maximum mass, sometime loss of mass (McCarthy and Coombe, 1999, Sadras and McCarthy, 2007). It could be generalised from a study of 32 growth curves of Shiraz during four different seasons, that each phase of berry growth has a characteristic sugar accumulation rate and increase in berry fresh mass rate (McCarthy and Coombe, 1999). The last phase also correlates with a loss of cell vitality in some cultivars (Tilbrook and Tyerman, 2008) that may or may not be correlated with loss of mass (Fuentes et al, 2010).

1.3.3.1 Loss of berry mass (shrivel)

The physiological causes of berry mass loss remains unclear and there is no suggestion regarding how to ameliorate this problem (Greer and Rogiers, 2009). This is probably because no single symptom has been described. There are similar berry shrivel phenotypes in bunch stem necrosis, sugar accumulation disorder and late shrivel. However, they differ in the distribution in the vine and bunch, in the inception and in the resulting quality of the grape (Krasnow et al., 2010).

Late berry shrivel is widely described as a process later in development associated with high sugar accumulation and additional softening typified by Shiraz (McCarthy, 1997, 1999, McCarthy and Coombe, 1999, Tyerman et al., 2004, Sadras and McCarthy, 2007, Tilbrook and Tyerman, 2008, Greer and Rogiers, 2009, Tilbrook and Tyerman, 2009). In contrast, an early disorder that occurs at lower sugar content, has been observed in other cultivars in North America, for instance Cabernet Sauvignon (Krasnow et al., 2009, Bondada and Keller, 2012). Shrivelled berries in Shiraz appears to be a consequence of a net loss of water from the berry (McCarthy and Coombe, 1999).

Concurrently, cell vitality declines in the mesocarp cells of Shiraz berries around the time when the berry reaches maximum fresh mass (Tilbrook and Tyerman, 2008). This apparent degeneration of cell membranes in the mesocarp has been proposed to cause the negative osmotic solute potential of sap in the berry to become incapable of balancing the xylem tension created by the transpiring canopy (Tilbrook and Tyerman, 2008, Bondada and Keller, 2012). On the other hand, this decrease in the vitality of the mesocarp cells in Shiraz seems to reduce the potential of the berry to generate turgor, making berries less vulnerable to splitting (Clarke et al., 2010).

Apparently, the shrivel disorder described in Cabernet Sauvignon differs from the late shrivel in Shiraz. Shrivelled berries of Cabernet Sauvignon are characterized by low sugar accumulation, low fresh berry mass, and the first symptoms start around the time of veraison (Krasnow et al., 2009, 2010, Bondada and Keller, 2012). Conversely, Fang et al. (2011) reported a higher concentration of sugar, among many other changes observed in shrivelled Cabernet Sauvignon and a dissimilar observation was made for the Zweiglt variety, where shrivelled berries did not exceed 14 °Brix at harvest (Knoll et al., 2010). It has been suggested that this disorder be called sugar accumulation disorder (SAD) making reference to the lower level of sugar (Krasnow et al., 2009, Krasnow et al., 2010). Interestingly, this shrivel has also been observed to be associated with a decrease in cell vitality as also presented in Shiraz shrivel (Krasnow et al., 2008, Krasnow et al., 2009, Clarke et al., 2010, Fuentes et al., 2010, Bondada and Keller, 2012, Bonada et al., 2013a). Additionally, a previous cessation of phloem translocation is seen to be also a common characteristic shared by shrivelled berries in both cultivars (Coombe and McCarthy, 2000, Greer and Rogiers, 2009, Krasnow et al., 2009, Knoll et al., 2010), and the cessation of translocation could be interpreted as the berry ceasing to be a sink, involving other adjustments in the vine (Coombe and McCarthy, 2000).

Since berry shrivel may indicate more than one disorder, there are also different shrivel distribution patterns. Some authors report that shrivel is observed in all the bunches in a vine as visible or non-symptomatic (Krasnow et al., 2009, Krasnow et al., 2010). Other studies have found normal and affected bunches in the same vine and interestingly no change in photosynthesis or transpiration rate of leaves opposite normal and shrivelled bunches were detected (Knoll et al., 2010). In general terms the mass loss phenomenon is a varietal property. For instance, Chardonnay shares the cell

death pattern sometimes, however, it does not present shrivel (Tilbrook and Tyerman, 2008, Fuentes et al., 2010, Bonada et al., 2013a) and Thompson Seedless does not show cell death and does not shrivel (Tilbrook and Tyerman, 2008).

In spite of the previous findings, shrivelled berries seem to have normal and functional seeds (Hall et al., 2011, Bondada and Keller, 2012). It is important to note that there are few studies regarding how phenolic profiles develop in the shrivelled berry. Nevertheless, Fang et al. (2011), contrast the phenolic profile of shrivelled and normal berries of Cabernet Sauvignon, showing that increased shrivel could be associated with a decrease in anthocyanin synthesis. The authors also found an increment of total polyphenol profile in shrivelled berries.

1.3.4 Transpiration of berries at different stages

A deficiency of inflow to the berry compared to berry transpiration has been suggested as the most probable cause of berry mass loss in grapes ((McCarthy and Coombe, 1999, Coombe and McCarthy, 2000, Rogiers et al., 2004, 2006). For grape berries late in ripening the transpiration rate decreased to 16 % of the level before veraison. This rate would account for a daily water loss of about 15 mg (Rogiers et al., 2004). Similarly, grape berry transpiration rate in potted Shiraz vines was assessed by a gas exchange chamber, where the maximum daily transpiration rate at the early stage of berry development was 300 mg, around veraison was 30 mg and at late ripening decreased to 15 mg (Greer and Rogiers, 2009). These authors argued that, due to the fact that there is also an important decline in the inflow rate of water to the berry at late maturity, the berry water balance becomes negative, and this was proposed as the main cause of mass loss in late ripening Shiraz berries.

The berry skin, which could be considered to be the main barrier to water loss by transpiration, shows changed behaviour during berry development based on the decline in transpiration. However, in a study by Rogiers et al. (2004) there was no variation in the amount of cuticular wax from the time that maximum berry mass was reached to later stages of ripening when shrivel was observed, and no correlation was found between the structure or amount of cuticle wax and the level of shrivel or transpiration rate. In addition, no great differences in the transpiration rate were observed between detached berries of Chardonnay, Shiraz and Grenache at the time when the Shiraz berries begin to shrivel (Scharwies, 2013). Therefore, it is difficult to account for the difference in berry mass loss between these cultivars to berry transpiration. Moreover, a diverse backflow capacity given the differential xylem resistance amount these cultivars (Scharwies, 2013), seen to be a more likely explanation for the lack of shrivel berries in Chardonnay berries compared to Shiraz at low cell vitality in late ripening stages (Tilbrook and Tyerman, 2008, Bonada et al., 2013a).

1.3.5 Cell vitality in berries

The finding that there was no osmotic potential differences between the xylem and berry juice at ripening stages, suggested that cell compartmentation was damaged (Lang and Düring, 1991). A decrease in cell vitality is widely documented as a phenomenon that precedes berry mass loss (Krasnow et al., 2008, Tilbrook and Tyerman, 2008, Krasnow et al., 2009, Tilbrook and Tyerman, 2009, Clarke et al., 2010, Fuentes et al., 2010, Bondada and Keller, 2012). The loss of membrane functionality under stress may be linked to membrane oxidation and/or depletion of energy in the cells (Trippi et al., 1989).

The use of dyes to differentially visualise living from non-living tissues is the current technique to appraise berry cell vitality. The fluorescein diacetate dye (FDA) has been an effective tool to assess cell vitality, discerning between artificial killed tissues in the expected way (Krasnow et al., 2008, Tilbrook and Tyerman, 2008, Fuentes et al., 2010). However, this technique presents limitations in terms of quantification (Puértolas et al., 2012). A previous study by Tilbrook and Tyerman (2008) referred to the result of this technique as a relative measurement of the cell vitality in the berry. The practical usage of this technique could be restricted due to the slow laboratory process and the subsequent data interpretation (Tyerman et al., 2012). Therefore, in order to incorporate the degree of berry cell vitality as part of viticultural assessment of fruit, a more practical technique is required.

Based on vital staining there appears to be different patterns in the progression of cell death between Shiraz and Chardonnay, being earlier and faster in Shiraz (Tilbrook and Tyerman, 2008). Furthermore, a different pattern of cell death between cultivars in the brush area at similar total proportions of nonliving tissue in the flesh was reported by Fuentes et al. (2010). They recognized living areas in the brush of Chardonnay, while Shiraz berries showed dead areas. This observation could be associated with the contrasting shrivel behaviours of these two cultivars. The cell death process started around the seed locule and at harvest non shrivelled berries presented a minor degree of cell death restricted around this area (Krasnow et al., 2008)**.** As a consequence of cell death in the mesocarp tissue of Shiraz, it is highly likely that there would be a lysing and mixing of the different cell components, which would modify the resulting flavour present at harvest (Tilbrook and Tyerman, 2008).

1.3.6 Impedance properties and applications to assess cell death

Electrical impedance is the effective resistance to an alternating current and reflects the combined effects of ohmic resistance and reactance that is a function of capacitance or inductance. It is dependent on the frequency of the alternating current (AC) and its characteristics as a function of frequency can reveal components of the underlying circuit. The impedance of plant tissues seems to be able to detect the degree degradation and other alterations within the tissue, and with relative ease of measurement (Hildebrandt and Thielecke, 2010). Impedance describes the passive electrical behaviour of any material in terms of its capacity of dissipating and preserving the electrical energy. In this respect, each material could work as a resistor, a capacitor or as an inductor (Pliquett, 2010). From this perspective, a biological membrane could be represented as a resistor and capacitor (Angersbach et al., 1999).

The impedance in tissues involves the electric interaction of cell components and the extracellular space. At both sides of the cell membranes the apoplasmic and the symplasmic space act as electric resistors due to their electrolyte content. The membranes due to their high resistance behave as an electric capacitor (Pliquett, 2010). There are different approaches to explain the electrical behaviour of tissues, and different models are described in the literature, which address this relationship. Four electrical models or circuit equivalent models were evaluated by Zhang and Willison (1991) working on potato and carrot. They vary in terms of the complexity of the included parameters, for example the double shell model developed by (Zhang et al., 1990) explains the bioimpedance in tissues including apoplasm, symplasm and vacuole resistance and plasma membrane and tonoplast interior capacitance. Varlan and Sansen (1996) suggested that the impedance in fruits should work as two skin disks connected in series, since the voltage could partially be lowered by the fruit

skin. Due to the fact that, electrical impedance in tissues involves the electrical behaviour of several cells as a group, a time constant distribution is generated, and this has to be incorporated in the electrical circuit as a constant phase element (Ando et al., 2014).

Impedance has been experimentally used in plant tissues to assess the degree of cell deterioration (Varlan and Sansen, 1996, Angersbach et al., 1999, Vozary et al., 1999, Euring et al., 2011, Ando et al., 2014). Cell deterioration caused by artificial damage leads to degradation of cell membranes, which was reflected as a decrease in low frequency resistance due to solute leakage (Varlan and Sansen, 1996). Angersbach et al. (1999) commented that, when tissues present cells with an irreversible damage in the membranes, there is an increment of the conductivity at low frequency. While, at high frequency the cells are not capable of causing a significant resistance to this current. Further, there is no fluctuation in the conductivity of intact cells with respect to the conductivity of damaged tissue in the high frequency range.

Some practical impedance assessments on fruits have confirmed the above points. Over ripening mango fruits seem to have less fruit resistance (Kohm) and high fruit capacitance (nF) compared with ripened fruits (Rehman et al., 2011). Measurements of low frequency resistance shows the apoplasm changes during nectarine ripening (Harker and Maindonald, 1994). Cell wall destruction in bruised tissues of apples was indicated as a decrease in the ratio of apoplasm resistance to symplasmic resistance, the ratio was 30% lower in bruised apples. The apoplasm resistance declined in apple tissue as a consequence of an increase in the charged solutes leaked from cells to the apoplasm after cell membrane rupture (Vozary et al., 1999). Similarly, cell wall resistance and vacuole resistance decrease by 60 and 26 per cent respectively as fruit ripened in the study of Harker and Maindonald (1994). However, they argue that there

was no cell deterioration, since membrane capacitance was unaffected during the whole ripening process.

The morphological characteristics of fruit to be measured by impedance have to be considered. For instance, the impedance properties were affected by the fruit mass (Euring et al., 2011, Rehman et al., 2011). Also there was a varietal effect in apples that could be associated with different shapes (Euring et al., 2011). Impedance spectroscopy (i.e. impedance as a function of the AC frequency) has been shown to be a useful technique to detect the level of cell deterioration in fruit, being a practical tool in terms of time and degree of complexity (Azzarello et al., 2012). However, although there have been promising results at the research level, there has been little practical application of this technology (Pliquett, 2010). There appears to be a gap in calibrating this technique in order to develop a simple and rapid assessment of tissue vitally in fruit.

1.3.7 Berry water balance.

It would be expected that under warming conditions the evaporative demand on vines would increase as indicated by vine transpiration rates in warmer areas of Australia being mostly influenced by high VPD rather than by soil moisture status (Rogiers et al., 2011). Climate warming is also expected to increasingly distress berry development as explained in the opening sections. This is indicated through artificial increase in the mean daily temperature of only 1.5° C causing earlier onset of cell death and increasing the rate of cell death in the mesocarp (Bonada et al., 2013a). Additionally this study, observed that there was an increase in the shrivel process in artificially heated vines.

Shrinkage and berry mass loss seems to be very dependent on weather conditions after maximum berry mass is achieved. For instance, berry mass loss in Shiraz could continuously progress after maximum berry mass if there is no significant rain events, while a plateau or even berry mass gain could be observed after significant rain (Rogiers and Holzapfel, 2015). In addition, deficit irrigation could accelerate berry cell death and shrivelling in Shiraz (Bonada et al., 2013b).

The literature related to berry shrivel includes several studies that aimed to determine the cause of berry mass loss. It would appear that the consensus is that xylem can conduct water during late ripening stages (Chatelet et al., 2008, Tilbrook and Tyerman, 2009, Becker et al., 2012), which means that backflow is possible (Tyerman et al., 2004) and it may be required to dispense with surplus water inflow from the phloem during berry ripening (Keller et al., 2015). The cell death process starts at approximately the same time when loss of mass begins (Krasnow et al., 2008, Krasnow et al., 2009, Clarke et al., 2010, Fuentes et al., 2010, Bondada and Keller, 2012, Bonada et al., 2013a, Bonada et al., 2013b), and simultaneously with slowed or ceased sugar translocation to the berry (Coombe and McCarthy, 2000, Greer and Rogiers, 2009, Krasnow et al., 2009, Knoll et al., 2010). The loss of berry mass and decrease in berry cell vitality are clearly linked with heat stress in Shiraz and Chardonnay (Bonada et al., 2013a).

1.3.7.1 Canopy cooling systems

There are published reports on the effects of artificial shade during heat stress. For instance, fruit surface temperature decreased and solar injury was ameliorated in pear trees protected with shade cloth (Gindaba and Wand, 2005). The use of shade-net on grape vines from flowering to harvest decreased canopy temperature (Greer et al.,

2010). However, the timing of bunch shading treatments has been shown to influence the effects on vine and berry development (Dokoozlian and Kliewer, 1996, Koch et al., 2012, Li et al., 2013). Therefore, it is highly likely that shading over a defined period during berry ripening may mitigate the effects of heatwaves on berry degeneration and shrinkage. It seems reasonable to hypothesise that an improvement in the water balance of vines under high evaporative demand would lead to an ameliorated effect on fresh berry mass loss and senescence processes.

Micro sprinklers have been used as an evaporative cooling system in apple trees to reducing maximum air and fruit surface temperature (Parchomchuk and Meheriuk, 1996, Iglesias et al., 2005). Furthermore, Parchomchuk and Meheriuk (1996) delivered 2 mm h^{-1} causing a decrease in maximum fruit surface temperature of 8° C and 2° C in air temperature. In contrast, Iglesias et al (2005) reported a more significant decrease in terms of air temperature (-5.2-5.9° C), with a decrease in fruit temperature by almost 6.1 °C. The rate of water application in the sprays was 3.4 mm h^{-1}. The application of an evaporative cooling system on grape vines was earlier assessed by (Gilbert et al., 1971, Kliewer and Schultz, 1973, Aljibury et al., 1975), where a decrease in fruit, air and canopy temperature was observed. This caused an increase in fresh berry mass (Kliewer and Schultz, 1973, Aljibury et al., 1975). However, they did not suggest further application of this technique since limited results on berry composition were observed. Recently, Semillon vines were watered from overhead when air temperatures exceeded 35 ^{o}C from flowering to harvest. This resulted in canopy temperatures dropping by 7-8^{o} C and fresh berry mass was increased (Greer and Weedon, 2014). To date there is no reported study of the favourable effects of misting within the canopy at bunch height from veraison to harvest on berry properties and composition.

1.4 Research questions

Can berry cell death be detected by changes in berry impedance?

Can the negative effects of global warming on berry ripening (mass loss, early berry senescence and high level of sugar) be controlled by modifying vine microclimate, either by various canopy shading or by misting within the canopy, from veraison to harvest?

Is wrapping arms in cordon establishment a stressful practice for grapevine?

1.5 Research Objectives

In order to answer the questions above, this research has the following specific objectives and hypotheses:

1.5.1 Development of a more practical technique for viticulturists to assess berry cell death**.**

Hypotheses:

Impedance behaviour of berries is correlated with berry cell death.

Electrical capacitance of the berry is reduced with increasing cell death due to breakdown of membranes within the berry.

Extracellular resistance is reduced with increasing cell death due to leakage of electrolytes from the dying cells.

1.5.2.1 Test the probable beneficial effect of shade cloth protection.

Hypotheses:

Shading from veraison to harvest using shad cloth will reduce canopy and fruit maximum temperature during heat waves.

Shading will decrease the rate of loss of berry mass.

Shading will decrease the rate of berry cell death progression.

As a consequence of a lower temperature during ripening, a better assemblage between the onset and maturity of sugar and colour would be expected, resulting in less alcohol concentration in wine.

1.5.2.2 Developing a heatwave control system based on inside-vine misting as an evaporative cooling system. Which would be capable of dealing with berry mass loss in a warming climate.

Hypotheses:

Evaporative cooling will reduce rate of loss of berry mass.

Evaporative cooling will delay and decrease berry cell death process.

1.5.3 Test if wrapping canes on to a wire for cordon establishment represents a vine stress.

The arm constriction created by wrapped canes, would progressively restrict water flow and will exacerbated water stress conditions.

Hypotheses:

1) The constriction of the cordon wire on the arms reduces growth (arm transversal area and pruning mass).

2) The constriction negative effects the water relations of the distal shoots on the arm.

3) The storage of carbohydrate might be affected in wrapped vines.

1.6 Significance/Contribution to the discipline

An evaluation of cell vitality in berries would improve viticultural decisions on harvest timing since cell death integrates the heat stress and water stress imposed on the vine. This information if it can be easily obtained should be incorporated as a standard part of viticultural assessment.

This is the first attempt to combine knowledge of berry mass loss with techniques to mitigate effects of heat stress. In this respect, two possible techniques were assessed and related to berry development, berry mass loss and berry cell death.

A reduction in the concentration of alcohol in the resulting wine would be expected resulting from reduced loss of berry mass and to a better assemblage between sugar and colour maturity.

Dead arm is a most relevant factor affecting grapevine longevity in Australia, and evidences of its increasing incidence in the past decade that may be linked to an escalating stress on the vine vasculature in wrapped cordons, will be the first step for correcting this management. Furthermore, if a water stress is imposed by restriction of vasculature due to the cordon wire, it is likely that the added stress on the vine during heatwave events will exacerbate the negative impacts of climate warming.

Chapter 2

Electrical impedance of berries correlates with decreasing cell vitality during ripening in Shiraz

Australian Journal of Grape and Wine Research, 10.1111/ajgw.12157

Statement of Authorship

Title of Paper	Electrical impedance of Shiraz berries correlates with decreasing cell vitality during ripening
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Australian Journal of Grape and Wine Research, 10.1111/ajgw.12157

Principal Author

Name of Principal Author (Candidate)	Luciano Caravia Bayer
Contribution to the Paper	Developed the research idea, designed and conducted all research experiments, analysed the data and drafted and constructed the manuscript.
Overall percentage (%)	75
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 21/09/2015

Co-Author Contributions

Name of Co-Author	Cassandra Collins
Contribution to the Paper	Contributed to editing the manuscript
Signature	Date 21/9/15

Name of Co-Author	Stephen Tyerman
Contribution to the Paper	Contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.
Signature	Date 21/9/15

Caravia, L., Collins, C. & Tyerman, S.D. (2015). Electrical impedance of berries correlates with decreasing cell vitality during ripening in Shiraz.
Australian Journal of Grape and Wine Research, 21(3), 430-438.

Chapter 3

Application of shade treatments during Shiraz berry ripening to reduce the impacts of high temperature

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Statement of Authorship

Title of Paper	Application of shade treatments during Shiraz berry ripening to reduce the impacts of high temperature
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Luciano Caravia Bayer
Contribution to the Paper	Developed the research idea, designed and conducted all research experiments, analysed the data and drafted and constructed the manuscript.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 21/09/2015

Co-Author Contributions

Name of Co-Author	Cassandra Collins
Contribution to the Paper	Contributed to the research ideas, edited the manuscript and provided advice for the winemaking process and berry and wine quality assessments.
Signature	Date 21/9/15

Name of Co-Author	Paul Petrie
Contribution to the Paper	Contributed to the research ideas and edited the manuscript.
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Name of Co-Author	Stephen Tyerman
Contribution to the Paper	Contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.
Signature	Date 21/9/15.

Abstract

Background and Aims: The impacts of heatwaves on grapevine function are serious problems in our warming climate. We tested the impact of shading on vine physiology, berry cell death and other components of berry development and the resultant wines in order to understand grapevine physiology and the ripening process in shaded conditions and to assess shading as a mitigation strategy for heatwaves.

Methods and Results: Several shading treatments on Shiraz vines were imposed from veraison to harvest during three seasons at two locations. An overhead-shade (OS) treatment (62% absorption) was the most consistent in high temperature mitigation. Compared to controls (no shading) or soil shade the leaves of OS vines had higher chlorophyll content, higher net CO_2 assimilation at saturating light, higher ψ but similar sap flow and leaf transpiration rates. OS berries accumulated sugars per berry at a similar rate but had lower TSS due to higher water content. Berry cell vitality measured with impedance or vital stains was higher in OS and mass loss was delayed. Wines had lower alcohol with no significant difference in anthocyanin. However, tannins, polyphenol, and some of the colour properties were affected.

Conclusion: Overhead shade can ameliorate the impacts of heatwaves through a significant reduction in berry cell death and berry shrinkage, resulting in reduced alcohol content in wines and with only minor effects on wine quality.

Significance of Study: Shading from veraison to harvest may be a viable mitigation strategy against heatwaves during ripening.

Keywords: berry ripening, heatwaves, cell death, shade treatment, lowered alcohol

Introduction

Several studies have examined the impact of global warming on grapevine quality, and there is wide agreement that vine phenology has moved earlier in the season (Duchêne and Schneider 2005, Jones 2007, Petrie and Sadras 2008, Sadras and Petrie 2011, Sadras and Petrie 2012, Webb, et al. 2011). Among the observed changes in berry quality, increased sugar concentration at harvest in red cultivars is commonly observed (Mira de Orduña 2010, Petrie and Sadras 2008, Sadras and Moran 2012, Sadras and Petrie 2011), which can increase alcohol in wines as indicated by the trend observed in the last decades (Godden and Muhlack 2010). Higher pH values and a decrease in total acidity of juice are also reported (Anderson, et al. 2008, Spayd, et al. 2002). Anthocyanin content is likely to decline (Downey, et al. 2006, Sadras and Moran 2012, Spayd, et al. 2002), and an increase in berry shrinkage in mature fruit has been predicted (Bonada, et al. 2013, Bonada, et al. 2013b, Fuentes, et al. 2010).

Heatwaves are predicted to increase in frequency as a result of global warming (Rahmstorf and Coumou 2011). Shading may be used to mitigate the impacts of heatwaves and has been examined for some cultivars and climatic conditions. Shade cloth to partially or completely cover grapevine canopies from budburst to harvest has been tested in Sangiovese and Semillon (Cartechini and Palliotti 1995, Greer 2012, Greer and Weedon 2012, Greer, et al. 2011, Greer, et al. 2010). This had a sustained effect throughout the whole season on canopy growth and berry development, which led to a decrease in berry weigh, total phenolic accumulation; an increase in total acidity and a detrimental effect on photosynthesis. A partial side-canopy shade treatment that included basal leaf removal in both control and shade treatment was tested from 2 weeks before veraison to harvest on Cabernet Sauvignon and Shiraz

(Joscelyne, et al. 2007, Rochfort, et al. 2010). Wine made from these shade treatments had lower aromatic and flavour profiles. Other studies have tested canopy shading treatments from 2 weeks after flowering in Cabernet Sauvignon (Rojas-Lara and Morrison 1989). Berry weight, sugar per berry and anthocyanin content was negatively affected, however, in (Rojas-Lara and Morrison 1989) the air temperature was higher under the shade treatment than control and no difference was observed in berry temperature.

To our knowledge no investigations have examined the effects of shading treatments from veraison to harvest in Shiraz and the interaction with heatwave events. Shiraz has a strong tendency to undergo cell death in the berry and berry weight loss that makes it particularly sensitive to high temperature conditions (Bonada, et al. 2013,Bonada, et al. 2013b). Here a range of shading treatments were applied to Shiraz grapevines in the field over three seasons and at two locations to test the type of shading that gave the most consistent effects and mitigated heatwave events. We examined how shading affected vine leaf gas exchange, whole vine water relations, berry development and berry composition measures, including changes in the degree of cell death and weight loss. Wines were made from treated and control vines at two harvest dates the second season and at harvest the third season to test impacts on some components of wine quality.

Material and methods

Experimental sites

Shiraz vines (clone BVCR12) on own roots planted in 1992 were studied at the Coombe vineyard, located at the University of Adelaide Waite Campus (34°58'03.12" S and 138°38'00.21" E). Vines were trained to a vertical shoot positioned (VSP) trellis with rows oriented north-south and vine and row spacing 2.7 m x 3 m respectively. A second trial was conducted in the 2012-2013 and 2013-2014 seasons in McLaren Vale in a premium quality commercial Shiraz vineyard (34°14'10.94" S and 138°34'00.61" E) planted in 1997, with north-south row orientation and vine and row spacing 2.0 m x 3.3 m respectively. Vines were trained to a sprawl canopy (with one foliar wire at 0.3 m above the cordon). Treatments were conducted during the ripening period (from veraison to harvest). For each site standard irrigation and viticulture practices typical for the region was applied. Bunch thinning was performed in season 2013-2014 over all the treatments at Coombe vineyard at 63 DAA, approximatley 5 bunches m^{-1} were removed at veraison (the upper bunches on shoots carrying two bunches, large shoulders and green bunches located on weak shoots were targeted for removal).

Treatments

All shade treatments used a 62% (exclusion) shade cloth (Premium Hortshade, medium grade, Pacific^{TM},Braeside, Victoria, Australia). Table 1 provides a summary of the shade treatments used at each site and season. Over the three seasons, S1 (2012-13), S2 (2013-14) and S3 (2014-15), an overhead shade (OS) treatment (Figure 1a) was imposed from around veraison at the Coombe vineyard. The treatments were imposed in four replicates each comprising of one panel (2 vines), located in different

rows, with the adjacent panels as controls. For the OS treatment the cloth was suspended 50 cm above the top of the canopy, the cloth dimensions were 6.0 x 7.0 m whereby the shade cloth covered a complete panel, shading 50 % of the east inter row side of the canopy and completely shading the western side of the canopy. In S2 side-canopy shade (CS) and soil shade (SS) treatments were also tested in the Coombe vineyard (Figure 1d). Here adjacent panels were tested with one of these treatments in four replicates with each pair situated in a different row.

Table 1. Shade treatments tested during three seasons (S1, S2, S3) and at two sites.

Treatment	**Location**	**Replicates**	**2012-2013**	**2013-2014**	**2014-2015**
			S1	***S2***	***S3***
C, Control	Coombe	4	X	X	X
	McLaren Vale	3	X	X	
OS, Overhead shade - roof	Coombe	4	X	X	X
CS, Canopy shade - side	Coombe	4		X	
	McLaren Vale	3		X	
SS, Soil shade	Coombe	4		X	
FCS, Full canopy shade	McLaren Vale	3	X		
BS, Bunch shade	McLaren Vale	3	X		

The treatments were installed on 26/12/2012, 09/01/2014 and 05/01/2015 corresponding to 47 days after anthesis (DAA), 61 DAA and 71 DAA respectively

(5.2, 9.1 and 14.4 ^{o}Brix respectively). For the Coombe site veraison stated (20 % of berries colouring change) at 62 DAA in S1 and S2 and at 67 DAA in S3. Treatments were removed after harvest (119, 118) and 144 DAA for S1, S2 and S3 respectively.

Figure 1. Shade treatments tested during three seasons. (a) Overhead shade (OS) treatment tested at Coombe vineyard in S1, S2 and S3, (b) full canopy shade (FCS) treatment and (c) bunch shade (BS) treatment tested at McLaren Vale in S1, and (d)

soil shade (SS, foreground) and side-canopy shade (CS, background) treatments tested in S2. The CS treatment was tested in both locations and SS only at the Coombe vineyard. Bird netting also shown in (d) had no effect on radiation.

During S1 full-canopy shade (FCS) and bunch shade (BS) treatments were imposed at the second trial site in McLaren Vale (Figure 1b,c), The treatments were imposed in three replicates comprised of one panel (3 vines) each, located in three different rows. These treatments were imposed from 56 DAA until harvest (99 DAA). For S2 a side-canopy shade (CS) treatment (Figure 1d) was tested at McLaren Vale where veraison occurred at 67 DAA and the treatment occurred from 69 DAA until harvest at 113 DAA.

Canopy temperature and vapour pressure deficit

For each field replicate canopy temperature was monitored using temperature data loggers with built-in sensors (Tiny Tag Transit 2 Data Logger thermometers, model TG-4080, Gemini Data Loggers, West Sussex, UK) that were positioned in the middle of the canopy at bunch level. The sensors were protected by a shell made of foil bubble wrap following the design described by (Tarara and Hoheisel 2007). In two of the replicates at the Coombe vineyard (OS and C) Tiny Tag model TPG-4017 were used. The sensors recorded temperature every 15 minutes. For S3, Tiny Tag model TPG-4500 were used to record the temperature and relative humidity inside the canopy every 10 minutes. Data from the Kent town weather station located 5 km away from the Coombe vineyard (34°55'16.5" S and 138°37'10.2" E) was used as a reference for historical data analysis at Coombe vineyard site and Norlunga weather station (34°08'39.5" S and 138°28'22.2" E) located at 13 km away from the McLaren Vale trial was used as a reference (http://www.bom.gov.au/climate/data/).

Total solar radiation and photon flux density

Two photosynthetically active radiation (PAR) sensors (400-700 nm, model LINPAR), and two radiometers (model PYR, both sensors from ICT International Pty Ltd, Armidale, NSW, Australia) monitored the photon flux density (PFD) and solar radiation across one of the field replicates at the Coombe vineyard for two treatments (OS and C) every 10 minutes. The sensors were mounted on a wood frame placed on the western side of the canopy at 40 cm above the cordon and 20 cm from the canopy edge. The radiometer was positioned facing the ground for 9 days (from 91-99 DAA) in order to check changes in soil irradiation to the canopy between the treatments.

Chlorophyll content

The relative chlorophyll content was measured using a portable chlorophyll content meter (model CCM-200, APOGEE INSTRUMENTS, INC. Utah, USA). To randomly select leaves, a rope with 10 labelled points at 0.5 m between them was tied at an intermediate height within the canopy ±35 cm above the cordon wire on the western side of the canopy. At each of these ten-labelled points one leaf immediately surrounding the label was measured at three different positions on the leaf. The measurements were done for each field replicate and the data was obtained as chlorophyll content index (CCI, units).

Leaf gas exchange

For S1, stomatal conductance (g_s) was measured using a porometer (Model AP4, DeltaT Devices, Cambridge U.K.). For S2 more comprehensive gas exchange measurements were obtained at the Coombe vineyard site (net CO_2 assimilation at light saturation A_{sat}, leaf evaporation E, and g_s) using an infrared gas analyser (LCpro-

SD Portable Photosynthesis System, ADC BioScientific Ltd., UK). Measurements were performed between 11:00 to 13:00 (Australian CST) in S1 and between midday and 16:00 in S2. The measurements were done on the western side of the canopy on four of the central fully exposed labelled leaves used for chlorophyll assessment. OS, CS, SS treatments and C over the same row were performed sequentially (no more than 15 minutes elapsed between the measurement of each treatment in the same row), in that way it is expected to make the data between each pair of treatments more comparable (OS to C and CS to SS), since gas exchange parameters can change rapidly with changes in environmental conditions. The IRGA was operated under ambient temperature (ranged from 29-45 ° C), ambient CO_2 and humidity. Incident light on the measured part of the leaf was controlled by on-board LED lights set at a photon flux density of 919 μmol $m^{-2}s^{-1}$. The measurement was recorded after 3 minutes of enclosing the leaf. A preliminary experiment identified the light saturation point using leaves from both sides of the canopy (supplementary Figure 5). The western side was selected for further measurement since this side is exposed to the direct solar radiation during the measurement period and is under more stressful conditions during heatwaves.

Sap flow

Sap flow sensors using the heat ratio method (model SFM1, ICT international Pty Ltd, Armidale, NSW, Australia) were installed at each replicate for OS and C vines in the Coombe vineyard in S2. The sensors were calibrated to wood properties according to supplier specifications. Briefly, a stem core sample was collected at the sensor entry point, sapwood, heartwood and bark thickness were determined. Sapwood volume was recorded with a digital caliper, fresh sapwood weight was recorded and

after drying 7 days at 60° C, sapwood dry weight was recorded. Trunk diameter was also recorded at probe access height. The data was obtained as total flow rate (cm^3 h^{-1}).

Soil water profile

Soil water profiles were measured in S2 at the Coombe vineyard site using two different types of soil probes. Thirteen of the 16 replicates in the Coombe vineyard were monitored using a portable capacitance technique (Diviner 2000, Sentek technologies, Australia). The 13 access tubes for the Diviner probe were positioned 30 cm from the trunk and to a depth of 1.0 m. These were measured 10 times during the treatment period. In addition enviroSCAN probes (Sentek technologies, Australia) were used in the other three remaining replicates (two OS and one C), with the tubes located as described above but recording soil moisture at 5 depths between 10 to 50 cm, every 15 minutes for the entire season. During the data analysis using IrriMAX 9.1 (Sentek technologies, Australia) no significant differences were found between the enviroSCAN probes. Therefore, the values recorded (EnviroSCAN) at the same time as the Diviner measurements were extracted and analysed as part of the Diviner pool of data. The data was grouped in two depth ranges, from 10 cm to 50 cm depth (which include the EnviroSCAN data, 4 replicates in all the treatments) and from 60 cm to 90 cm depth (2 replicates for OS, 3 replicates for C).

Stem water potential

Stem water potential (ψ) was measured on one occasion in S1 and more intensively during S2 at the Coombe vineyard. Three leaves from the middle of the panel at an intermediate height within the canopy (30-50 cm from the cordon) were randomly

selected and enclosed in foil covered zip-locked bags for 1 hour before the leaf water potential was measured. It was assumed that this time allowed equilibration with the stem. The measurement was done between 11:30 to 14:00 Australian CST using a pressure chamber, (PMS Instrument Company model 1005, Albany, OR. USA. www.pmsinstrument.com). No more than 5 seconds elapsed between the time the leaves (inside the bag) were removed from the vine and placed in the chamber.

Berry sampling

Berries were sampled in the three seasons for the Coombe vineyard, and S1 and S2 for the McLaren Vale site. Berries were sampled from the proximal portion of labelled bunches for each replicate and treatment by cutting the pedicel with scissors. Berries were collected from the entire treated replicate except the area 0.2 m from each end. Samples were stored in a sealable plastic bag, and then placed in an insulated box cooled with ice in the field (the ice was not in contact with the samples), once in the laboratory, subsamples were taken to measure fresh and dry berry mass, cell vitality tests (impedance spectroscopy and fluorescein diacetate, FDA), total soluble solids (TSS), total anthocyanin + total phenolics, pH and total acidity.

At the Coombe vineyard during S1, 30 berries were collected from each of the replicates five times between veraison and harvest while for S2, 120 berries were collected on ten occasions. For S3 the samples were taken twice; where 120 and 200 berries were collected respectively. At the McLaren Vale site berry measures were made 3 times between veraison and harvest in S1, and 6 times during this period in S2.

Berry total soluble solids (TSS)

During S1, 10 halves of berries from each replicate (see FDA staining below) were individually crushed and the TSS (°Brix) was determined from the juice for each berry using a temperature compensated digital refractometer (Model PR101, ATAGO, Tokyo, Japan). For S2 a sub-sample of 50 berries from each replicate was crushed, juice collected and then centrifugated for 5 minutes at 2000 rpm before the measurement was taken on the clarified juice (Iland, et al. 2004). For S3, TSS was measured in a similar manner to S2 on 50 and 100 berries at the two sample dates (respectively) for each replicate.

Berry anthocyanin, polyphenol and tannin concentration

Berry anthocyanin and polyphenol concentrations were measured in triplicate each season at the Coombe vineyard site. Sub-samples of 50 berries were taken from each field replicate and berry fresh mass was recorded prior to storage at -20° C until analysis. The total anthocyanin content and total polyphenols were estimated from absorbance at 280 nm and 520 nm respectively on homogenised thawed berries according to (Iland, et al. 2004). Total grape tannin was determined from homogenate extracts according to (Mercurio et al. 2007).

pH and titratable acidity (TA)

Subsamples of 50 berries from the Coombe vineyard were used for analysis of pH and TA during S2 and S3. Samples were crushed and the juice was collected into a 10 ml tube, and centrifuged for 5 minutes at 2000 rpm. The 10 ml of juice was diluted in 40 ml deionised water for pH and titratable acidity (TA) measurements using an autotitrator (CRISON, CompacT titrator, Crison Instruments, S.A., Barcelona, Spain).

Berry fresh mass and water content

Berry fresh mass were obtained for other measures (anthocyanins and impedance) and in addition a group of 10 berries was sub sampled from both sites to determine water content for each replicate. Each berry was carefully cut from the pedicel using a razor and weighed before and after drying for 7 days (75° C). The mass differences correspond to the berry water content. For the harvest measurement in S2 this measurement was performed over 5 groups of 10 berries per replicate. For the harvest measurement in S3 berry fresh mass was measured for 200 berries per replicate.

Berry cell vitality; electrical impedance and vital staining.

A group of 10 berries were randomly selected from each field replicate during S1 and both field sites and from the Coombe vineyard site in S2. Impedance spectroscopy was performed on each berry as described in Caravia, et al. (2015). Briefly a slice of skin (2-3 mm diameter) was removed on opposite sides of the berry equator using a razor blade. The skinless portion of the berry was covered with electrolyte solution (0.1 M KCl) and the berry was gently clamped between two disc electrodes with the distance between the electrodes recorded. Impedance was measured on the berry at frequencies between 1 Hz and 2 MHz in S1 and 1 Hz and 1 MHz in S2 using an impedance meter (Bode 100, Vector Network Analyzer, OMICRON LAB, Kowloon, Hong Kong). After the impedance measurement, berries were weighed and stained with fluorescein diacetate (FDA) on the cut surface of a medial longitudinal section (half) of the berry (Tilbrook and Tyerman 2008), the other half was used for determination of TSS. The fluorescence signal was captured under ultraviolet light using a Nikon SMZ 800 dissecting microscope (Nikon Co., Tokyo, Japan) (Tilbrook and Tyerman 2008). FDA-images were analysed using a Matlab script MATLAB

R2008a (Mathworks Inc., Natick, MA, USA) (Fuentes, et al. 2010). Berry cell vitality was determined from impedance using the equation from Caravia, et al. (2015).

Leaf area index and pruning weight

During S2 leaf area index (LAI) was estimated at the Coombe vineyard using an iPad App (Fuentes, et al. 2012, Fuentes, et al. 2014). Two pictures were obtained from two vines for each field replicate at 125 DAA using a iPad model A1460 (Apple Inc., 1 Infinite Loop, Cupertino, CA 95014 USA). Upwardly direct photographs under the canopy for analysis were taken consistently at 20 cm from the trunk, the distance between the iPad screen and the cordon was recorded (0.69 m ± 0.09 SEM) and was not different between treatments ($P<0.05$). The Images were taken between 10:00 to 12:00 to avoid sun reflections. The pruning weight was also recorded after S2 and S3 at the Coombe vineyard by cutting all the shoots to 2 nodes and the values are presented as weight per meter of row length. The numbers of shoots were also counted per meter of row.

Harvest

For S1, Coombe vineyard treatments were harvested on 08/03/2013 (119 DAA), and at McLaren Vale on 19/02/2013 (99 DAA). For S2, due a to an abrupt change in weather during ripening, a partial harvest of 1.3 kg per field replicate was taken at 96 DAA from all the treatments in the Coombe vineyard, and a final harvest was done 3 weeks later at 118 DAA (07/03/2014). McLaren Vale treatments were harvested on 26/02/2014 at 113 DAA. For S3 the treatments were harvested on 17/02/2015 at 114 DAA at the Coombe vineyard only.

Microvinification and chemical analysis

Small ferments to make wine were made on S2 and S3 from every replicate and treatment from the Coombe vineyard. Briefly, the fruit was de-stemmed and crushed by a manual stainless steel crusher and the juice and skin was collected. Potassium metabisulphite (0.1 g l^{-1}) and diammonium phosphate (0.3 g/l^{-1}) was added to the juice before it was inoculated with yeast (0.2 g l^{-1}, Maurivin AWRI 796, Mauri Yeast Australia). The fermentation vessels consisted of plastic containers (2.25 or 5 L, circular base, with 14.5 or 18 cm of diameter respectively) with open top, which was covered with cloth. The ferments were maintained at approximately 21° C and were plunged manually every 24 h. When the ferments reached 0 Baume (8 days), they were pressed by a manual press and wine was collected in 0.5 L bottles. Potassium metabisulphite (0.1 g l^{-1}) was added to each bottle. For S2 wine was made twice for all treatments in the Coombe vineyard at 96 DAA and after harvest. On average 1.3 kg and 5 kg of berries were crushed at 96 and 118 DAA respectively. For S3, each field replicate (3 kg) from the Coombe vineyard was collected for wine making at 114 DAA. From these wines, approximately 10 days after bottling a sample was taken for colour, phenolic and tannin assessment following the Modified Somers assay technique described by Mercurio, et al. (2007), the measurement was done in triplicate. The alcohol content was determined as alcoholic strength by ebulliometry following (Iland, et al. 2004). No additions or corrections were made to these wines.

Statistical analysis

Significance differences in the data between treatments and control were examined by two way ANOVA (repeated measures where appropriate), and Fisher's LSD. Differences in wine quality were tested by one-way ANOVA and Fisher's LSD at

each wine making time. Mean values are given in the text and in figures +/- SEM. Analysis was performed using Prism 6 (Graphpad Software Inc. La Jolla, CA 92037 USA).

Results

Seasonal phenology and mesoclimate at the Coombe vineyard site

At the Coombe Vineyard site, full anthesis (stage EL 23) (Coombe 1995) occurred on the 10th November in both S1 and S2, and on the 27th October in S3. Veraison occurred at 62 DAA in S1 and S2 and at 67 DAA in S3 and are indicated in the relevant figures. From veraison to harvest for control vines the total accumulated growing degree days (base 10 °C, GDD) were 810, 845 and 659 day °C in S1, S2 and S3 respectively. From veraison until 95 DAA it was significantly warmer in S2 with an average maximum temperature of 35.8°C compared to S1 and S3, where the average maximum temperature was 28.6 °C and 28.3 °C respectively (Figure S1a, b). The GDD accumulated from veraison to 95 DAA increased from 410 day °C during S1 to 612 day °C in S2, and dropped in S3 to 434 day °C (Figure 2a).

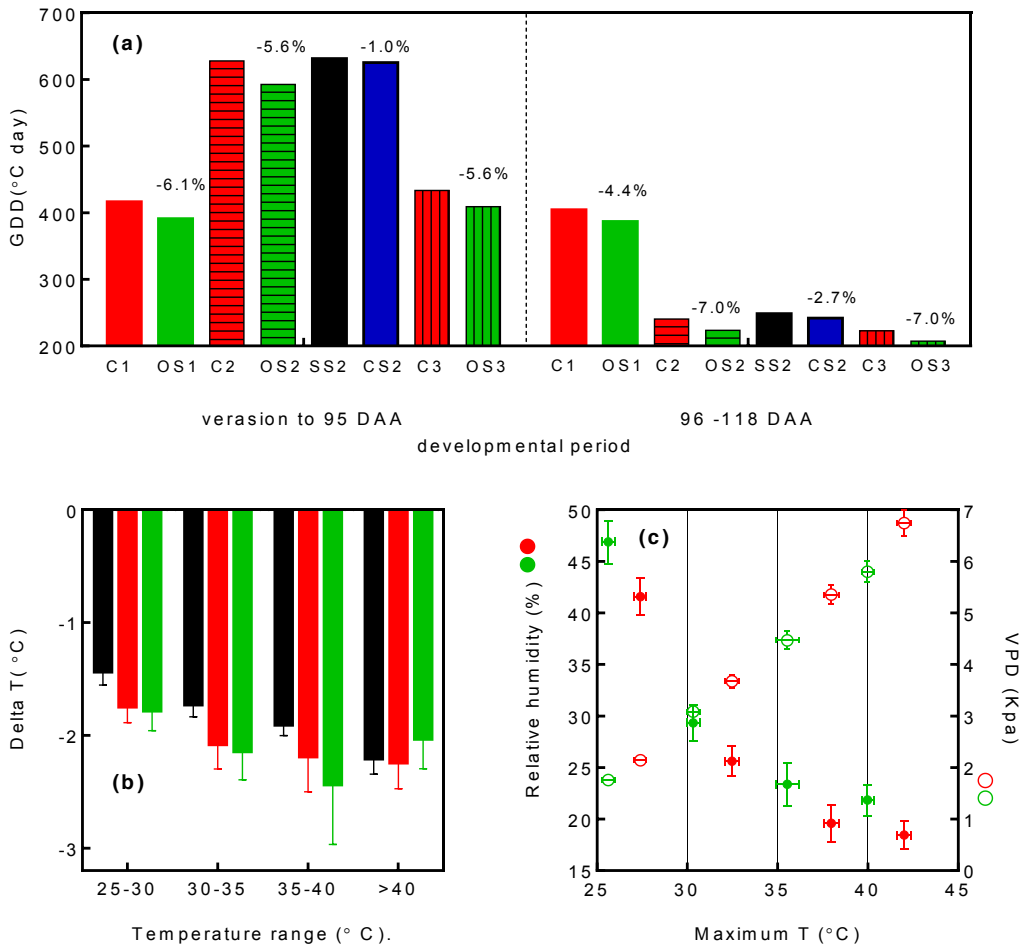

Figure 2. Growing degree days and canopy microclimate data for shade treatments at the Coombe vineyard site. (a) Growing degree days (base 10 °C) in all shade treatments at the Coombe site over three seasons (Indicated on x-labels 1-3). The ripening period (veraison to harvest) was divided for comparison between seasons based on the rainfall event that occurred in S2 at 95 DAA. S1 and S2: veraison to 95 DAA and from 96 DAA to 118 DAA. S3: veraison to 100 DAA and 101 DAA to harvest. (b) Reduction in maximum temperature in OS compared to control at four ambient temperature ranges from S1 (black bars), S2 (red bars) and S3 (green bars). (c) Mean relative humidity (left y-axis) and mean VPD (right y-axis) as a function of mean maximum temperature in four maximum temperature ranges for OS and control during S3.

There was a large rain event (100 mm) at 96 DAA during S2 and the period between this event and harvest at 118 DAA was much cooler with an average maximum temperature of 25.8 °C compared to 32.9 °C observed during the same period in S1, and 33.9 °C in S3 (in S3 this period was considered from 101 DAA to harvest). Also

the GDD accumulated during this period was lower during S2 (233 day °C) compared to S1 (400 day °C). For S3, GDD was also lower than for S1 (225 day °C) between 96 DAA and harvest (10 days shorter than S1 and S2). The number of days with a maximum temperature above 40° C during the ripening period was similar between S1 and S3 (4 and 5 days respectively) which contrasts with 11 days for S2 which is generally above the long term average (Figure S1a, b). At the McLaren Vale site during the treatment period the maximum temperature was above 40° C only 2 days during S1 and 8 days in S2.

Effect of shading treatments on vine microclimate

The overhead shade (OS) treatment decreased the maximum temperature by approximately 2.16°C (Figure 2b) compared to control when the maximum temperature in control vines was above 35°C. The decrease in maximum temperature caused by the OS treatment was consistent across the three seasons and over a wide range of temperatures (Figure 2b). There was no difference in the minimum temperature between shading treatments (data not shown). Based on the data collected in S3 relative humidity was increased in the OS treatment at the daily maximum temperature (Figure 2c), corresponding to a decrease in vapour pressure deficit (VPD) in shaded vines during the hottest period of the day (Figure 2c).

There was no significant difference in the average maximum temperature between control and the CS or SS treatments tested at the Coombe vineyard (Figure S2). At the McLaren Vale site where there was no OS treatment, canopy maximum temperatures were not different between treatments (Figure S3). Moreover, the full-canopy shade and bunch shade treatment tended to be hotter during the afternoon and morning

respectively compared to the control (Figure S3c). Lower GDD by around 6% was also observed during three seasons in the OS shade treatment compared to the control (Figure 2a) with no significant differences in GDD for other treatments compared to control.

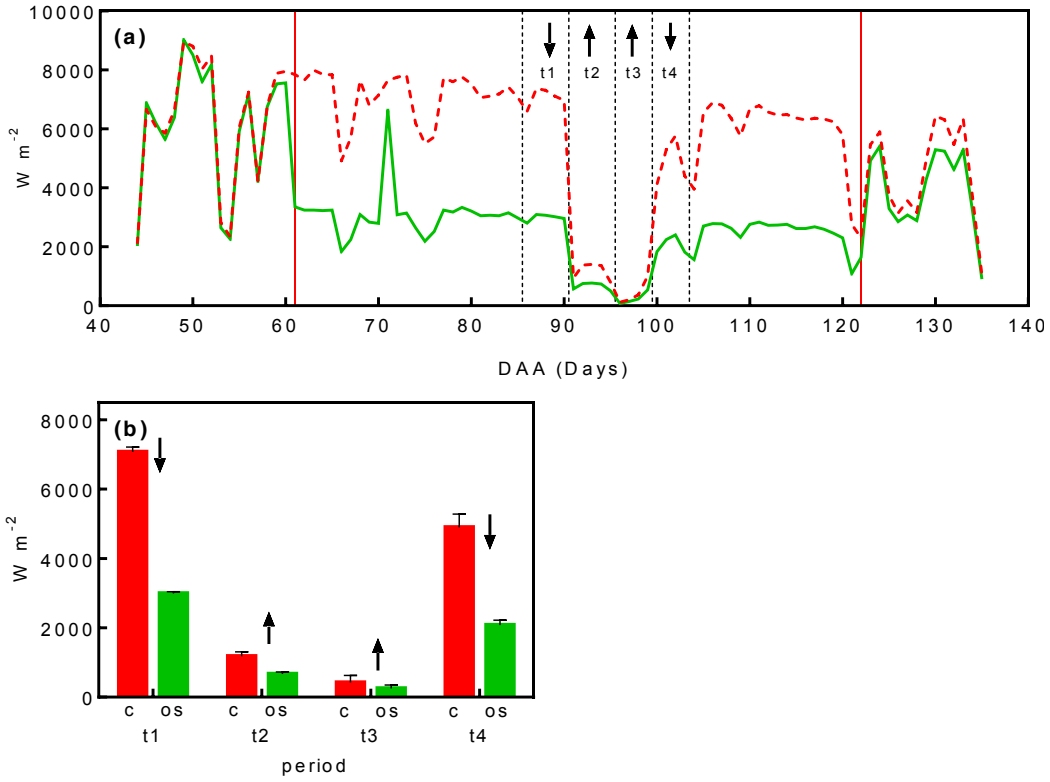

Figure 3. Effect of OS treatment on solar radiation relative to controls. (a) Total solar radiation in OS (green solid line) and control (red dashed line) vines during S2. Sensors were positioned at bunch height on the western side of the canopy. The start and end of shading is indicated by red vertical lines. The dashed black vertical lines indicate when the sensors were oriented to test differences in ground radiation; t2 and t3 sensors facing soil, t1 and t4, were used as comparative sky values in hot and cool conditions. Arrows above graph indicate direction of the radiation detected. (b) Mean ground radiation (replicated over time), reaching the canopy, in t1 and t4 from the sky and from the ground in t2 and t3. The corresponding % reduction of radiation that reached the canopy due to OS treatment was 67.7, 43.2, 41.5 and 67.6 % in t1, t2, t3 and t4 respectively.

The shade cloth used in this study blocked on average 62% of the total solar radiation (Figure 3a, for OS in S2) with the photon flux density (PFD) also decreased (Figure

S4) to daily maximum values lower than 600 μmol m^{-2} s^{-1} under the OS treatment compared to 1500 μmol m^{-2} s^{-1} in the control vines. From 86 to 95 DAA the radiometer was placed facing the ground from the western side of the canopy at bunch level, with the aim to examine the effect of the OS shading on reflected ground radiation. During this period there was a significant change in the weather so the data was split in to two portions (t2 and t3) of 5 warm days and 4 cool days respectively. T2 was compared to a 5 day period before this test (t1), and t3 was compared to a 4 day period where the radiometer was facing upwards (t4) (Figure 3b). Reflected ground radiation was reduced by the OS treatment being around 40 % lower than the values recorded in the control treatment in both periods (t2 and t3).

Leaf gas exchange

Net CO_2 assimilation as a function of PFD saturated between 500 to 900 μmol m^{-2} s^{-1} (Figure S5a) so A_{sat} was routinely measured at a PFD of 919 μmol m^{-2} s^{-1}. Additionally leaves from OS and C vines did not show an apparent shift in saturation during the treatment period (Figure S5b). Figure 4 shows A_{sat}, E, g_s and WUEi over time for S2 at the Coombe site for OS treatment and control. The reduction in A_{sat} and g_s for both control and OS treatments during the ripening period corresponds to periods of higher temperature (Figure 4a). The OS treatment had higher A_{sat} than control after 3 weeks from the beginning of the treatment. There were no significant differences between OS treatment and control in E or g_s in S2, but g_s measured using the porometer was higher in the OS treatment in S1 (Figure S5c). The higher A_{sat} but similar g_s resulted in higher WUE_i in the OS and CS treatment compared to their paired treatment (control and soil shade respectively) during periods at high temperature (Figure 4, Figure S6). A_{sat} versus g_s for OS and C (from 83 DAA, 3

weeks under treatment) could be fitted to an exponential curve, where both the rate constant and plateau are higher for OS compared to C (Figure 4f). Comparison of gas exchange parameters between CS and SS treatments in S2 showed no significant differences in A_{sat} though initially E was lower in the shade treatment (Figure S6).

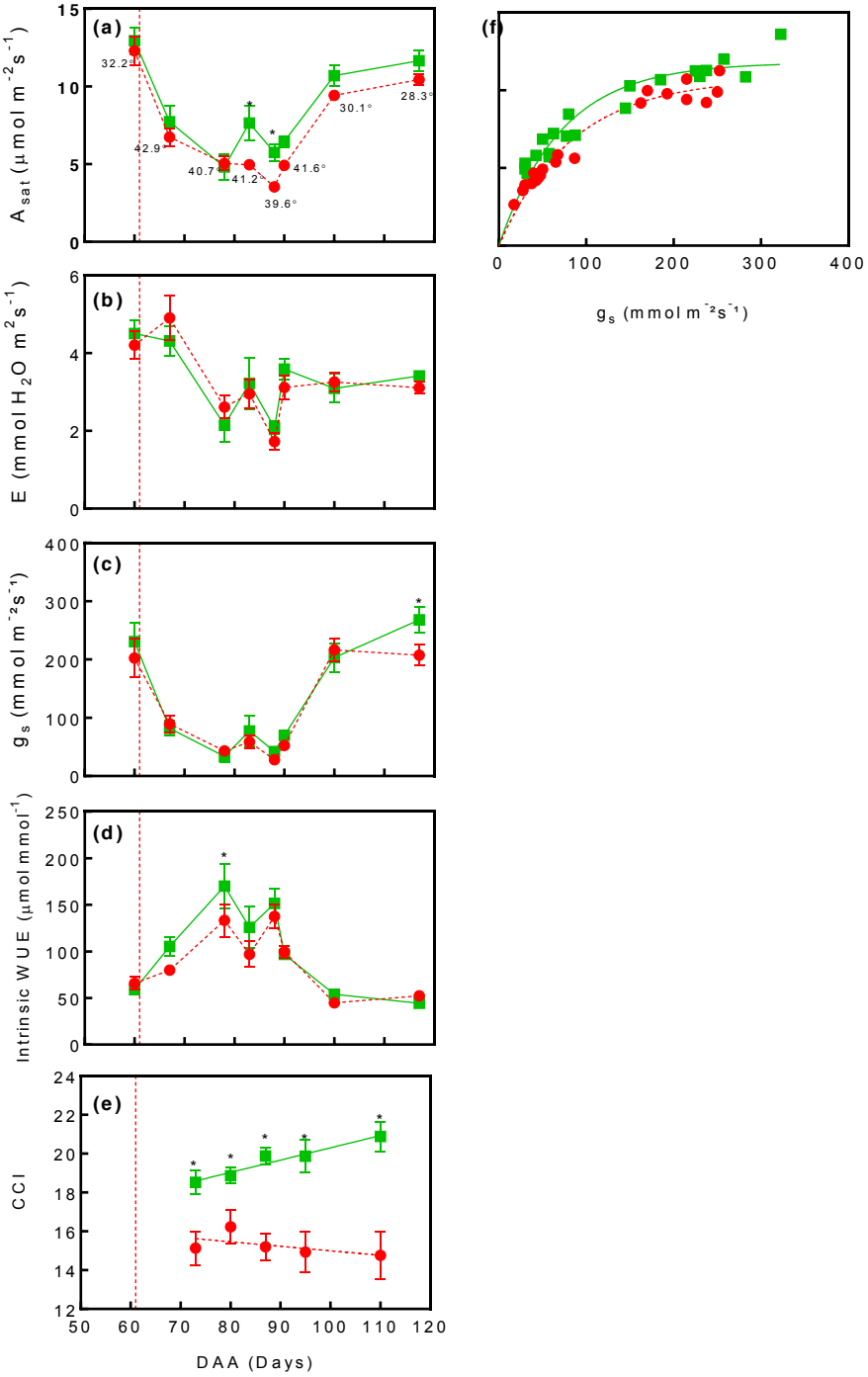

Figure 4. Gas exchange properties and relative chlorophyll during the treatment period in S2 for the OS treatment (green filled squares) and control (red filled circles, dashed lines) at the Coombe site. (a) Net CO_2 assimilation at light saturation (A_{sat}). Values under points indicate leaf temperature in the control vines during the gas exchange measurements. (b) Transpiration rate (E), (c) stomatal conductance (g_s), (d) intrinsic water use efficiency (WUE = A_{sat}/gs), (e) chlorophyll content (chlorophyll content index CCI, units). (f) A_{sat} versus g_s, from data after 83 DAA. The fitted curves ($A_{sat} = MaxA_{sat}*(1-exp(-K*g_s))$ are different for the rate constant (K) and the maximum A_{sat} ($MaxA_{sat}$) ($p<0.0002$). OS: K = 0.0139 $mmol^{-1}$ m^2 s^1, $MaxA_{sat}$ = 11.79 μmol m^{-2} s^{-1}. C: K = 0.0119 $mmol^{-1}$ m^2 s^1, $MaxA_{sat}$ = 10.79 μmol m^{-2} s^{-1}. Vertical dotted line in a,b,c,d,e indicate start of shade treatment. For a,b,c,d,e, n=4 with 4 technical replicates for each biological replicate, and stars above data points indicate significant difference ($P < .05$) within a particular time.

Relative chlorophyll content

Some differences in canopy appearance were detected late in S1 in the Coombe OS treatment where the leaves of control vines were pale green while in the OS treatment the leaves were bright green. This observation was corroborated by a measurement of relative chlorophyll content at 116 DAA, where it was found that control vines had on average 75.5% of the chlorophyll content of shaded vines (C =19.96 ± 0.7 CCI units; OS = 26.45 ± 0.7 CCI units, n=48). This trend was confirmed in S2 (Figure 4e), where it was found that the relative chlorophyll decreased in control vines and increased in OS vines. No differences in relative chlorophyll of leaves were found for the other shade treatments at the Coombe site (Figure S6e). However, for the McLaren Vale treatments there was also higher relative chlorophyll in the CS treatment relative to the control in S2.

Vine water relations

There was no significant difference between C and OS treatments in daily sap flow measured at the Coombe vineyard site during S2, and there was no significant interaction between treatment and time (2-way repeated measures ANOVA) (Figure 5a) as after OS shade had begun the difference in sap flow rates was not altered.

There was also no significant difference in maximum daily sap flow rates between the treatments (2-way repeated measures ANOVA).

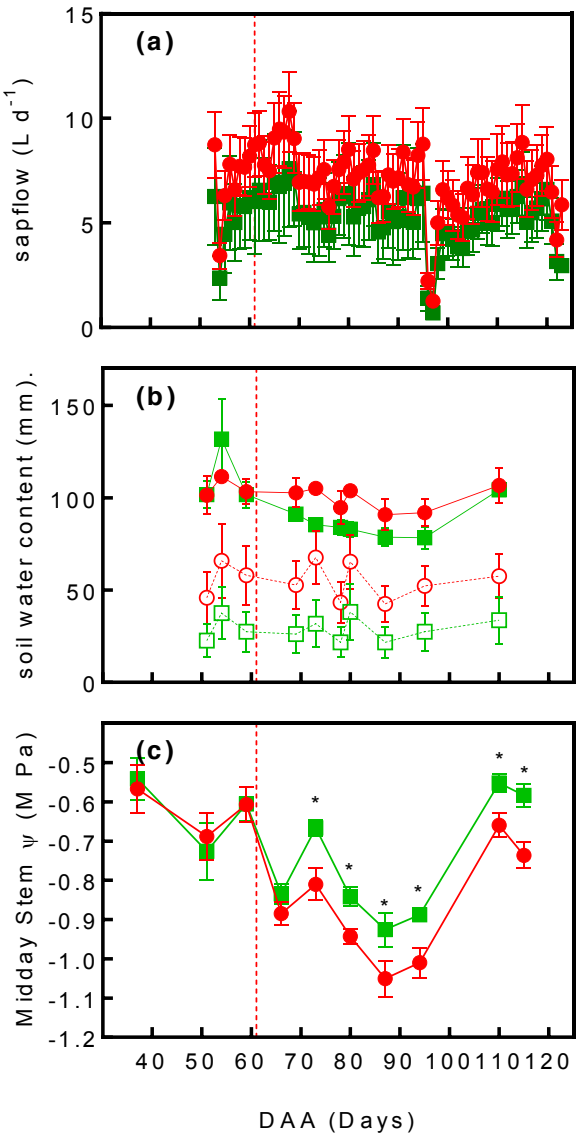

Figure 5. Vine and soil water relations for the OS treatment (green squares) and control (red circles dashed lines) at the Coombe site in S2. (a) Daily sap-flow (n=3 and 4, in OS and C). (b) Soil water contents at two depth ranges 0.1 to 0.5 m (open symbols), and 0.6 to 0.9 m (solid symbols) (n=4). (c) Midday stem water potential (n=4). Vertical dotted line indicates start of shade treatment. Data in (b) and (c) are, stars above points indicate level of significant (* $p < .05$) within a particular time (DAA).

The SS and OS treatments were expected to have a higher water content in upper layers due to lower soil evaporation by the end of the treatment. However, there was no significant difference in soil water content comparing OS with C (Figure 5b) and SS with CS (Figure S7) at the Coombe vineyard site.

Midday stem water potentials (ψ) during S1 (75 DAA) were not different between treatments at the Coombe site, although a higher value in the OS-treated vines was the trend (-0.84 ±0.02 MPa in OS compared to -0.91 ±0.03 MPa in C). For S2 when temperatures were higher, OS treated vines showed higher ψ compared to controls (Figure 5c). The CS treatment compared to SS showed a similar trend, but ψ was higher in CS only at 80 DAA (Figure S7b). The SS treatment was set up to examine the possible influence of cooler surface layers of soil and water content on vine water relations, and as a control for the effect of soil shading in the OS treatment. However, no significant differences in ψ were detected between the SS treatment and control treatment.

Berry maturation

The OS treatment at the Coombe site showed the largest effect of shade on berry maturation and composition compared to controls over the three seasons (Figure 6). Total soluble solids (TSS) were lower in berries from OS vines compared to the control in the three seasons, being on average 1.5 °Brix lower (Figure 6a). The linear phase of TSS accumulation for S1 and S2, determined by segmented linear regression ($R^2 > 0.9$) showed that OS and CS (Figure S8a) shade treatments had a lower rate of TSS accumulation in S2 compared to C and SS respectively (OS = 0.491, C = 0.549, CS = 0.493, SS =0.547 ^{o}Brix day^{-1}, $P<0.004$).

Fresh berry mass was increased by the OS treatment compared to control in S1 and S2, however loss in mass was observed in both seasons in OS treatment and C around 90 DAA (Figure 6b). No difference in the rate of decrease in fresh mass was detected between OS and C in S1, but in the warmer S2 the drop in berry mass in the control occurred earlier and to a greater extent than that of the OS treatment. A recovery in fresh berry mass after the rain event in S2 was observed in both treatments. A similar trend in TSS and fresh berry mass was observed between CS and SS treatments (Figure S8a,b). Only at one time point was there a significant difference in sugar content per berry between OS and C in each of S1 and S2 (Figure 6c). However, berries from the OS treatment had higher water content compared to controls, with this trend being consistent and significant for the three seasons (Figure 6d). Note also the increment in water content after the rain in S2. Except at one time point (100 DAA), no difference in berry water content was found between CS and SS (Figure S8d). No significant differences in TSS, fresh berry weight, sugar per berry, and berry water content were observed between the different shade treatments at the McLaren Vale site for the seasons examined (Figure S9).

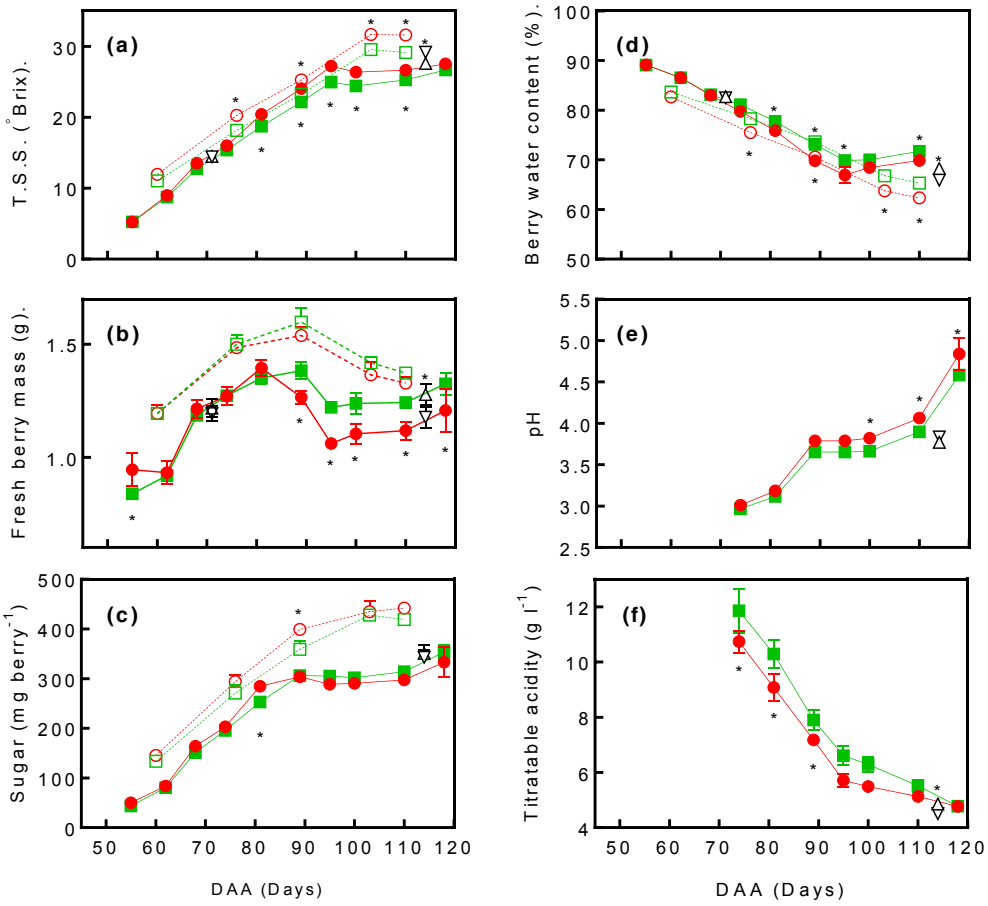

Figure 6. Berry composition during ripening over three seasons for the OS treatment (□,■,Δ) and control (○,●,▽) at the Coombe site in S1 (□,○ dashed lines), S2 (■,● solid lines) and S3 (at harvest: Δ,▽). (a) TSS, (b) fresh berry mass, (c) sugar per berry, (d) berry water content, (e) juice pH (S2 only) and, (f) titratable acidity (S2 only). Data are means ± SEM (n=4). For some data points errors bars can be smaller than symbols. Stars adjacent to data points indicate significant difference ($p < .05$) within a particular time (DAA) in a season.

Berries from OS treated vines had lower pH and higher TA compared to controls, and while pH increased as berry development advanced the titratable acidity decreased (Figure 6 e,f). At harvest in S3, TA was also higher in OS. Interestingly the decline in TA showed a clear linear trend up to about 95 DAA then a sudden decrease in rate corresponding to the cooler weather in S2 at this time. This could be well fit by a segmented linear regression. For OS and controls the break point occurred at 93.6 and

95.1 days respectively but were not different. Both lines had slopes that were not different between OS and C (-0.255 g L^{-1} day^{-1} and after 94 days -0.061 g L^{-1} day^{-1}). A similar trend in pH and TA was observed in CS and SS treatments (Figure S8e,f), where pH from SS berries were higher than CS berries three times in S1 and TA was higher in CS compared to SS only once.

There was a trend for total anthocyanins, phenolics, tannins and epicatechin concentrations in fresh berries at the Coombe site to be lower in the OS treatment compared to controls (Figure 7). A plateau in anthocyanin accumulation can be seen after 100 DAA in the control during S1 while the accumulation was seen to persist in the OS treatment. However, only on four occasions in the OS treatment were there lower values in anthocyanin concentration; 91 and 98 DAA in S1, and at 74 and 81 DAA in S2. For both seasons no differences were detected at harvest in anthocyanin concentration. For S3, lower anthocyanin accumulation occurred compared to the first two seasons but no significant differences were detected between treatments. No differences were detected between CS and SS treatments at the Coombe site (Figure S10a).

For S2 sufficient data was obtained for OS and C to analyse the relationship between anthocyanin concentration and TSS. This was linear and there was no significant difference between the regression lines for the OS ($R^2 = 0.97$) and C ($R^2 = 0.93$) data sets. The combined equation had the following parameters:

Anthocyanin = -0.783 (mg g^{-1}) + 0.0987 mg g^{-1} $^{o}Brix^{-1}$ * TSS ($R^2 = 0.95$).

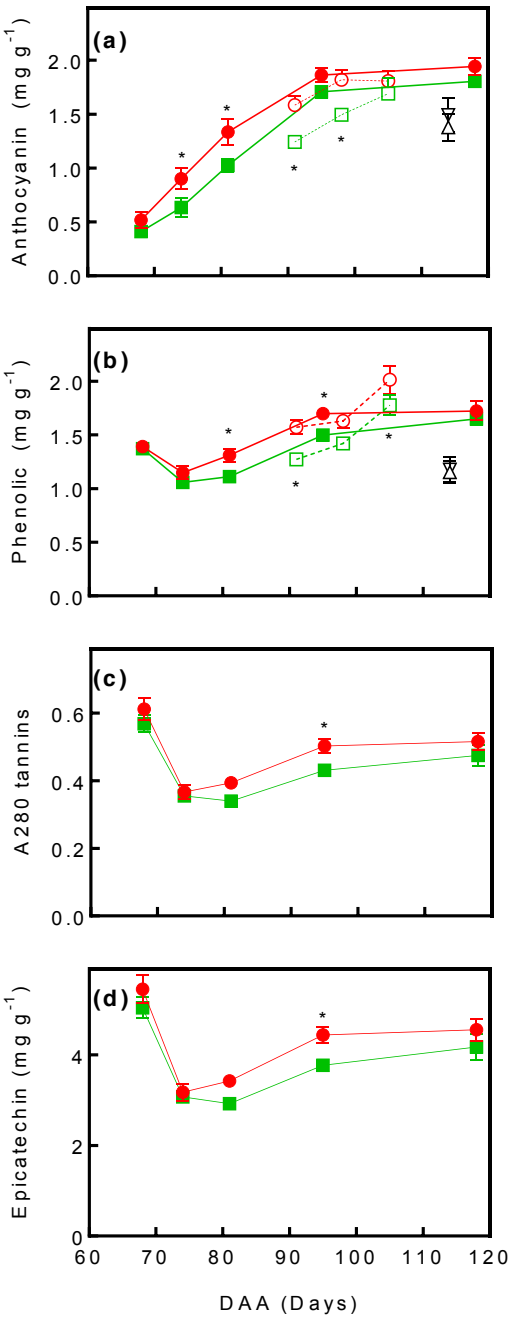

Figure 7. Berry colour, phenolic and tannin compositions during ripening in response to the OS treatment (□,■,△) and control (○,●,▽) at the Coombe site in S1 (□,○ dashed lines), S2 (■,● solid lines) and S3 (at harvest: △,▽). (a) Total anthocyanin concentration, (b) total phenolics concentration, (c) tannins and d) epicatechin concentration (S2 only). Data are means ± SEM (n=4). Stars adjacent to data points indicate significant difference ($p < .05$) within a particular time (DAA) and season.

In S2 no further increase in anthocyanin concentrations occurred for OS and C after the rain event (95 DAA). A similar cessation of accumulation can be seen in the phenolics, tannins and epicatechin concentrations during S2 (Figure 7). However, the phenolics accumulation persisted after 105 DAA in S1. Harvest in S3 showed the lowest phenolic accumulation over this study. Tannin and epicatechin content measured in S2 showed lower content in OS compared to C at 95 DAA only (Figure 7). A lower amount of tannin and epicatechin was detected in the CS treatment compared to the SS treatment at harvest (Figure S10).

Berry cell vitality

Berry cell vitality was consistently higher under OS than control after about 90 DAA in both seasons (S1, S2) that were measured (Figure 8a,b). The decline in berry cell vitality was higher in control berries than OS berries in both seasons. The data from the shade treatments was also predicted by the electrical impedance technique (Caravia et al, (2015) (Figure 8). The berry cell vitality predicted by impedance in the CS and SS treatments at the Coombe vineyard showed a similar trend to that observed for OS and C (Figure 8c). However, the CS treatment was only different from SS treatment at 95 DAA. No differences between shade treatments were observed in living tissue using impedance at the McLaren Vale site (Figure S9e) during S1. Examination of the relationship of living tissue with thermal time and combining the data for S1 and S2 (Figure 8d), indicated that after 990 °Cd the percentage living tissue declined linearly with thermal time with the slope steeper for C compared to OS treatment (C = -0.097 % $°Cd^{-1}$, OS = 0.059 % $°Cd^{-1}$, P=0.014). Also shown for comparison are the ranges in slopes of % living tissue with thermal time observed in another study on Shiraz berries (Bonada et al. 2013a).

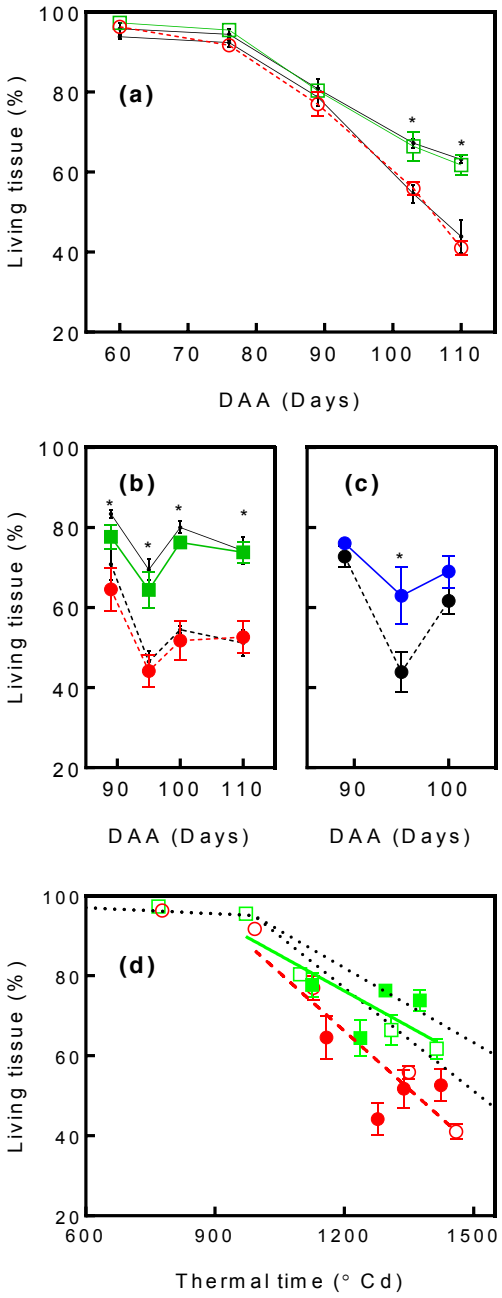

Figure 8. Berry cell vitality during ripening observed in the shade treatments using the FDA vital stain and electrical impedance. (a) OS treatment (□, green solid line) and C (○, red dashed line) treatments for S1. Black symbols and lines show % living tissue determined from impedance for OS and C. (b) OS treatment (■ green solid line) and C (● red dashed line) for S2, (c) impedance determination of % living tissue for CS (● solid blue line) and SS (● dashed black line) treatments. (d) Berry cell vitality as a function of thermal time for OS and C in S1 and S2. Fitted lines for each treatment (OS, green solid line; C red dashed line) combine two seasons, dotted lines are extreme values of the range in slopes i.e. heated and control from (Bonada, et al. 2013a), the onset was adjusted from Bonada et al to match our data (1080 GDD to

990 GDD). Data are means ± SEM (n=4). Stars above data points indicate significant difference ($p < .05$) within a particular time (DAA).

Yield and vine growth

Despite higher fresh berry mass being observed in the OS treatment compared to C, no differences were observed in yield per meter of vine row for this comparison, nor in any of the treatments by location and season combination. For the OS and C comparison, this may be explained by OS having a lower bunch number in S2 and S3 (Table S1). Pruning weight per metre of vine row was also not different between OS treatment and control (Table S1) and no differences were detected between these two treatments in leaf area index.

Wine characteristics

For S2 wine from all treatments (OS, CS, SS and control) at the Coombe site were made twice; 96 DAA before the rain event and at 118 DAA. For S3 wines were made from fruit harvested at 114 DAA. For S2, 96 DAA wine from the OS treatment had a lower alcohol concentration compared to SS and C (Figure 9). For the later harvest (118 DA) there was no significant difference in alcohol concentration. Although no significant differences were detected in S3, the lower alcohol trend was also observed.

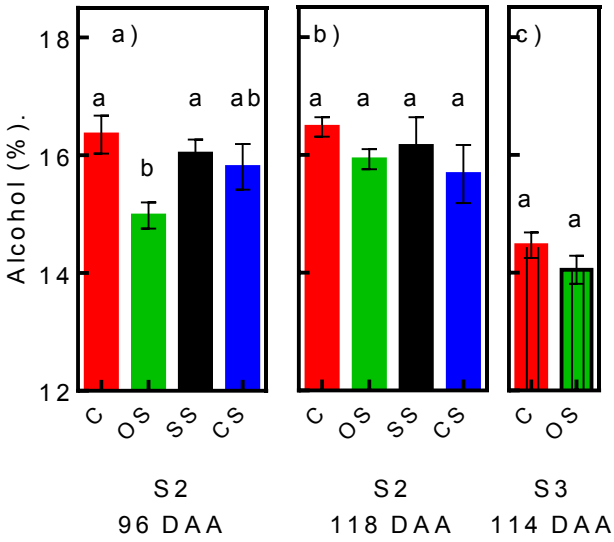

Figure 9. Alcohol concentration of wines made from all shade treatments for the Coombe site. In S2 at two harvest dates (a) and (b) (96 and 118 DAA) and (c) wine made at harvest date (114 DAA) in S3. Where the treatment are represented as Control (C), Overhead Shade (OS), Soil Shade (SS) and Side-Canopy-Shade (CS). Different letter indicates significant difference ($P<0.05$) within each harvest date. Data are means ± SEM (n=4).

Significant differences were observed between OS and C at bottling in several of the wine composition properties (Table 2,3,4). Chemical Age 2, Colour Density, Colour Density SO_2 corrected, were consistently lower in OS compared to control in S2 (both harvest dates) and S3. For CS in S2 similar trends, though not always significant were observed in these properties (Table 2 & 3). Total Phenolics was also lower in OS compared to control for both S2 and S3 (ignoring harvest dates in S2). This trend was also observed for CS compared to SS. Berry anthocyanin concentration was not different between OS and C at harvest and this is reflected in total anthocyanin concentration in wine not being different between OS and C.

Table 2. Wine composition for each shade treatment measured in the early (96 DAA) harvest in season 2013-2014 at the Coombe site. Data are means ± SEM (n=4). In each row, mean values followed by different letters are significantly different ($p < 0.05$).

	Control	**Overhead shade (OS)**	**Side-canopy shade (CS)**	**Soil Shade (SS)**
Chemical age 1 (au)	0.27±0.008^{ab}	0.24±0.004^{c}	0.25±0.009^{ac}	0.30±0.007^{b}
Chemical age 2 (au)	0.08±0.004^{ab}	0.07±0.002^{c}	0.07±0.004^{ac}	0.09±0.005^{b}
Degree of ionisation of anthocyanins (%)	21.02±2.46	20.98±0.5	19.74±0.45	21.58±1.1
Total anthocyanins (mg L^{-1})	544.02±27.92	495.68±23.5	537.71±18.5	486.17±19.23
Colour density (au)	13.18±0.76^{a}	11.23±0.57^{b}	11.60±0.32^{bc}	13.05±0.68^{ac}
Colour density: SO_2 corrected (au)	15.19±0.83^{ab}	12.20±0.39^{c}	13.20±0.59^{bc}	14.72±0.54^{b}
Hue	0.59±0.014^{a}	0.58±0.012^{a}	0.57±0.007^{a}	0.64±0.008^{b}
Total phenolics (au, 10 mm)	46.59±3.14^{a}	38.20±2.21^{b}	42.84±2.13^{ab}	45.25±1.42^{ab}
SO_2 resistant pigments (au)	2.61±0.19^{a}	1.92±0.066^{b}	2.10±0.15 ^{b}	2.69±0.121^{a}
A280 (tannin)	0.246±0.03^{a}	0.175±0.013^{b}	0.247±0.021^{a}	0.260±0.016^{a}
Epicatechin [mg L^{-1}]	1,088.8±150^{ab}	743.5±79^{a}	1,121.5±166^{ab}	1,159.6±71.5^{b}

Table 3. Wine composition for each shade treatment measured in the late (118 DAA) harvest in S2 at the Coombe site. Data are means ± SEM (n=4). In each row, mean values followed by different letters are significantly different ($p < 0.05$).

	Control	**Overhead shade (OS)**	**Side-canopy shade (CS)**	**Soil Shade (SS)**
Chemical age 1 (au)	0.19±0.009^{ab}	0.17±0.004^{a}	0.18±0.008^{ab}	0.19±0.008^{b}
Chemical age 2 (au)	0.05±0.004^{a}	0.04±0.002^{b}	0.05±0.003^{ab}	0.05±0.004^{ab}
Degree of ionisation of anthocyanins (%)	13.07±0.34	11.81±0.494	12.25±0.313	12.76±0.996
Total anthocyanins (mg L^{-1})	927.31±18.2	862.17±40.7	871.23±46.1	963.54±24.2
Colour density (au)	13.07±0.75^{ab}	10.51±0.39^{c}	11.30±0.61^{bc}	13.46±0.94^{a}
Colour density: SO_2 corrected (au)	19.59±1.03^{ab}	16.17±0.64^{c}	17.31±1.01^{bc}	20.58±1.24^{a}
Hue	0.53±0.005^{ab}	0.52±0.004^{a}	0.52±0.002^{a}	0.53±0.002^{b}
Total phenolics (au, 10 mm)	67.10±3.25^{ab}	58.75±2.5^{a}	62.07±3.115^{ab}	71.38±2.1^{b}
SO_2 resistant pigments (au)	2.49±0.245^{ab}	1.86±0.095^{c}	2.12±0.158^{bc}	2.650±0.19^{b}
A280 (tannin)	0.301±0.04	0.336±0.07	0.287±0.01	0.321±0.03
Epicatechin [mg L^{-1}]	1,804.6±259	2,000.7±432	1,707.8±85	1,912.6±160

Table 4. Wine composition for overheat shade treatment and control measured in wine made at (18 DAA) harvest in S3 at the Coombe site. Data are means ± SEM (n=4). In each row, mean values followed by different letters are significantly different ($p < 0.05$).

	Control	**Overhead Shade (OS)**
Chemical age 1 (au)	0.389±0.008	0.370±0.004
Chemical age 2 (au)	0.119±0.003[a]	0.099±0.001[b]
Degree of ionisation of anthocyanins (%)	22.07±0.537[a]	19.01±0.458[b]
Total anthocyanins (mg L^{-1})	440.40±21.610	408.40±15.93
Colour density (au)	13.53±0.321[a]	10.28±0.388[b]
Colour density: SO_2 corrected (au)	13.93±0.281[a]	10.65±0.445[b]
Hue (au)	0.67±0.014	0.65±0.006
Total phenolics (au, 10 mm)	45.79±1.718[a]	39.00±1.088[b]
SO_2 resistant pigments (au)	3.26±0.140[a]	2.39±0.088[b]
A280 (tannin)	0.410±0.04	0.347±0.02
Epicatechin [mg L^{-1}]	1288±139	1046±91.4

Discussion

Overhead shade had the largest effect on vine microclimate

In this study the overhead shade treatment (OS) caused a decrease in maximum temperature by an average of 2.16 °C. The maximum reduction in temperature occurred during heatwave conditions and may afford some degree of protection to heat sensitive physiological and biochemical processes in both leaves and berries. Similar microclimate effects of shading have been reported also in other studies on grapevines (Cartechini and Palliotti 1995, Greer and Weedon 2012, Greer and Weedon 2013, Greer, et al. 2010, Rana, et al. 2004). The decrease in maximum

temperature ranged from 1.1 °C (Cartechini and Palliotti, 1995) to 4-5 °C in the other studies. No significant decreases in maximum temperature were observed in the other shade treatments tested, and in some cases the effect of the placement of the shade cloth appeared to increase temperature during morning and afternoon (full-canopy shade and bunch shade treatments). Nevertheless, some physiological and berry development measures indicated an amelioration of the impact of heat stress in the CS treatment as indicated by similar trends in ψ, chlorophyll content, cell death, berry TSS and pH.

Shading has a significant positive effect on leaf photosynthesis and water use efficiency

Gas exchange measurements performed in S2 revealed that A_{sat} was increased relative to controls during heatwave events 23 days after the OS shading began. A_{sat} was on average 46 % higher (31-63%) in the OS shaded vines when the temperature was above 40 °C compared to the values observed in control vines. The side-canopy shade (CS) treatment did not show differences in gas exchange including A_{sat} compared to exposed vines in S2 (soil shade, SS) consistent with the lack of differences in internal canopy maximum temperature. We measured A_{sat} rather than A at ambient light to obtain a more consistent effect of shading on photosynthesis and to reduce the variation due to variable light intensities during field measurements. The light response curves we obtained are similar to those obtained in other studies (e.g. (Zufferey, et al. 2000)). It should be noted that light saturation of A occurred in exposed vines at a similar light intensity and near the same maximum intensity measured under the OS treatment. The higher A_{sat} under shading in our experiment was unexpected given that the generally reported response to shading is for saturation

of *A* to occur at a lower PFD and with a lower A_{sat} (e.g. Greer et al., 2011, Cartechini and Palliotti 1995). However these studies applied shading from budburst so that full leaf development occurred under shaded conditions. In our study the application of shade occurred at veraison when most leaf expansion and canopy growth had ceased. Therefore, it is likely that shading vines from as early as budburst involved different type of acclimation of the canopy, which could be exemplified by a decline in leaf biomass observed in Semillon (Greer, et al. 2011). The decrease in A_{sat} that we observed for all treatments and controls on days with temperatures near or above 40 ^{o}C is in accord with the approximately 50% reduction in *A* from its maximum near 35 ^{o}C that has been observed for Sultana leaves grown in the field (Kriedemann 1968). From this the higher A_{sat} under OS treatment may be simply due to the lower canopy temperature compared to controls, since at these extreme temperatures photosynthesis drops off rapidly with temperature (Kriedemann 1968). It is also likely that the higher relative chlorophyll concentration on an area basis observed in OS shaded vines (26-29%) compared to controls may account for the higher A_{sat} in the OS treatment. Relative chlorophyll content tended to decrease in control vines as the season progressed, while it increased in shaded vines. Similarly, higher chlorophyll concentration was reported for leaves before harvest in Sangiovese vines under shade (Cartechini and Palliotti 1995).

There were small differences in *gs* between OS and control, although, there was a trend of having a higher intrinsic water use efficiency in OS. A_{sat} versus *gs* showed a saturating relationship that could be fit to a single exponential for the purposes of testing differences between control and OS. This revealed a significant difference in the fitted curve ($P<0.0002$) so that at any given stomatal conductance the OS treatment gave higher A_{sat}. During the first season of this study porometer

measurements also showed higher *gs* in OS treatment compared to the control. An increase in *gs* has been reported for shading of table grapevines (Rana, et al. 2004). No differences were found in *E* between OS and control, even when the temperature was above 40 C, which is contrary to the much higher *E* observed in exposed Semillon vines compared to shaded vines during a heat wave (Greer and Weedon 2013). The almost identical behaviour of leaf level *E* between OS and control in our study is also manifest in no significant difference being detected in whole vine sap flow rates; either maximum daily rates or mean daily rates. This is best explained by there being a slightly higher *gs* for OS treated vines that offset the lower VPD in OS compared to control.

Vine water status was improved by OS shading

The ψ consistently showed higher values in OS treated vines, which was evident after the second week of treatment and thereafter until harvest. This increase in ψ was not observed in the other shade treatments tested, although a similar trend could be observed comparing CS and SS. Given that the OS treatment also shades the soil we checked that treatment effects could be related to reduced soil evaporation and would be indicated by a higher soil water content in upper layers of the soil under shade. No significant differences in soil water content were detected with the DIVINER 2000 and furthermore the SS treated vines essentially behaved the same as control. A similar increase in ψ was reported for shaded Sangiovese vines (Cartechini and Palliotti 1995). The fact that no differences were observed in sap flow rate or *E* while ψ was consistently higher, suggests increased whole vine hydraulic conductance under OS shade treatment.

Berry composition was a sensitive indicator of shade treatments

Consistently berry composition was strongly affected by the OS and CS shading treatments. We found that the rate of increase in TSS was lower in OS and CS treatment compared to controls. However the rate of increase in sugar per berry was the same and reached similar final values. In contrast a significant decrease in the amount of sugar per berry was observed in Cabernet Sauvignon vines under shade (Rojas-Lara and Morrison 1989). In our study berry water content was lower in controls than in the OS treatment indicating that the higher TSS in the controls may have been a consequence of berry dehydration. A strong effect of canopy shading from bud-break on TSS at harvest has also been reported in Sangiovese (Cartechini and Palliotti 1995) where shading decreased TSS by almost 5 °Brix compared to control. In a glasshouse study, potted Cabernet Sauvignon under side-canopy shade treatments from 2 weeks before veraison showed that TSS progressively decreased as shading intensity increased (Smart, et al. 1988). Semillon berries under side-canopy shade from budbreak to harvest during a warm season also showed a significant decrease in TSS at harvest (Greer and Weedon 2013). However, the opposite is also reported, in the same variety where berries from shaded vines showed higher total soluble solid content at harvest compared to the control (Greer and Weedon 2012). A lower pH and higher TA in berries from the OS treatment is in agreement with previous studies reporting the effect of temperature on pH and TA (Greer and Weedon 2013,Smart, et al. 1988). Lower temperature during ripening also reduces malate degradation (Sweetman, et al. 2014).

Shade placed along the side of the canopy (CS) appears to have some protective effect based on the similar rate of TSS accumulation to the OS treatment even though we could not detect a reduction in canopy temperature in the CS treatment. This is similar

to the results found in a previous study where Cabernet Sauvignon berries under side-canopy shade treatment (92 % block) applied from two weeks after flowering accumulated less TSS and had lower anthocyanin contents (Rojas-Lara and Morrison, 1989). However using berry TSS to assess the effects of side-canopy shade revealed no protective effect in another study (Ristic, et al. 2007) and perhaps indicating that side-canopy shade, although easier to install, has a relatively minor effect.

Anthocyanin, phenolics, tannins and epicatechins concentrations in berries were all consistently lower in the OS treatment compared to controls, but as for TSS this may reflect partially a dehydration effect in the controls. We found that the slope of linear phase of anthocyanin concentration versus TSS was identical between the OS and C treatments and similar to those reported by Sadras and Moran (2012) where Shiraz vines were subjected to heating and other treatments including water stress and variation in source sink ratio. We could not detect a difference in the onset of the linear phase of anthocyanins versus TSS as was found for heated vines compared to controls by Sadras and Moran (2012).

Berries developing under natural shade generally show lower anthocyanin content, (Archer and Strauss 1989, Bergqvist, et al. 2001, Jogaiah, et al. 2012). Field Pinot noir vines under natural shade had decreased flavonols (Cortell and Kennedy 2006). Bunch light exclusion after veraison appears to have contrasting effects compared to shading during the whole berry development period. For instance, a significant increase in methoxy isobutylpyrazine was observed in Cabernet Sauvignon berries when the bunch enclosed treatment were imposed from fruit set until verasion or harvest, but there was no increase when bunches were enclosed from veraison to harvest (Koch, et al. 2012). Enclosed bunches developed berries with more total anthocyanin concentration in the red grape cv. *Jingxiu* when the treatment was

imposed from fruit set to one week before veraison, but there was no effect on anthocyanin content when it was applied from veraison to harvest or at veraison (Li, et al. 2013). Similarly, differences observed in berry weight, sugar accumulation and total anthocyanin when treatments started at fruit set tended to be smaller or absent when the same treatments are started at veraison (Dokoozlian and Kliewer 1996).

Berry cell death is a sensitive indicator of temperature and water status

Berry cell death can be triggered by high temperature and water stress during ripening (Bonada, et al. 2013a,b, Caravia, et al. 2015). The OS treatment resulted in berries with a larger proportion of living tissue at harvest, but we also observed less cell death in the CS shade treatment despite no detectable difference in internal canopy temperature. Possibly the shading of bunches in the CS treatment lowered their temperature as there was less solar heating. This may indicate that berry cell death is a sensitive indicator of heat stress. The impedance technique used to predict the level of berry cell death was able to accurately determine the values without significant differences between the modelled data and the actual values measured, confirming that this technique may be used reliably as a rapid measure of berry cell death (Caravia, et al. 2015).

Heat and water-stressed vines show greater berry cell death in proportion to the degree of stress (Bonada, et al. 2013b). We found that the difference in temperature alone was not enough to explain the higher proportion of living tissue in the OS treatment compared to controls as judged by examination of the proportion of living tissue versus thermal time (Bonada, et al. 2013a). The OS treatment had a greater proportion of living tissue at the same accumulated thermal time as the controls. This suggests that water stress was also playing a role, since controls had lower ψ. It is

also interesting to consider the degree of berry weight loss observed in this context, since previously it has been shown that there is a correlation between the degree of weight loss (or shrivel index) and the degree of cell death in the berry (Fuentes, et al. 2010). In this study we observed a greater degree of weight loss in the control compared to the OS treatment in S2, which corresponded also to a much larger degree of cell death. The differences observed in S1 were less dramatic both in terms of the degree of cell death and berry weight loss.

Impacts of shading on wine

Microvinifications indicated that the OS shading resulted in a significant reduction on the final alcohol concentration in wine at bottling. To a certain extent the CS treatment also showed the same pattern but this was not significant. A trend of lower anthocyanin and percentage of anthocyanin in coloured form was observed in both OS (compared to control) and CS (compared to soil shade). Other colour properties of the wine were reduced (Colour Density, Colour Density SO_2 corrected) in OS treatment compared to control, however these were still in the range of values observed for young commercial Shiraz wines (AWRI 2012). Reduced anthocyanin and other colour properties in wine from Shiraz grapes has been observed after light elimination from flowering (Ristic, et al. 2007). In our treatments not only minor shading occurred but also maximum daily temperature was reduced in the OS treatment and there was evidence of less water stress in the shaded vines. A study on the combination of heating and water stress on Shiraz vines showed that colour density and colour density SO_2 corrected in wines were reduced in response to heating for both water stressed and normally irrigated vines (Bonada, et al. 2015). From this study we would expect the opposite result to that which we observed; thus it would

appear that the interaction of shading, water stress and temperature results in more complex interactions that precludes prediction of final wine composition from shading alone of from separate effects of heating and water stress.

Short term shading does not affect vine growth or yield potential.

The short -term shading treatments from veraison to harvest used here, although having a significant effect on several important aspects of berry composition and vine physiology, did not change yield, leaf area index or pruning weight. This indicates that the canopy modifications experienced in the shade treatments were not enough to have broad effects on vine growth or yield potential. In contrast, a significant decreased in these parameters has been reported in shaded Sangiovese vines treated from budburst to harvest (Cartechini and Palliotti 1995). Therefore, not only the timing in which the treatments are imposed will be relevant in having an effect on grape composition, but also the length of the period under treatment that can cause significant changes in vine performance and compromise vine sustainability.

Conclusions

Overhead shade (OS) was the most effective treatment, having a consistent decrease in the maximum temperature and lower VPD at the bunch zone inside the canopy. The lack of differences in maximum temperature in the other treatments probably reveals the role that free air movement had on canopy microclimate. The overhead shade treatment was able to protect the vines during heatwaves, which was evidenced by a higher CO_2 assimilation rate, higher WUEi, less water stress and higher chlorophyll content. On balance berry composition was impacted in a positive way by the overhead shade treatment by reducing berry cell death and dehydration, which

lowered TSS resulting in lower alcohol wines without a large impact on other properties of wines. Therefore, overhead shade treatments at or before veraison (8 °Brix) to harvest has a beneficial impact in berry composition, vine performance and could assist in reducing the level of alcohol in wine.

Acknowledgments

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Supplementary material

Table S1. Yield, and vine growth components (leaf area index LAI_e, shoots per meter and prunning weight per meter) in shade treatments. Values are mean ± SEM of 4 field replicates at Coombe and mean ± SEM of 3 field replicates at McLaren Vale (McL.V.). Diferent letter shows significant diference at a location and within season.

Treatment	Location	Season	Yield (kg m^{-1})	Bunch m^{-1}	LAI_e	Shoot m^{-1}	Prunning (kg m^{-1})
C	Coombe	S1	1.53±0.27	14.5±2.1			
OS	Coombe	S1	1.93±0.20	17.4±1.6			
C	Coombe	S2	2.05±0.15^{a}	18.7±1.5^{a}	1.72±0.05	18.8±0.74	1.41±0.05
OS	Coombe	S2	1.98±0.20^{a}	14.1±1.1^{b}	1.66±0.07	18.25±0.51	1.49±0.11
CS	Coombe	S2	1.65±0.16^{ab}	12.4±1.0^{ab}	1.59±0.05		
SS	Coombe	S2	1.25±0.12^{b}	11.7±0.8^{ab}	1.73±0.09		
C	McL.V.	S1	1.07±0.05	16.1±1.2			
FCS	McL.V.	S1	1.14±0.19	18.8±1.1			
BS	McL.V.	S1	1.39±0.05	18.8±1.5			
C	McL.V.	S2	1.32±0.44	23.0±3.8		18.68±2.60	0.60±0.14
CS	McL.V.	S2	1.12±0.13	21.5±0.9		17.60±1.30	0.55±0.07
C	Coombe	S3	3.31±0.32	27.3±1.8^{a}		19.63±0.46	1.06±0.02
OS	Coombe	S3	2.83±0.17	22.9±1.6^{b}		19.54±0.86	1.07±0.07

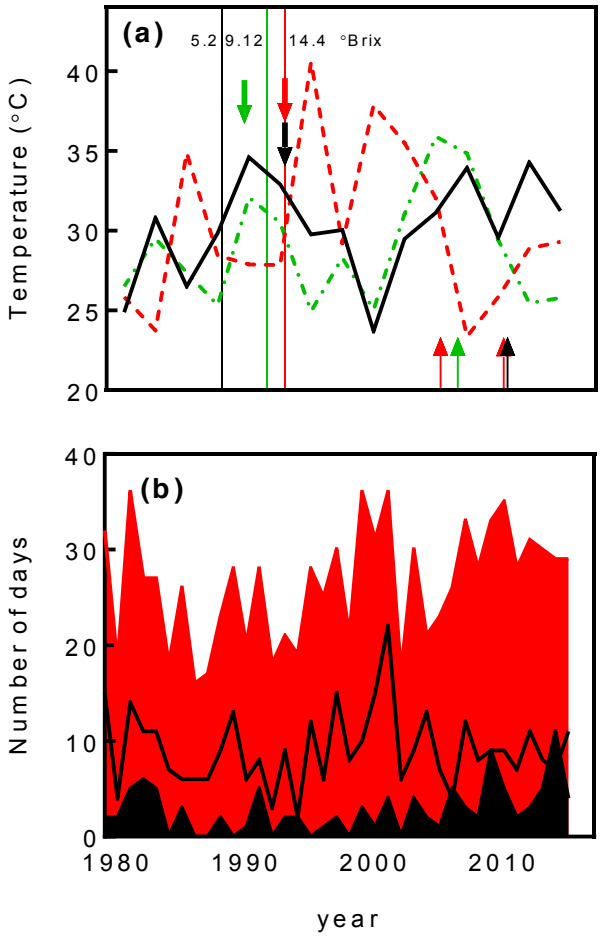

Figure S1. Frequency of heat wave conditions during berry ripening (Kent town weather station, Adelaide). (a) Maximum mean temperature (weekly mean) during ripening period (1/12 to 14/03) during three seasons. S1 (black line), S2 (red dashed line) and S3 (green dash-dotted line). Vertical lines indicate the start of shading; the numbers indicate the average TSS at the start of the treatments; arrows facing downwards indicate veraison and arrows facing upwards indicate harvest. (b) Number of days in the period between January and February since 1978 to 2015 with maximum temperatures above 30° C (red area), between 30° and 35° C (black solid line) and days with maximum above 40° C (black area).

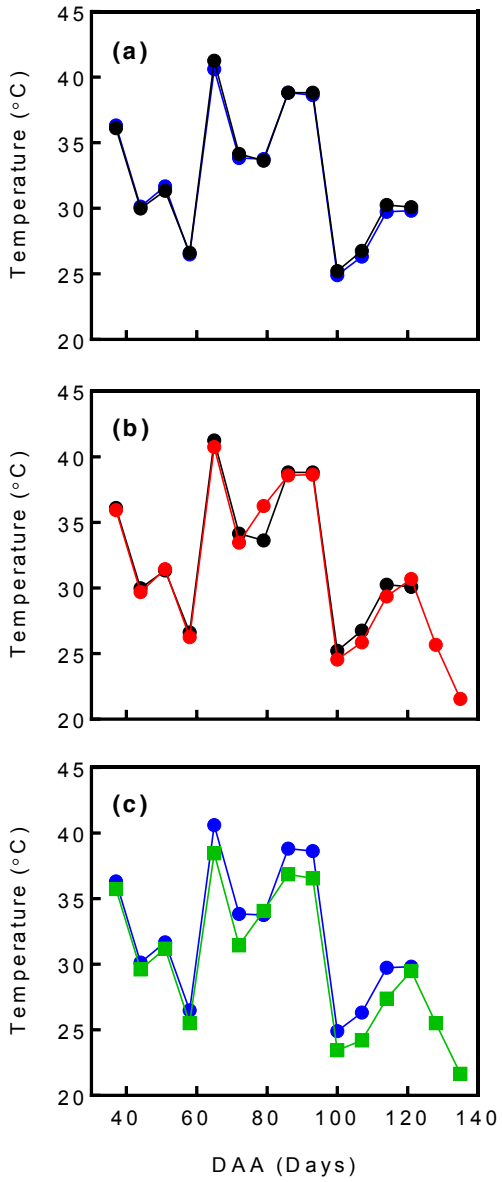

Figure S2. Daily maximum temperature inside the canopy in shade treatments at Coombe vineyard during S2, values are weekly means. (a) Soil shade (SS) (●) compared with side-canopy shade (CS) (●), (b) control (C) (●) compared with soil shade (SS) and, (c) overhead shade (OS) (■) compared with and side-canopy shade (CS).

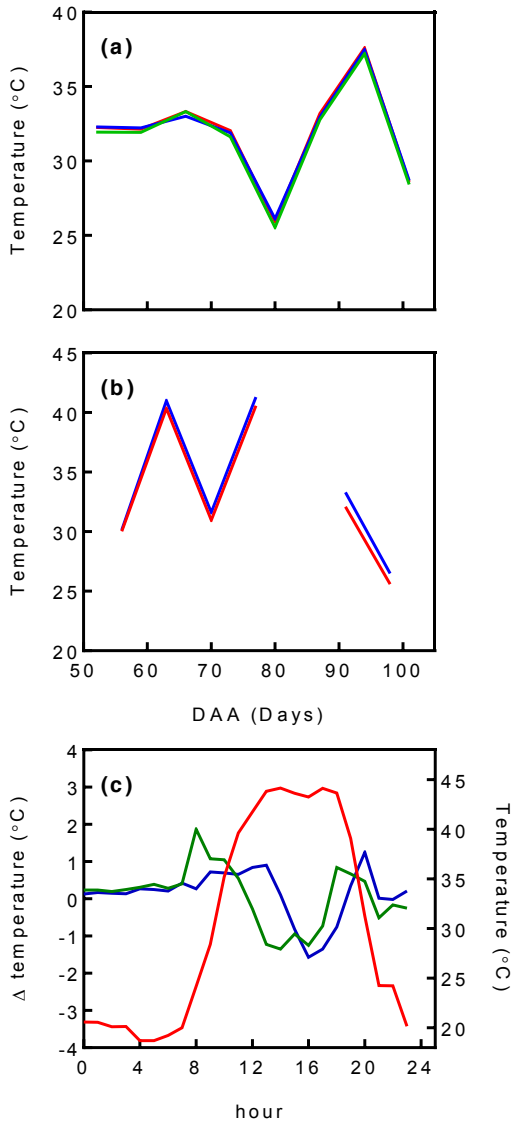

Figure S3. Daily maximum temperatures inside the canopy in shade treatments at McLaren Vale vineyard over two seasons (S1 and S2). (a) Full-canopy shade (FCS) (green), bunch shade (BS) (blue) and control (C) (red) in S1. (b) Side-canopy shade (CS) (blue), control (C) (red), values are weekly means. The gap in the data was due to equipment failure. (c) Hourly temperature difference from control for FCS (green) and BS (blue) on 07/01/2013. Also shown is the temperature observed in the control vine (red, right y-axes).

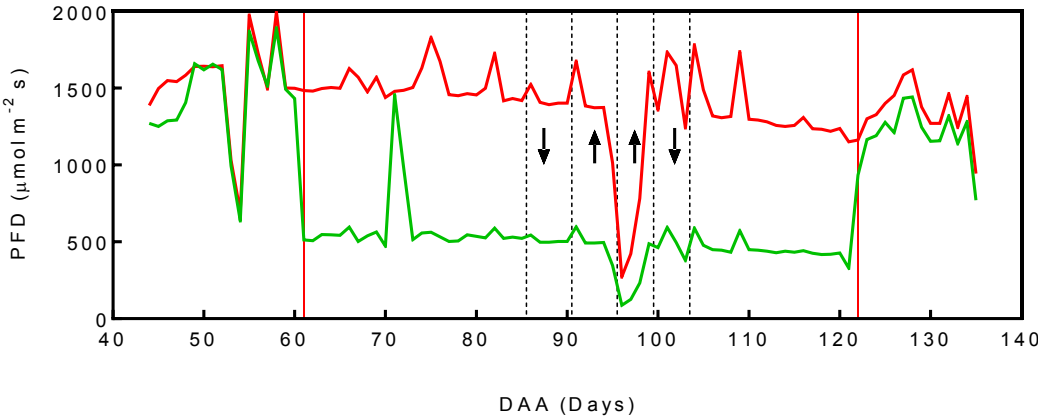

Figure S4. Changes in daily maximum photon flux density (PFD) in OH (green) and C (red) during S2. Vertical red lines indicated the start and end of the treatments. The dashed black vertical lines indicated the periods of time (t2 and t3) where the radiometer sensors where facing the ground in order to test differences in ground irradiation.

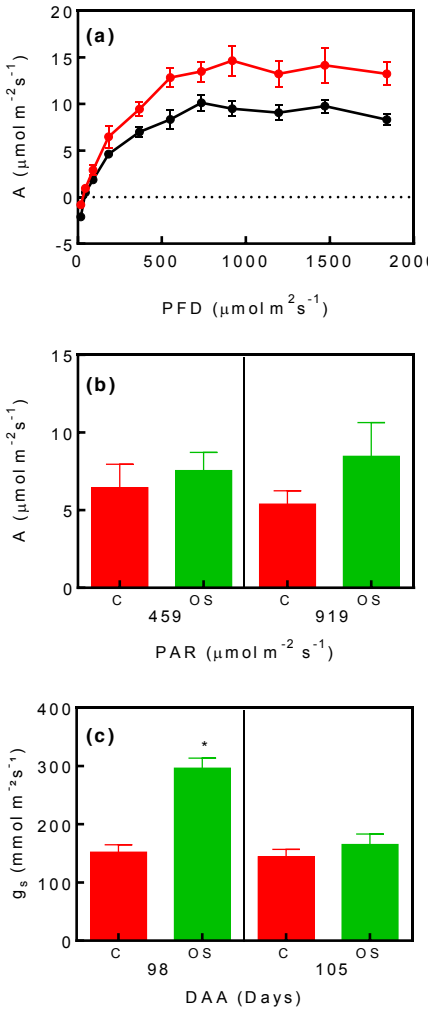

Figure S5. Preliminary gas exchange and porometer measurements on OS (green bars) and C (red bars). (a) Net CO_2 assimilation (A) in response to PFD on leaves from the western (●) and eastern side of the canopy (●) measured at 17 DAA in S2. Data are mean ± SEM (n=5). (b) A measured at 83 DAA in S2 at two PFDs (459 and 919 µmol m^{-2} s^{-1}) tested on one of the replicates. Data are mean ± SEM (n=4 leaves). (c) Stomatal conductance (g_s) measured with the porometer in OS and C treatments at 98 and 105 DAA in S1. Data are mean ± SEM (n=12 and 16). Stars above data points indicate significant difference ($p < .05$) within a particular time (DAA).

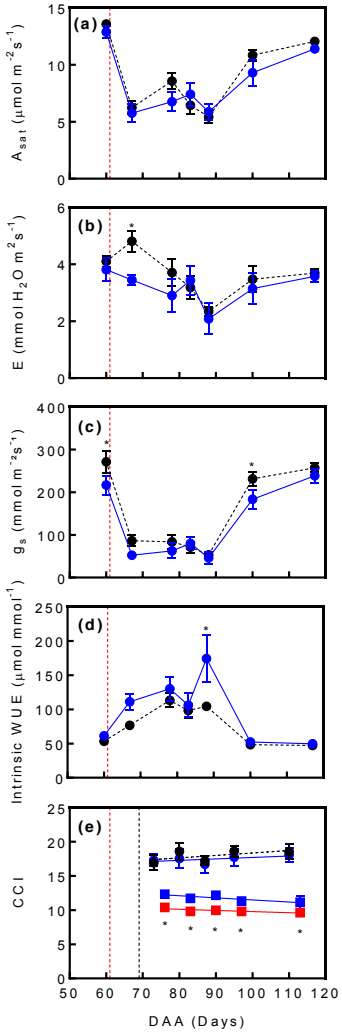

Figure S6. Gas exchange and relative chlorophyll during the treatment period in S2 for the SS (●, dashed line) and CS (●). (a) Net CO_2 assimilation at light saturation (A_{sat}, 919 µmol $m^{-2}s^{-1}$), (b) transpiration rate (E), (c) stomatal conductance (g_s), (d) intrinsic water use efficiency (WUE = A_{sat}/gs), e) chlorophyll content (CCI units), where side-canopy shade (■) and control (■) treatments from McLaren Vale in S2 are also included. Vertical dotted line in (a),(b),(c),(d), and (e) indicate start of shade treatment, and vertical line at 69 DAA in (e) indicates start of shade treatments at McLaren Vale. Data are means ± SEM (n=4) (n=3, McLaren Vale). Stars adjacent to data points indicate significant difference ($p < .05$) within a particular time (DAA).

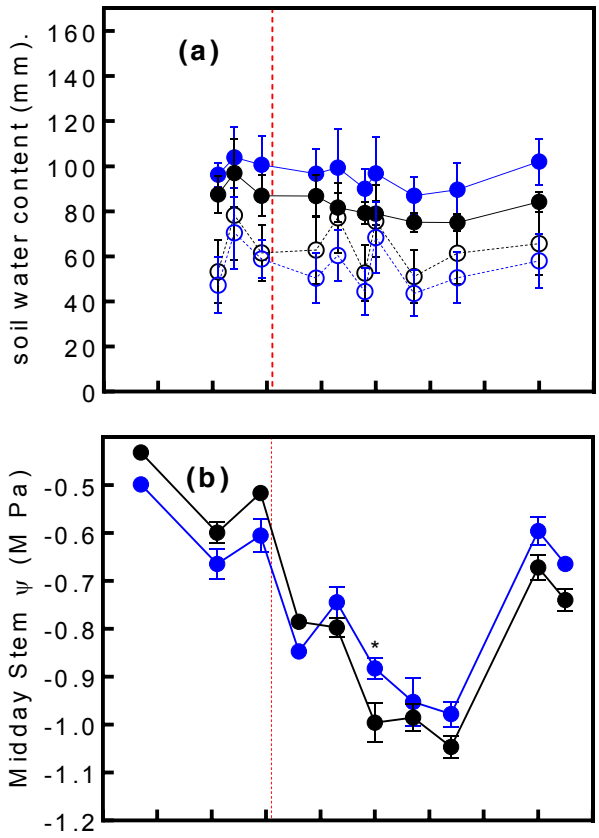

Figure S7. Soil water contents and midday stem water potential for CS and SS treatments. (a) Soil water contents in CS (●,○) and SS (●,○) treatments at two depth ranges, from 0.1 to 0.5 m (○,○) and from 0.6 to 0.9 m (●,●). (b) Midday stem water potential during treatments for SS (●) and CS (●). Data are mean ± SEM (n=4). Stars above data points indicate significant difference ($p < .05$) within a particular time (DAA). Vertical dotted lines indicate start of shade treatment.

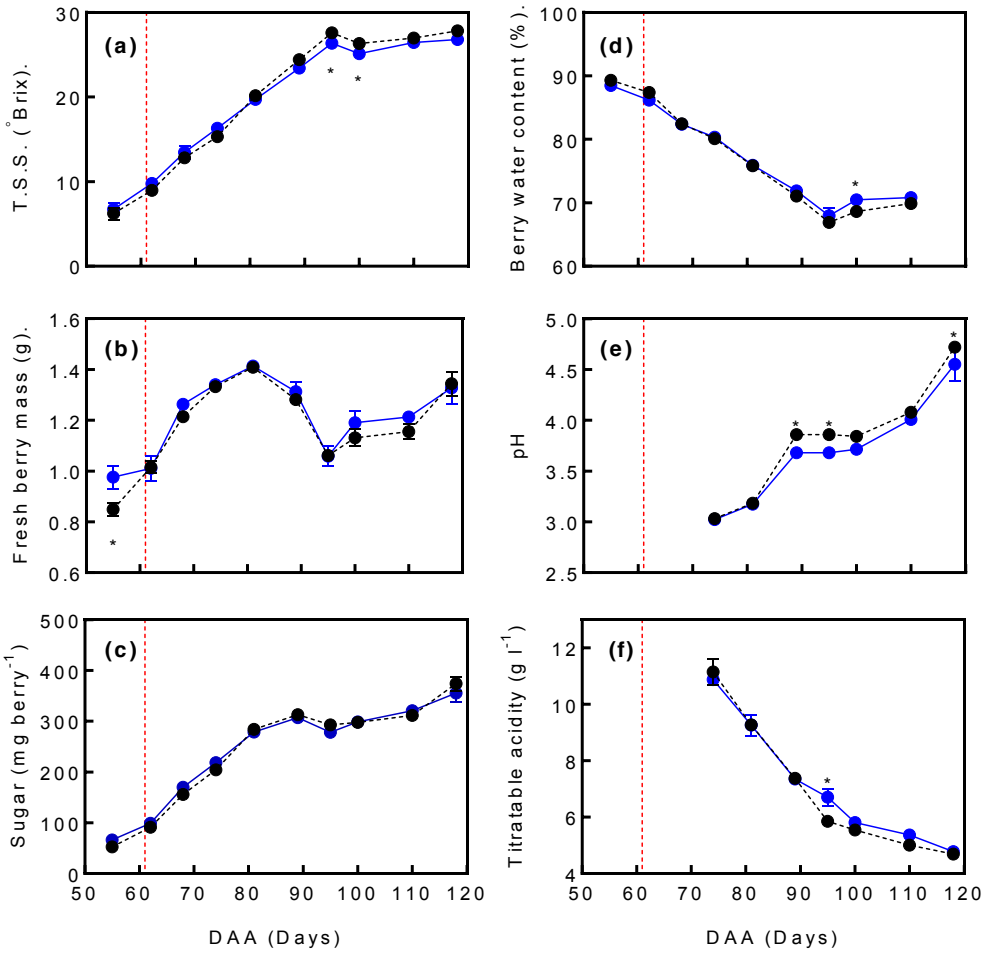

Figure S8. Changes in berry composition over S2 in shade treatments, CS (●) and SS (●, dashed line). (a) TSS, (b) fresh berry mass, (c) sugar per berry, (d) berry water content, (e) juice pH, and (f) titratable acidity. Data are means ± SEM (n=4). For some data points error bars are smaller than symbols. Stars adjacent to data points indicate significant difference ($p < .05$) within a particular time (DAA).

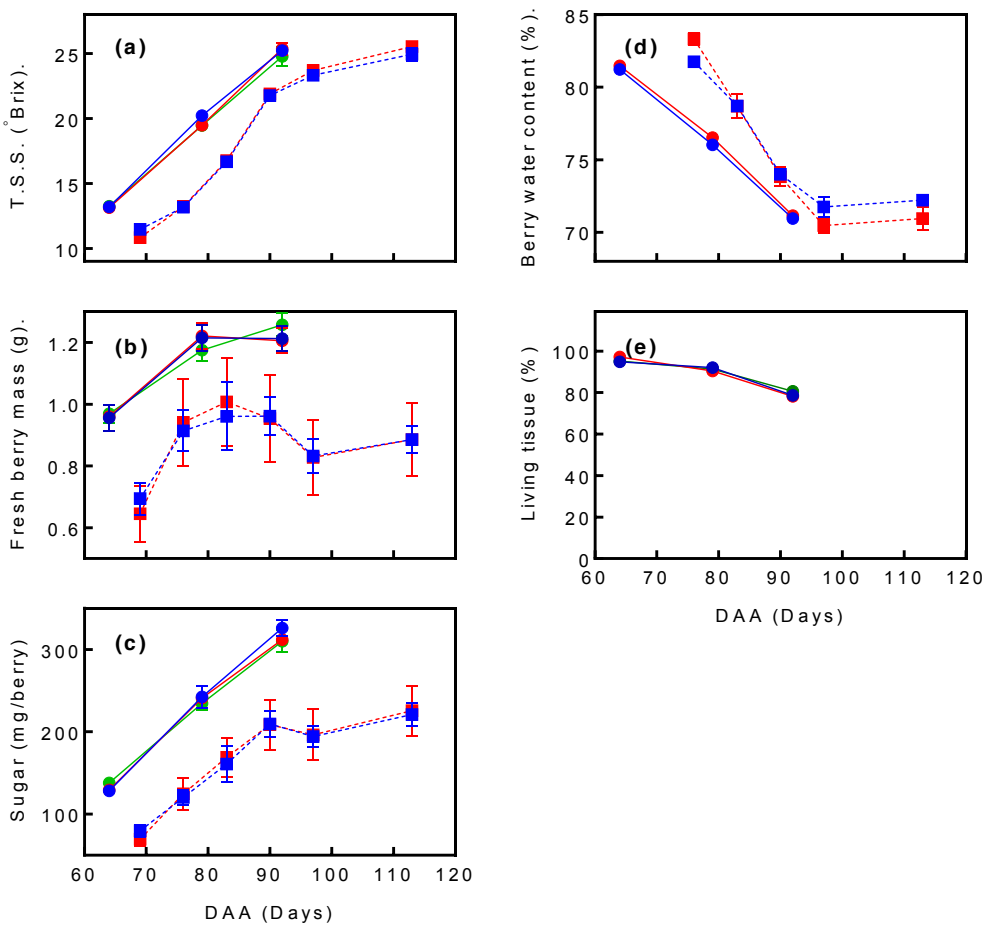

Figure S9. Changes in berry composition during ripening over two seasons (S1 and S2) at McLaren Vale in shade treatments. Full-canopy shade (FCS) (●), bunch shade (BS) (●) and control (C) (●) treatments from S1 (solid lines). Side-canopy shade (CS) (■) and control (C) (■) from S2 (dashed lines). (a) TSS, (b) fresh berry mass, (c) sugar per berry, (d) berry water content and (e) proportion of living tissue in berries from S1, based on impedance. Data are mean ± SEM (n=3).

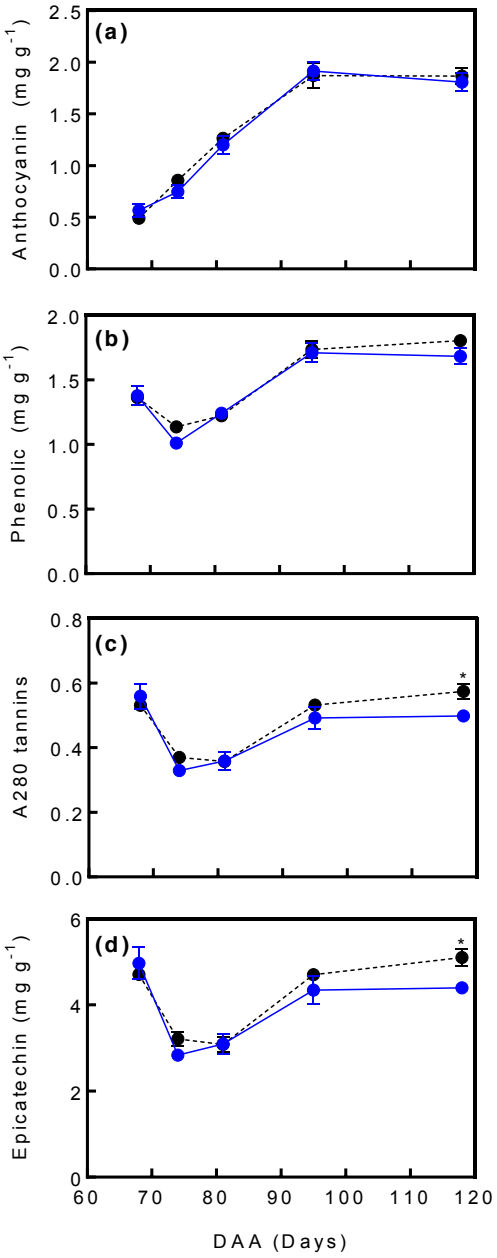

Figure S10. Berry colour and phenolics during ripening in response to the side-canopy shade CS (●) and soil shade SS (●, dashed line) in S2. (a) Total anthocyanin concentration, (b) total phenolics concentration, (c) tannins and (d) epicatechin concentration. Data are means ± SEM (n=4). Stars above points indicate significant difference ($p < .05$) within a particular time (DAA).

Chapter 4

Application of hydro-cooling sprinkler treatment during Cabernet Sauvignon ripening to reduce the impacts of high temperature

Manuscript submitted on 11 September 2015 to Australian Journal of Grape and Wine Research. AJGWR-15-115

Statement of Authorship

Title of Paper	Application of hydro-cooling sprinkler treatment during Cabernet Sauvignon ripening to reduce the impacts of high temperature
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Principal Author

Name of Principal Author (Candidate)	Luciano Caravia Bayer
Contribution to the Paper	Developed the research idea, designed and conducted all research experiments, analysed the data and drafted and constructed the manuscript.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	21/09/2015

Co-Author Contributions

Name of Co-Author	Vinay Pagay
Contribution to the Paper	Contributed to editing the manuscript and to data analysis by developing the energy balance equation, the modelling presented in this chapter and provided a draft of this section.
Signature	Date 21/9/15

Name of Co-Author	Cassandra Collins
Contribution to the Paper	Contributed to editing the manuscript, supervised the winemaking, berry and wine assessments.
Signature	Date 21/9/15

Name of Co-Author	Stephen Tyerman		
Contribution to the Paper	Contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.		
Signature		Date	21/9/15.

Abstract

Background and aim: Sugar concentration in harvested berries is steadily increasing throughout the world. This increase has been positively correlated to high temperatures during ripening, loss of berry mass and water stress. This study aimed to assess the impact of an evaporative cooling system located inside the grapevine canopy to mitigate heatwave events during ripening.

Methods and Results: During three seasons from 2012 to 2015 a misting system was tested on Cabernet Sauvignon vines. The system cyclically misted 20 seconds every 10 minutes when the air temperature was above 38° C, cooling the air inside the canopy by approximately 3° to 5° C. This was in agreement with an air cooling simulation based on a model presented in this paper. The relative humidity in the canopy also increased in the sprinkler-cooled canopy. No differences were detected in TSS, although sugar per berry was higher in treated vines because of increased berry mass. Anthocyanin and polyphenol per berry were also higher in cooled berries and higher anthocyanin concentration was observed in wine from cooled fruit.

Conclusion: Despite the lack of differences in TSS concentration between cooled and uncooled grapes, the increase in berry mass and yield, suggest the sprinkler system could be a useful tool for warm wine regions without a detrimental impact on fruit quality and wine.

Significance of the study: This research demonstrates the potential of a sprinkler cooling system as a tool to mitigate heat stress during ripening.

Keywords: berry ripening, heat stress, cell death, evaporative cooling treatment, climate change.

Introduction

Climate warming and its impact on winegrapes and resultant wines have been widely reported from many wine regions around the world (Webb et al., 2005, Jones, 2007a, Jones, 2007b, Webb et al., 2007a, Webb et al., 2008, Hall and Jones, 2009, Jones, 2009, Duchêne et al., 2010, Keller, 2010, Tomasi et al., 2011). An increase in the level of sugar at harvest in red cultivars due to elevated temperatures is widely accepted (Petrie and Sadras, 2008, Mira de Orduña, 2010, Sadras and Petrie, 2011, Sadras and Moran, 2012). An increase in juice pH and lower acidity have also been linked to this warming trend (Spayd et al., 2002, Anderson et al., 2008). An increase in the level of berry shrivel negatively affecting yield is expected (Fuentes et al., 2010, Bonada et al., 2013a, Bonada et al., 2013b) and berry anthocyanin content can be negatively affected if berry ripening occurs in very high temperatures (Spayd et al., 2002, Downey et al., 2006, Sadras and Moran, 2012). A threshold of 37.8° C was suggested to be the maximum limiting temperature for berry development, beyond this maximum value sunburn (for exposed berries), berry desiccation and constrained growth would arise (Aljibury et al., 1975). Berry cell vitality is widely linked to the degree of heat stress and water stress on vines over the ripening period (Bonada et al., 2013a, Bonada et al., 2013b, Caravia et al., 2015a), but its use as an indicator of berry health has been restricted due to time demanding vital staining and imaging techniques. Here we examine berry cell vitality using impedance spectroscopy, which is a more rapid and easily applied technique that has been shown to be able to accurately determine berry cell vitality in Shiraz (Caravia et al., 2015a) and impacts of heat stress (Caravia et al., 2015b).

There are a limited number of practical tools to mitigate heat stress in the vineyard. Shade cloth as an overhead layer was effective at lowering maximum temperature

compared to several other canopy shading options (Greer et al., 2010, Caravia et al., 2015b), and its application during the ripening period had a positive impact on Shiraz berry cell vitality, sugar concentration and berry mass, resulting in reduced alcohol concentration in wine (Caravia et al., 2015b) . However, its practical use in a commercial vineyard could be limited by cost, so other options for cooling of canopies during heat waves need to be further explored. Heat stress protection through overhead evaporative cooling systems have been tested previously in a wide range of horticultural crops, such as apples (Iglesias et al., 2005), pears (Dussi et al., 1997, Wand et al., 2004), oranges (Brewer et al., 1979), potatoes, bush beans (Hobbs, 1973) and strawberries (Chesness and Braud, 1970). In all the above cases, significant decreases in air or canopy temperatures were observed. This hydro-cooling technique has also been tested in grapevines. Higher fresh berry mass, larger berries and lower TSS concentration were observed in Chardonnay, Riesling and Chenin Blanc vineyards when a sprinkler system was operated for air temperatures above 32° C from veraison to harvest, (Aljibury et al., 1975). Similarly, fresh berry mass was higher in Cardinal, Carignane and Riesling (potted vines), when a sprinkler system watered the canopy for air temperatures above 30°C (Kliewer and Schultz, 1973). In this case the cooling effect was tested from flowering to veraison and from veraison to harvest; differences in sugar concentration were only observed for the flowering to harvest treatment. Berry ripening was faster in Semillon vines when they were cooled from flowering to harvest when the canopy temperature exceeded 35° C (Greer and Weedon, 2014). These previous studies used overhead sprinkler systems that mainly watered the outer canopy layer, with some water potentially reaching the internal canopy and bunch zone.

The aim of the present study was to assess the effects of applying the sprinkler cooling system inside the canopy at the bunch level from veraison to harvest when air temperatures exceeded 38° C and to examine whether cooling would reduce the occurrence of cell death in berries, which has been associated with high temperatures and water stress. We also examined the factors that contribute to the relative efficiency of cooling with respect to the water used and the cooling of the canopy.

Material and methods

Experimental site and design

Own-rooted Cabernet Sauvignon grapevines (*Vitis vinifera* L.), planted in 1997 at the Alverstoke vineyard at the Waite Campus of the University of Adelaide (34°58'04.06" S and 138°38'13.15" E) were trained to a sprawl canopy, with north-south row orientation. Vines were planted at 2 m between vines and 3.3 m between rows. Standard irrigation and viticulture practices typical for the region were applied. During the ripening period, vines were irrigated for 6 h twice a week, dripper distance was 0.75 m and dripper water rate of 1.6 L h^{-1} (8.5 mm $week^{-1}$).

Treatments

Over the three seasons, S1 (2012-13), S2 (2013-14) and S3 (2014-15) an evaporative cooling system using four sprinklers CoolNet Pro™ "tee" configuration per panel (Netafim Australia Pty Ltd. Adelaide, Australia, see http://www.netafim.com.au/product/coolnet-pro-) with a water use rate of 11 L h^{-1} per sprinkler unit (44 L h^{-1} per panel of four vines) were located inside the canopy at bunch height (30 cm above the cordon arm) distributed in four panel replicates and another four panels without sprinklers were used as the control (Figure 1). The system

was distributed over two rows (2 sprinkled and 2 controls panels of 4 m each per row), the treatments were interleaved leaving one panel free as a buffer. Potable water from the mains water supply was stored in a 1000 L plastic tank and a pump provided the 0.5 MPa pressure required in the system. The system was operated manually and was turned on when the forecast suggested a chance of warmer temperatures for days above 40° C (target temperature). The air temperature before turning on the system was around 35° C. Water was applied for 1 minute every ten minutes (0.333 mm h^{-1}) during S1. Due to some water runoff to the ground in S1, the application time was reduced in S2 and S3 to 20 seconds every 10 minutes (0.111 mm h^{-1}). Anthesis (EL Stage 20, Coombe 1995) occurred at 02/11/12 (S1), 25/10/13 (S2) and 26/10/2014 (S3), veraison occurred at 71, 78 and 68 days after anthesis (DAA) in S1, S2 and S3, respectively. Each field replicate was harvested separately at 131, 139 and 129 DAA in S1, S2 and S3, respectively, when bunch numbers and yields per vine were recorded.

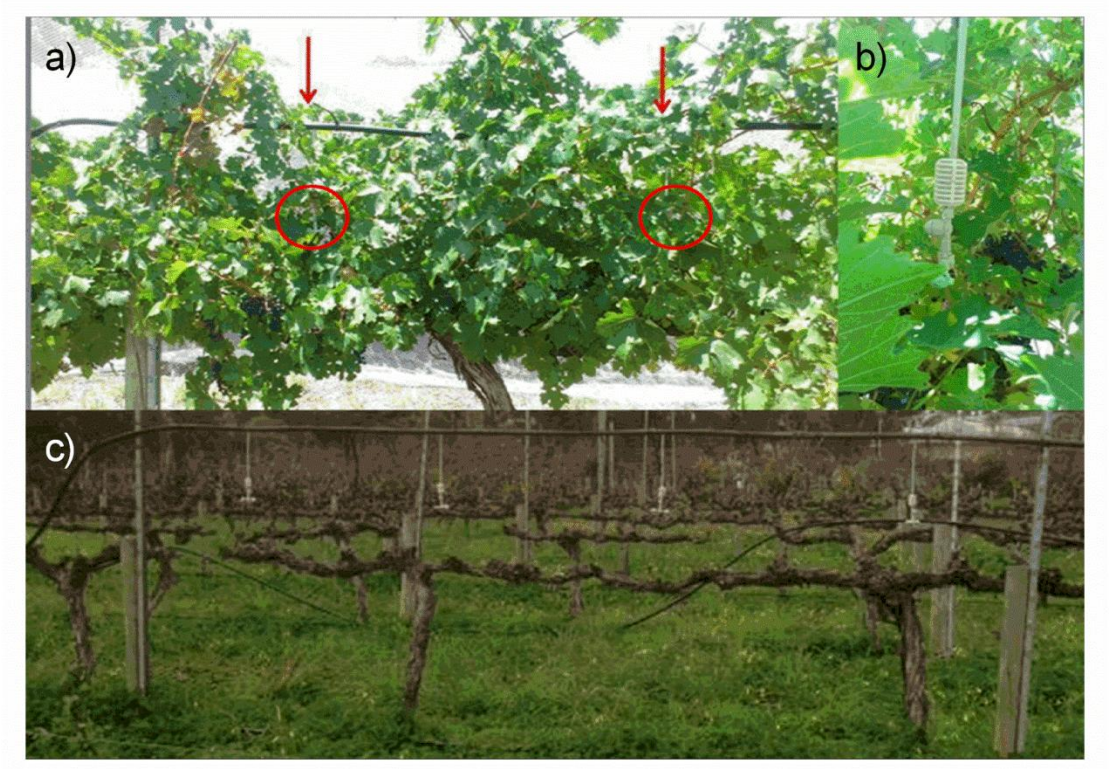

Figure 1. Sprinkler treatment layout in Cabernet Sauvignon. In a) panel view showing at the top of the canopy a 25 mm poly line that feeds the sprinkler allocated inside the canopy at bunch level. Red arrows indicated the sprinkler connection point to pipe and red circles indicate the sprinkler location (4 per panel). In b) sprinkler view from inside the canopy. In c) panel view after pruning.

Canopy temperature

Two temperature data loggers per treatment were installed in the middle of the canopy. The sensors were protected by a shell made of foil bubble wrap following the design described in Tarara and Hoheisel (2007). The sensors recorded temperature (Tiny Tag model TPG-4017), and temperature and relative humidity (Tiny Tag model TPG-4500, Gemini Data Loggers, West Sussex, UK) every minute. A thermal imaging camera (Model T360, FLIR, Wilsonville, USA) was used once to collect canopy temperature during S2 (14/01/2014) when air temperature was around 40° C. Growing degree days (base 10° C) was calculated based on the air temperature inside the canopy according to equation 1. Where

$T°_{min}$ and $T°_{max}$ are daily minimum and maximum temperature respectively.

$$GDD_{10°C} = \sum((T°_{min} + T°_{max})/2)\text{-}10) \quad \text{Eqn. 1}$$

Due to the suggestion that 37.8° C is an upper threshold for grape berry development (Aljibury et al., 1975), and in order to isolate the extreme heat ($HE_{>37.8°C}$) effects on berry ripening, a second growing degree day $GDD_{37.8°C}$ (Equation 2) which incorporated an upper threshold of 37.8° C was calculated. Thus, if the daily maximum temperature exceeds 37.8° C, then the real value was replaced by 37.8° C. Otherwise, equation 1 was used. $HE_{>37.8°C}$ was calculated by subtracting $GDD_{37.8°C}$ from $GDD_{10°C}$ (Equation 3) and represents relative quantification of the extreme heat above the threshold selected.

$$GDD_{37.8℃} = \sum((T°_{min} + 37.8℃)/2) - 10, if\ T°_{max} > 37.8℃ \quad \text{Eqn. 2}$$

$$HE_{>37.8℃} = GDD_{10℃} - GDD_{37.8℃} \quad \text{Eqn. 3}$$

Evaporative Cooling Model

A simple energy balance model was used to predict the evaporative cooling effect (canopy air temperatures) of the sprinklers. The model was based on conservation of specific enthalpy in which heat transfer occurs from sensible heat of air to latent heat of water vapour (Monteith, 1965, Rajput, 2007). It assumes constant air pressure and non-limiting heat input or incident radiation to the system. The enthalpy balance equation is given by Rajput (2007):

$$c_p T_1 + W_1 \lambda = c_p T_2 + W_2 \lambda \quad \text{Eqn. 4}$$

where c_p is the specific heat capacity of water (4.182 kJ g^{-1} $°C^{-1}$ @20°C), T_1 and T_2 [°C] are the initial and final temperatures of the canopy air, respectively, λ is the latent heat of vaporization of water (2.45 MJ kg^{-1} @20°C), and W_1 and W_2 are the humidity ratios of air before and during sprinkler operation, respectively. The humidity ratio (W=$mass_{vapour}/mass_{dry\ air}$) can be calculated using the relative humidity (RH, %) values of air and the saturation vapour pressure of air at temperature T_1, $p_{sat}(T_1)$ (Eqn. 5):

$$W = \frac{\varepsilon \times RH \times p_{sat}(T_1)}{P - [RH \times p_{sat}(T_1)]} \quad \text{Eqn. 5}$$

ε is the ratio of the molecular weight of water vapour to dry air (= 0.622) (Allen et al., 1998), P is the barometric pressure of air [bar]. $p_{sat}(T_1)$ [bar] is calculated using Antoine's equation using component-specific constants in the range of 1-100°C

(Thomson, 1946). The evaporative cooling model was developed using MATLAB programming software (v.R2013a, The MathWorks, Inc., Natick, MA, USA). The model was designed identical to the experimental setup with four dual-sprinkler heads located inside the grapevine canopy having a total nominal flow rate of 44 L h^{-1} within one panel of two vines having a total canopy volume of approx. 6 m^3 (4.0 × 1.5 × 1.0 m). The model was based on continuously-operating sprinklers, and a run-off volume fraction (water not used for evaporative cooling of air) was estimated at 30% as the canopies were observed to be wet during sprinkler operation (Figure 2cd). Predictions of air cooling as a function of sprinkler rate and ambient air temperature were made using the real values of temperatures and RH as measured inside the canopy. The Actual Water Use of Cooling (WUC_a) represents the volume of water misted that is needed to decrease the air canopy temperature by 1° C; these were computed from the data recorded. The Potential Water Use of Cooling (WUC_p) is the theoretical cooling simulated by the model as the maximum potential cooling capacity assuming no runoff, i.e. 100% of the sprinkled water is evaporated. The Actual Cooling Efficiency of Water (CEW_a) is the ratio of WUC_a and WUC_p and represents the air cooling efficacy of the sprinklers.

Maturity sampling

Berries from the top portion of shaded labelled bunches for each field replicate were collected by cutting the pedicel with scissors. Each replicate was comprised of berries collected from one panel (2 vines per panel) where berries were collected from the whole panel except the area 0.2 m from each end. During the first season 30 berries were collected from each of the field replicates and during the second season 80 berries. Samples were stored in a Ziploc plastic bag, and then placed in an insulated

box cooled with ice in the field (the ice was not in contact with the samples). Once in the laboratory subsamples were then taken to measure fresh and dry berry mass, berry impedance (Caravia et al., 2015a), total soluble solids , total anthocyanin, total phenolics, total tannin, pH and total acidity. During S1 the maturity assessment was performed four times (70, 80, 94 and 111 DAA), in S2 samples were collected eight times (70, 78, 84, 89, 96, 106, 115 and 126 DAA) and in S3 the measurements were done at harvest (129 DAA).

Impedance assessment and berry cell death

A group of 10 berries were randomly selected from each field replicate during S1. Impedance spectroscopy was performed on each berry as described in Caravia et al. (2015a). Briefly, a slice of skin (2-3 mm diameter) was removed on opposite sides of the berry equator using a razor blade. The skinless portion of the berry was covered with electrolyte solution (0.1 M KCl) and the berry was gently clamped between two disc electrodes with the distance between electrodes recorded. Impedance was measured on the berry at frequencies between 1 Hz and 2 MHz using a computer controlled impedance meter (Bode 100, Vector Network Analyzer, OMICRON LAB, Kowloon, Hong Kong). After the impedance measurement the berries were weighed and crushed for TSS determination.

Berry total soluble solids, titratable acidity and pH

In S1 after the impedance test was conducted, 10 berries from each replicate were individually crushed and the TSS was determined from the juice for each berry using a temperature compensated digital refractometer (Model PR101, ATAGO, Tokyo, Japan). For S2 and S3, a sub-sample of 50 berries were crushed, juice collected and

then centrifuged for 5 minutes at 2000 rpm before the measurement was taken on the clarified juice (Iland et al., 2004). Ten ml of clarified juice was diluted in 40 ml deionised water for pH and total titratable acidity (TA) measurements using an Autotitrator (CRISON, CompacT titrator, Crison Instruments, S.A., Barcelona, Spain). This measurement was performed 4 times during the ripening period (96, 106, 115 and 126 DAA) in S2 and at harvest in S3.

Anthocyanin and polyphenol berry content

Fifty berries were sub sampled from each field replicate at 115 DAA in S2 and at 129 DAA in S3. Berries were detached by removing the peduncle using a razor blade, the fresh berry mass was recorded and the sample stored at -20° C until the measurement was conducted. The total anthocyanin content expressed as mg of total anthocyanin per gram of berry and the total phenolic content expressed as mg of total phenolic compound per gram of berry were estimated from absorbance at 280 nm and 520 nm respectively on homogenised thawed berries following the technique described by (Iland et al., 2004).

Dry berry mass and berry water content

A group of 10 berries was sub-sampled to determine fresh and dry berry mass. Each berry was carefully cut from the pedicel using a razor and weighed before and after drying for 7 days at 75° C. The mass differences corresponded to the berry water content (expressed as percentage of fresh berry mass).

Fresh berry mass

During S1 10 individual berries were weighed immediately after the impedance test and another group of 10 berries were weighed for dry berry estimation. Both values were considered. For S2 and S3 50 berries were collected for anthocyanin and phenolic determination and this sample was used to determine mean fresh berry mass.

Microvinification and chemical analysis

Small lot wines were made from fruit harvested in S3 (129 DAA) from every field replicate and treatment. Briefly, fruit (~3 kg for each treatment/replicate) was de-stemmed and crushed by a manual stainless steel crusher and the juice and skin collected. Potassium metabisulphite (0.1 g/L) and diammonium phosphate (0.3 g/L) was added to the juice before it was inoculated with yeast (0.2 g/L, Maurivin AWRI 796, Mauri Yeast Australia). Fermentation was conducted in food grade plastic containers (5 L, circular base, with 18 cm of diameter, Menzel Plastic, Adelaide, Australia) with open top, which was covered with mesh to prevent spoilage. The ferments were maintained at approximately 21° C and plunged manually every 24 h. When the ferments reached 0 Baume (8 days), they were pressed using a manual press and wine stored in 0.5 L bottles. Potassium metabisulphite (0.1 g/L) was added to each bottle. From these wines, approximately 10 days after bottling a sample was taken for anthocyanin, phenolic and tannin assessment following the modified Somers assay technique described by Mercurio et al. (2007), the measurement was performed in triplicate. The alcohol content was determined as alcoholic strength by ebulliometry following (Iland et al., 2004). No additions or corrections were made to these wines.

Statistical analysis

Significant differences in the data from sprinkler treatments were tested using two-way ANOVA (repeated measurements where appropriate), and Fisher's LSD. Differences at harvest were tested by one way ANOVA and Fisher's LSD, both tests were performed in Graphpad Prism 6 (Graphpad Software Inc., La Jolla, CA, USA).

Results

The sprinkler was activated on various occasions between 01 January to 20 February during the three seasons of this study. During this period daily maximum temperature (based on control canopy temperature) was on average 33.6°, 33.4° and 31.0° C in each season. The number of days with a maximum temperature above 37.8° C during the sprinkler treatment (01 January to 20 February) was 14, 18 and 10 days in S1, S2 and S3 respectively. Moreover, the number of days with a maximum temperature above 40° C during the sprinkler treatment was 8, 12 and 4 days in S1, S2 and S3 respectively. During S1 the system was turned on 7 times (Figure S1). The sprinkler system failed 4 times (16/01; 24/01; 17-18/02) when air temperature was above 40° C due to a failure in the pump controller. In S2 the system was activated 15 times (when temperature was above 39° C). In S3 the sprinkler system was activated 10 times (when temperature was above 37° C). During the days the sprinkler system was activated, the average air maximum temperature in control vine canopies were 41.25°, 43.4° and 37.1° C while canopy air temperatures in misted vines were 39°, 40.7° and 35.3° C respectively. There was no difference in the minimum temperatures between treatments across the three seasons (data not shown). Over the period of cooling in each season a total of 59.7 mm of water was applied through dripper irrigation. The misting system added an additional 32 %, 25 % and 15% (19.3, 15 and 8.9 mm) above

the irrigations in S1, S2 and S3 respectively, at least 50 % of this volume was evaporated in S1 and around 75 % in S2 and S3 (Table 1).

Table 1. Effects of ambient temperature and sprinkler set up; inside the canopy (S1, S2) and overhead sprinkler (¶,*) on: Actual Water Used for Cooling WUC_a, Potential Water Used for Cooling WUC_p and Actual Cooling Efficiency of Water CEW_a.

Season this Study/References	**Mist rate (mm h-1)**	**Air temp. Control (°C)**	**RH % control vines**	**VPD (kPa)**	**Drop in air temp. misted vines**	**Δ RH % Misted vines**	***WUC_a* (mm °C-1)**	***WUC_p* (mm °C-1)**	***CEW_a* (%)**
S1	0.333	35.7	17.6	4.84	-4.3	+13.8	0.077	0.039	51
S1	0.333	38.1	14.7	5.71	-5.2	+18.2	0.064	0.035	55
S2	0.111	35.9	18.8	4.82	-1.0	+3.8	0.111	0.044	40
S2	0.111	38.3	16.7	5.64	-2.4	+6.0	0.046	0.037	79
S2	0.111	41.1	14.9	6.69	-3.0	+5.9	0.037	0.030	81
S2	0.111	43.3	13.9	7.60	-3.0	+5.8	0.037	0.026	70
S2	0.111	45.5	12.2	8.68	-3.7	+5.9	0.030	0.022	74
Gilbert et al 1971¶	0.254	32.9	19	4.07	-2.6	+12.7	0.175	0.045†	46
Aljibury et al 1975¶	0.458	35.8	21.8	4.62	-2.3	+17.0	0.196	0.040†	20
Iglesias et al 2005*	3.4	36.8	47	3.31	-5.2	+30.0	0.654	NA	
Iglesias et al 2005*	3.4	40.0	42	4.30	-5.9	+31.0	0.575	NA	

¶ overhead sprinkler in grapevines. *overhead sprinkler in ‘Mondial Gala’ apples. † assuming an air volume of 6 m3.

Figure 2a,b show the changes in air temperature and relative humidity recorded every minute inside the canopy on contrasting days when the system was activated. In the control vine canopies air temperature reached a peak of 38.5° and 45° C (Figure 2a and 2b respectively), while in misted vines the air temperature was around 5° C cooler in the first case and 2.4° C in the second case. At the start of each cycle the relative humidity inside the canopy of misted vines reached up to 80% or 30% in Figure 2a and 2b respectively, then dropped to lower values being always higher than the values recorded in control vine canopies. The predicted air temperature based on the simulation (which includes 30 % of volume applied as run-off) in S1 (Figure 2a) was slightly lower than actual; however, in S2 the simulated values matched actual values (Figure 2b). This is in agreement with the *Actual Cooling Efficiency of Water* (CEW_a) for the sprinklers which was higher in S2 and defined as the ratio of *Actual Water Use of Cooling* (WUC_a) to the *Potential Water Use of Cooling* (WUC_p) Table 1.

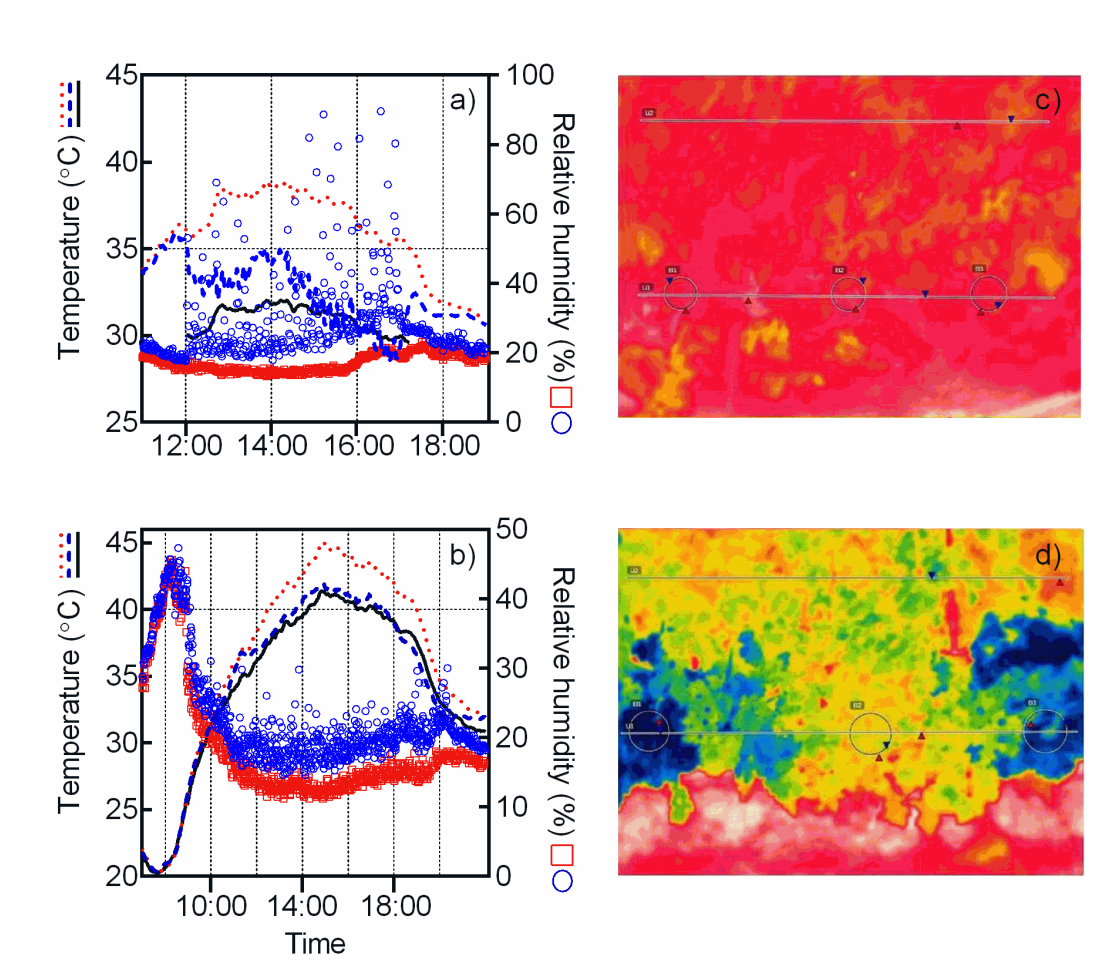

Figure 2. Air temperature and relative humidity (lines and open symbols respectively) from sprinkler treatment (blue dashed lines, ○), control (red dotted line, ○) treatment and modelled values based on Eqn 4. (assuming 30% run-off, black line). Data was recorded every 1 minute. In a) the data recorded on 06/02/2013 between 11:00 to 19:00 in S1 is reported, dashed horizontal black line indicates 35 °C and in b) the data recorded the 11/02/2014 between 7:00 to 22:00 in S2 is reported, dashed horizontal black line indicates 40 °C. In c and d thermal images are shown. c) Control panel and d) a sprinkler panel. Images taken on 14 January 2014 at 14:15 in S2, when air temperature was around 39.5° C (1 hour before temperature was 41°. In c and d bottom line (Li_1) and top line (Li_2) in each image show the temperature across the line, being Li_1 and Li_2 39.5° C and 38.7° C in control vine, and 32.6° C and 35.7° C in the hydro-cooled vine. From right to left Ei_1,Ei_2 and Ei_3 spheres show the temperature in these specific points, being 39.7° C, 39.5° C and 39.1° C in control and 24.9° C, 35.8 ° C and 28.4 ° C respectively.

The hydro-cooling reduced the leaf temperatures measured by infrared thermography when the air temperature was ~ 40 °C (Figure 2c,d). The average leaf temperature in control vines was 41.1° C while in the misted vines it was 34.2 °C. The thermal

images showed that the drop in leaf temperature was affected by the sprinkler location. The leaf temperatures in the misted treatment ranged from 22.8° C in the wet area surrounding the sprinkler emitter to 41.2° C (small portion left to the right sprinkler). The thermal images were captured during S2 (14/01/2014 at 14:15), the camera setting was kept constant for both images.

The Actual Water Used for Cooling (WUC_a) was lower in S1 than S2 when air temperature was around 35.7° C (0.077 and 0.111 mm $°C^{-1}$ respectively, Table 1). However, when control vines were at ~38° C the lower volume misted in S2 (0.111 mm h^{-1}) showed a better WUC_a (i.e. lower, 0.064 and 0.046 mm $°C^{-1}$ in S1 and S2 respectively) than higher volume misted in S1 (0.333 mm h^{-1}). The WUC_a gradually decreased as ambient temperature increased in S1 and S2. For instance, in S2 when ambient temperature was 45.5° C the WUC_a was 35 % lower than at 38°C (0.03 and 0.046 mm $°C^{-1}$ respectively). Moreover, as it would be expected, VPD had a high influence in the WUC_a and WUC_p. For instance, in S2 (Table 1) was correlated with WUC_a and WUC_p with a correlation coefficient (r^2) of 0.624 and 0.97 respectively.

The cooling efficiency simulations showed that the *Potential Water Used for Cooling* WUC_p is a function of sprinkler rate and ambient temperature (Table 1). For instance, as it was observed in WUC_a in S2 (Table 1), the simulations suggested that WUC_p increased as the ambient temperature increases. The CEW_a was around 50% in S1, and decreasing the misting volume to one-third in S2 increased the sprinkler efficiency to around 75% (Table 1).

Growing degree day ($GDD_{10°C}$) accumulated in control vines over the sprinkler operation period were 734, 812 and 691.3 ° Cd in S1, S2 and S3 respectively (Figure 3). Only a small reduction in $GDD_{10°C}$ in misted vines was observed (4.6%, 2.9% and 4.0 %) compared to the values observed in control. On the other hand, $GDD_{37.8°C}$ in control vines was 708, 769 and 681 ° Cd in S1, S2 and S3 respectively and these values in misted vines were slightly lower (3.1, 0.3 and 3.3%). Extreme heat ($HE_{>37.8°C}$) in control vines was 26.1, 42.6 and 10.6° Cd in S1, S2 and S3 respectively, while in misted vines was 14.5, 21.9 and 5.3° Cd, which represents a substantial decrease in accumulated heat above 37.8° C ($HE_{>37.8°C}$) in misted vines (45, 49 and 50% in S1, S2 and S3 respectively) compared to control vines.

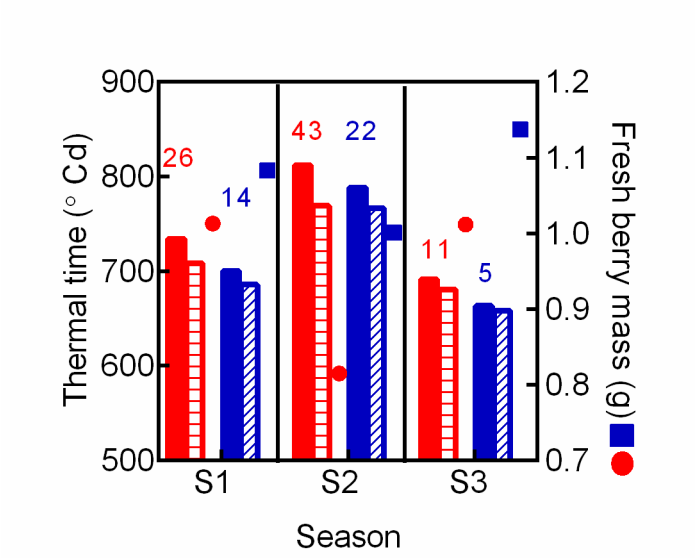

Figure 3. Growing degree days during ripening of Cabernet Sauvignon (01 January to 20 February, bar graph, left y-axis) and average fresh berry mass (right y-axis, points, ● control, ■ misted) during 3 seasons (S1, S2, S3). Growing degree day ($GDD_{10° C}$) without the upper threshold (solid bars) are compared to growing degree days including the upper threshold ($GDD_{37.8°C}$, pattern bars horizontal and diagonal for control and misted vines respectively). Numbers on the graph are the differences between the two thermal time calculations and provides a quantification of the extreme heat ($HE_{>37.8°C}$) that the treatments were exposed to. Fresh berry mass were measured at 111, 115 and 129 DAA in S1, S2 and S3 respectively. Data points are mean of 4 replicates.

Berry mass was increased in misted vines in all seasons (Figure 4b). Total soluble solids concentration (Figure 4a) was not affected by the misting, therefore, sugar per berry (Figure 4d) was higher in hydro-cooled vines compared to control in S2 and S3. Fresh berry mass was lower in S2 matching the hottest season, and larger berries were observed in S3 which was the coolest season (Figure 3,4c). There were no differences in berry water content as a proportion of fresh berry mass (Figure 4c), neither pH, TA nor the extracellular electrical resistance (Figure S2) as an indirect indication of berry cell death (Caravia et al., 2015a).

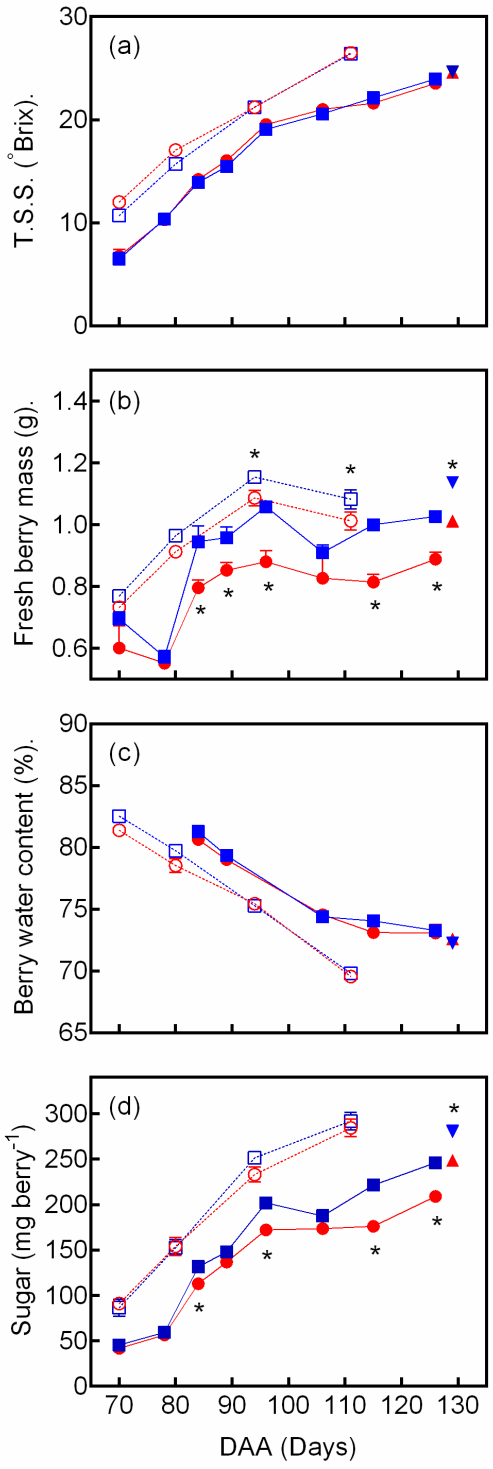

Figure 4. Berry development parameters over three seasons. S1 (open symbols, dashed lines), S2 (solid symbols, solid lines) and S3 at harvest (triangular symbols). Control (○,●,▲) and sprinkler treatments (□,■,▼). TSS (a), fresh berry mass (b), berry water proportion of fresh mass (%) in (c) and sugar as mg berry (d). Data points are mean ± SEM of 4 replicates (error bars are smaller than the symbols).

No differences were found in berry colour measures (Table 2). However, anthocyanin concentration and total phenolics expressed as mg g^{-1} of fresh berry mass were higher in the control in S2. The anthocyanin:TSS ratio (mg $g^{-1}Brix^{-1}$) was not altered by the misting treatment. The ratio was higher in S2 (the hottest season, 0.065) and lower in S3 (the coolest season 0.04). There were no significant differences at harvest in terms of bunch mass (g) and yield (kg m^{-1}) (Table 3). However, there was a trend of these values being higher in the misted vines (except in S3 when a large number of bunches were recorded in control vines). No significant differences were found in wine composition (at bottling) between treatments from wine made on S3 including alcohol content and anthocyanin concentration (Table 4). However, a trend of higher total anthocyanin levels was observed in wines from misted vines.

Table 2. Berry colour assessment at 17/02/2014 (115 DAA) in S2 and 03/03/2015 (129 DAA) in S3. Data points are mean ± SEM of four replicates. Different letter shows difference in same season.

	14/02/14		**03/03/15**	
	Sprinkler	Control	Sprinkler	Control
Anthocyanin (mg berry^{-1})	1.32±0.08	1.25±0.05	0.99±0.03	0.87±0.03
Anthocyanin (mg g-^{1})	1.32±0.09 ^{a}	1.53±0.08 ^{b}	0.88±0.04	0.88±0.04
Anthocyanin/TSS (mg g-$^{1^{o}}$Brix^{-1})	0.07±0.003	0.06±0.004	0.04±0.002	0.04±0.001
Total phenolics (mg berry^{-1})	1.59±0.09	1.44±0.03	0.94±0.01	0.86±0.05
total phenolics (mg g-^{1})	1.59±0.09 ^{a}	1.77±0.04 ^{b}	0.84±0.03	0.88±0.07

Table 3. Harvest information over three seasons in sprinkler and control treatments in Cabernet Sauvignon. Data represent mean ± SEM (n=4).

Treatment	Season	Bunch n^{o} (m^{-1})	Bunch mass (g)	Yield (Kg m^{-1})
Control	S1	22 ± 1.55	64.1 ± 8.46	1.39 ± 0.18
Sprinkler	S1	22 ± 1.61	75.3 ± 3.84	1.65 ± 0.09
Control	S2	30 ± 2.63	47.9 ± 7.18	1.43 ± 0.20
Sprinkler	S2	29 ± 2.26	61.3 ± 2.78	1.82 ± 0.21
Control	S3	32 ± 2.91	88.3 ± 11.01	2.91 ± 0.56
Sprinkler	S3	27 ± 2.03	95.0 ± 3.49	2.52 ± 0.13

Table 4. Wine quality parameters for sprinkler treatment and control measured in wine made at (129 DAA) harvest in season 2014-2015 at the Alverstoke. Data are means ± SEM (n=4). In each row, mean values followed by different letters are significantly different ($p < 0.05$).

	Wine 03/03/15 (DAA)	
	Control	Sprinkler
Total anthocyanins (mg L-1)	207.00±14.02	229.40±18.91
Colour density (au)	7.075±0.367	7.424±0.538
Colour density: SO2 corrected (au)	7.255±0.398	7.035±0.393
Hue (no units)	0.718±0.009	0.721±0.009
Total phenolics (au, 10 mm)	25.30±0.741	26.22±0.960
SO2 resistant pigments (au)	2.366±0.069	2.152±0.092
Epicatechin [mg L-1]	385.5±28	353.5±33
Alcohol (%)	11.85±0.33	11.6±0.54

Discussion

The misting cooling system successfully reduced the canopy air temperature by around 5°-7°C when the canopy air temperature in the control vines exceeded 38°C. However, the cooling effect was 2.4°-3.3°C when air temperature in the control vines was around 45°C. This cooling effect would be a combination of evaporative cooling and the lower temperature of the water being delivered into the canopy. This is cooler compared with a 2.5°C decrease in air temperature in sprinkler-cooled vines when air temperature in control vines was 33°C reported by Aljibury et al. (1975). The lower cooling capacity is difficult to explain since the overhead sprinklers also cover the inter row spaces. On the other hand, the vapour pressure deficit VPD at 33^{o} C should be less favourable for evaporation compared with our target temperature (40°C). The relative humidity increased during the sprinkler operation in our study. This increment was temperature related, being more significant when air temperature was 38° C compared to 45° C (Figure 2a). However, it was also related to the large volume misted in S1. Moreover, the increase in RH achieved in S1 was similar to the increase reported for overhead sprinklers (Table 1). Interestingly, these previous studies used two to four times more water compared with the 1.11 mm ha^{-1} h^{-1} used in S2 and S3. On the other hand, in an overhead sprinkler system the water is sprinkled also to the inter-row area, which assuming 3 m between rows (most commonly used row spacing in Australian vineyards) and potentially a canopy thickness of 1.5 m (sprawl canopy) could include an important proportion of ground surface. In contrast, when the sprinklers are located inside the canopy the amount of water required to cool the canopy environment is lower (Table 1).

Not all the volume of sprinkled water evaporated (Table 1). This was evidenced by the fact that the leaf temperature of wet leaves were up to 15° C cooler (revealing

some run-off) compared to canopy air temperature (39.5 °C), while dry leaves in misted vines were ~3.6 °C cooler (Figure 2c). In contrast, leaves in the control vines were slightly higher than the ambient air temperature (41.1 °C). These values are similar to what was reported previously in overhead systems in Semillon vines where canopy temperature dropped by 7°-8°C when air temperature exceeded 40° C (Greer and Weedon, 2014) and where 0.23 mm ha^{-1} h^{-1} were delivered when the temperature went above 35° C. A larger drop in leaf temperature of 22° C was reported on potted vines using 0.51 mm ha^{-1} h^{-1} when the control canopy temperature was 43° C (Kliewer and Schultz, 1973). Similarly, 16.5°C cooler canopy temperature was reported in Semillon when the control was around 40°C after 0.46 mm ha^{-1} h^{-1} of water was delivered (Aljibury et al., 1975). This is in agreement with the temperature drop observed in this study in the wetted areas around the emitters.

The WUC_a improved as ambient temperature increased (Table 1) and the cooling simulation (WUC_p) confirmed that cooling efficiency improved at higher temperature, having a high correlation with VPD (Table 1). Similarly, from data reported on overhead sprinkler application in apples trees, WUC_a was calculated as 0.654 mm $°C^{-1}$ when ambient temperature was 36.8° C decreasing 12 % (0.575 mm $°C^{-1}$ mm) at 40° C (Iglesias et al., 2005).

Misting inside the canopy used less water than overhead sprinklers as reflected in the lower WUC_a when compared at similar ambient temperature conditions. The CEW_a was better in S2 than in S1, increasing as ambient temperature gets warmer. Interestingly, calculations made from data reported on overhead sprinkler application in grapevines and assuming the same air volume ($6m^3$) (Table 1) showed that overhead sprinklers were less-effective for air cooling (CEW_a = 20-46%). The higher CEW_a for inside misting of the canopy could be explained by the misting being

confined to a specific air volume enclosed by the canopy as opposed to the larger coverage, including the inter-row, for overhead sprinklers. The air volume misted in overhead sprinklers is also less well-defined. Some runoff was observed in S1 in our system and may account for the lower CEW_a in S1. In S2, the canopy thermal image showed a significant decrease in canopy temperature in areas surrounding the sprinklers. This run-off (wetting of the canopy) in S2 might explain the observed difference between WUC_a and WUC_p.

Through the inclusion of an upper threshold into the thermal time calculation (Molitor et al., 2013) temperatures above a critical threshold can be excluded from the harmless and more useful temperatures for plant development. The misting caused a significant drop in the amount of extreme heat ($HE_{>37.8°C}$) during heatwave events by shortening the period above this threshold (Figure S3) and by temperature reduction. This threshold has been suggested as a limiting temperature for growth in grapevine (Aljibury et al., 1975). This is supported by our observation of cooled vines having larger berries and greater sugar accumulation per berry despite the relatively small amount of time that cooling occurred. Fresh berry mass was higher in the misted vines for each of the three seasons, which is in agreement with the increase in berry mass found previously (Kliewer and Schultz, 1973, Aljibury et al., 1975, Greer and Weedon, 2014). This perhaps indicates that high temperature events have a greater than proportional effect on processes leading to sugar and water accumulation in the berry. The misting treatment however, had no effect on TSS concentration (Figure 4), nor berry water content as percentage of fresh berry mass. In contrast, a shade treatment from veraison to harvest that caused a drop in maximum temperature of 2.1° C on average during the ripening period, reduced sugar accumulation and increased berry water content (Caravia et al., 2015b). The lack of effect in TSS and

berry water content in misted vines compared to shaded vines could be due to the intermittent cooling effect over the season compared to the more continuous cooling effect of shading. The absence of an effect on sugar accumulation is in agreement with previous studies using overhead sprinklers (Kliewer and Schultz, 1973, Aljibury et al., 1975, Greer and Weedon, 2014). There was also a trend of higher yield and bunch mass, most likely as a result of the increase in berry mass. The difference in berry mass led to a difference in sugar per berry (Figure 4d) in agreement with results reported for Semillon (Greer and Weedon, 2014).

Of particular interest was our observation that misting (or canopy cooling) had no significant effect on the extracellular electrical resistance of Cabernet Sauvignon berries. This measure is a surrogate for the degree of cell death in Shiraz berries (Caravia et al., 2015a). A lower electrical resistance was observed when cell death occurred probably due to ion leakage from damaged cells. Thus, although overhead shading reduced the degree of cell death in Shiraz berries that was mostly attributed to lower temperature (Caravia et al., 2015b) , mist cooling of Cabernet Sauvignon berries on a more stochastic basis when temperatures were high did not affect this berry property. It is not clear if this is a feature of the particular variety or the difference in the way cooling occurred.

The sprinkler treatment or cooler canopies showed a trend towards higher anthocyanin and phenolic content per berry. Anthocyanin and total phenolics concentrations were lower in misted vines in S2 in the hottest season of this study only when they were expressed as mg g^{-1} of fresh berry mass. This could be due to a dilution effect for larger fresh berry mass observed in mist-cooled vines. Contrastingly, a strong increase in colour was reported in Carignane and Cardinale berries from potted vines sprinkled from bloom to harvest (Kliewer and Schultz,

1973). The difference between control and cooled vines decreased after the cooling stopped and no difference was observed at harvest.

The anthocyanin:TSS ratio was not altered by misting. However, it has been reported that high temperature has a negative effect on this ratio in Shiraz where the canopy was heated convectively (Sadras and Moran, 2012). Microvinifications indicated that misting resulted in no significant reduction in the final alcohol concentration in wine at bottling and no differences were detected in wine phenolic components (Table 3). Anthocyanin concentration was higher in wine from misted vines and these were still in the range of values observed for young commercial Cabernet Sauvignon wines (AWRI, 2012).

The water supply for the hydro-cooling system in this study was potable water from the mains supply and, there was no visual evidence of salt burn on leaves, which can be a problem for sprinkler systems (Kliewer and Schultz, 1973). Because the sprinklers were turned on when air temperature frequently exceeded 38° C at a low relative humidity, the 10 minutes between wetting cycles allowed all the water to evaporate and no visual evidence of bunch rot was found in the misted treatment. In contrast, hydro-cooling systems started when air temperature reach 32° C could lead to an increase in fungal disease (Aljibury et al., 1975), mostly attributed to the large increase in relative humidity achieved by this sprinkler set-up (Table 1).

Based on these results, further research is required, exploring differences between combinations of on/off cycle depending on the temperature thresholds to have a longer lasting effect on maximum temperature control, and in order to enhance berry composition. The effect of this tool on Shiraz, which is vulnerable to heat stress, and other cultivars needs to be further examined. A fully-automated sprinkler system that could provide more detailed information and continuous measurements using for

instance NIR, IR images or energy balance approaches could offer more useful knowledge about this cooling methodology and its field calibration.

Conclusions

Misting sprinklers placed inside the canopy successfully decreased maximum temperature (canopy and air) and may effectively protect vines during the ripening process from severe heatwave events with relatively high water use efficiency. This system used a small fraction of water compared to the overhead sprinkler tested in previous studies and thus have a high WUC_a and CEW_a. The cooling prediction model maybe useful for growers to estimate sprinkler volume rates required during heatwaves. When sprinkler cooling was imposed from veraison to harvest there was no effect on sugar concentration in Cabernet Sauvignon berries, nor on the degree of cell death, which normally increases at higher temperatures. However, sprinkler cooling increased berry mass, sugar per berry and anthocyanin per berry despite cooling being applied for only a small fraction of the total ripening period. This indicates that heat-wave events have a strong negative effect on berry growth even when their occurrence is relatively infrequent. Further research is needed to fully understand this tool to maximize the benefits.

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Supplementary material

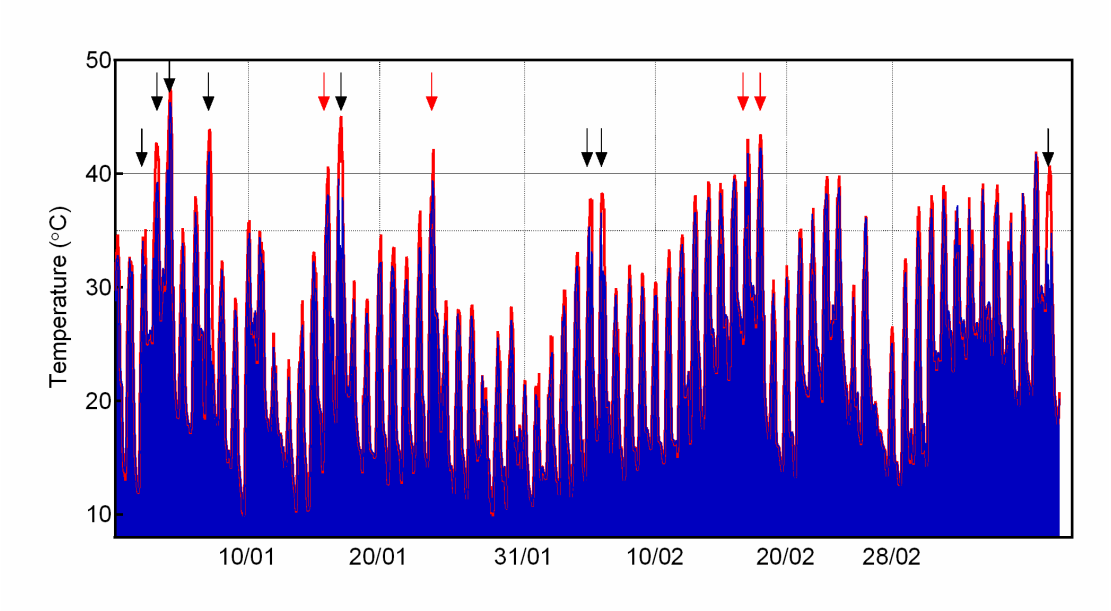

Figure S1. Hourly change in air temperature (° C) from 01/01/2013 to 12/03/2013. Red line shows data from control vines and in blue area data from the sprinkler. Horizontal dotted line indicated 35° C and horizontal solid line shows 40° C, data downloaded, the black arrow indicated days in which sprinkler were turned on and red arrow the days where the system was not working.

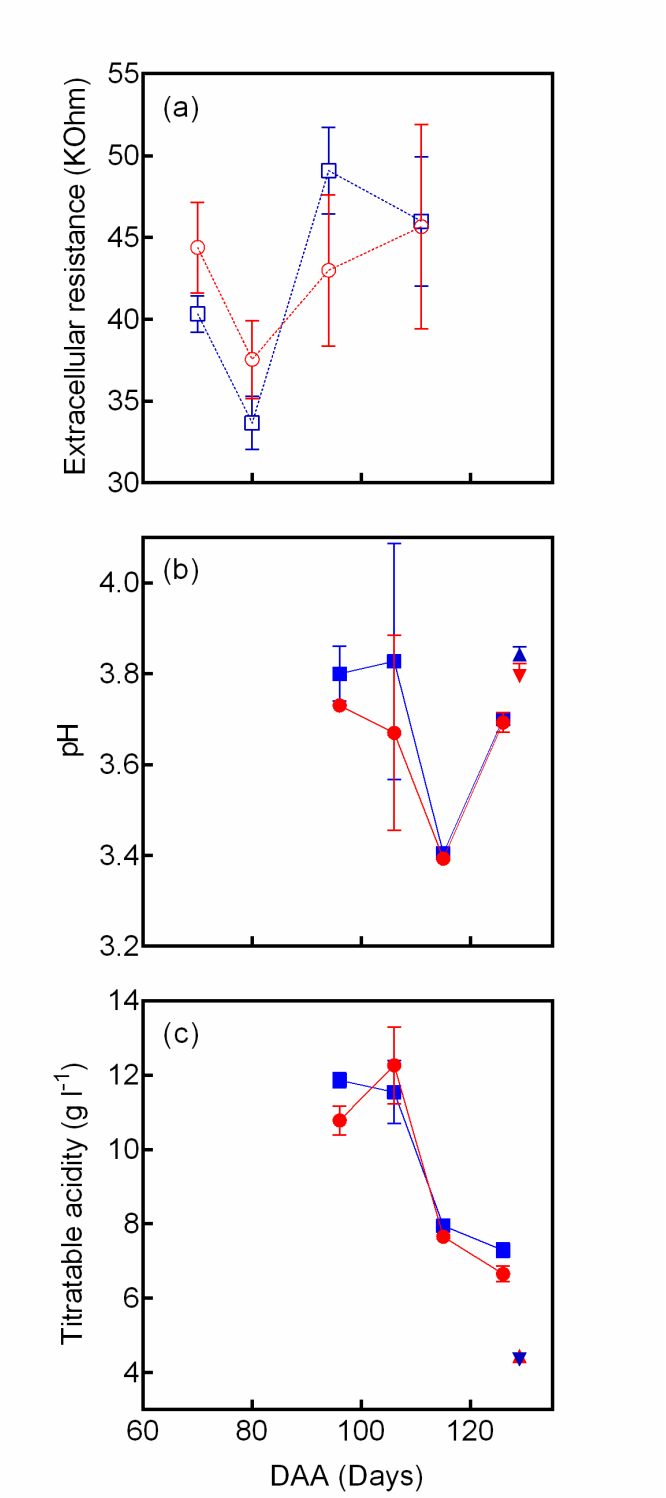

Figure S2. Changes in extracellular resistance *Re* in berries under sprinkler treatments (□) and control (○) during S1 (a). Changes in pH (b) and TA (c) in berries under sprinkler (■,▼) and control (●,▲) treatments during S2 (■,●) and at harvest (▼,▲) in S3. Data points are mean ± SEM of 4 replicates.

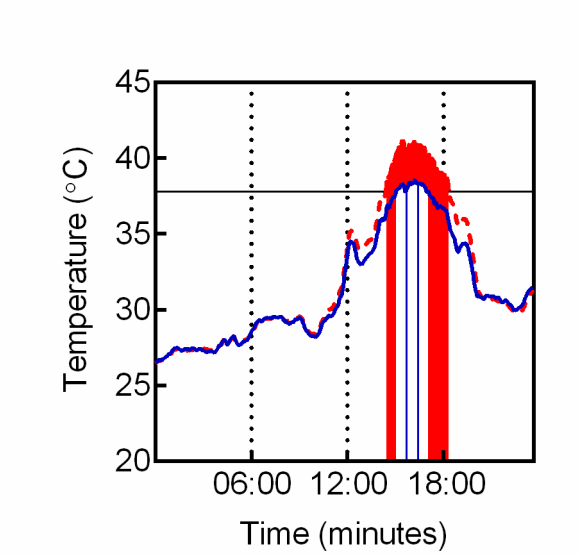

Figure S3. Data recorded the 14/02/2015 (24 hours in S3) where the red detached line represent control and blue solid line the misted vine. The red area and the blue vertical solid line represent the temperatures above the upper threshold of 37.8° C in control and misted vine, above which harmful heat occurs, the horizontal line indicated the threshold.

Chapter 5

Wrapping arms for cordon establishment could be a stressful practice for grapevines.

Wine and Viticulture Journal, Vol. 30, No. 6, Nov/Dec 2015: 46-50.

Statement of Authorship

Title of Paper	Wrapping arms for cordon establishment could be a stressful practice for grapevine
Publication Status	☑ Published ☐ Accepted for Publication
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Principal Author

Name of Principal Author (Candidate)	Luciano Caravia Bayer
Contribution to the Paper	Developed the research idea, designed and conducted all research experiments, analysed the data and drafted and constructed the manuscript.
Overall percentage (%)	
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	21/09/2015

Co-Author Contributions

Name of Co-Author	Cassandra Collins
Contribution to the Paper	Contributed to the research ideas and editing the manuscript.
Signature	Date 21/9/15

Name of Co-Author	Jana Shepherd
Contribution to the Paper	Assistance with the vineyard site and help in the field.
Signature	Date 17 Sep 15

Name of Co-Author	Stephen Tyerman	
Contribution to the Paper	Contributed to the research ideas and experimental design, supervised the research, contributed to data analysis and the editing of the manuscript.	
Signature		Date 21/9/15.

Caravia, L., Collins, C., Shepherd, J. & Tyerman, S.D. (2015). Wrapping arms for cordon establishment could be a stressful practice for grapevines.
Wine and Viticulture Journal, *30*(6), 46-50.

Chapter 6

General Discussion

Summary of findings and Future research

As an overall view, the research presented in this thesis has three main themes in the context of heat wave mitigation and impacts of heat on vine physiology and berry development. Firstly, in chapter 1, a method using impedance spectroscopy to objectively monitor berry cell vitality is presented. This technique was required to more rapidly and objectively measure the impacts of heat waves and cooling techniques on the process of berry cell death and berry mass loss, which has previously been shown to be strongly dependent on temperature and water stress. Secondly, Chapter 3 and 4 attend to understand vine physiology, berry ripening dynamics and wine composition, with practical adoption of two possible heat mitigation strategies. Finally, in Chapter 5, wrapping arms in cordon establishment, a widespread practice in Australian viticulture, is questioned based on its possible role in vascular restriction, which can exacerbate the impact on vines of water and heat stress. The more significant findings are summarised below.

A model of berry cell death based on impedance spectroscopy.

- Impedance spectroscopy reflects changes in berry vitality in artificially damaged Cabernet Sauvignon berries.
- Before the onset of berry cell death, impedance increases as sugar accumulation progress. This means, that this technique could be also useful for measuring berry composition.
- An equivalent electrical circuit can be retrieved from the berry impedance spectroscopy data, providing a singular value for Extracellular Resistance (*Re*), Intracellular Resistance (*Ri*) and Membrane Capacitance (*C*).

- Two models for predicting berry cell death in Shiraz berries based on impedance data were developed (r^2 >0.83).
- Membrane capacitance drops abruptly in artificially damaged berries. However, there was not a significant decrease in capacitance in berries that had low cell vitality. This suggested that cell membranes actually remain structurally stable but might become leaky on the basis of the observed decrease in extracellular resistance.

Application of overhead shade during Shiraz berry ripening as a heat mitigation strategy.

- Overhead shade that blocked 62 % of total solar radiation caused a significant and consistent decreased in air maximum temperature at canopy level by approximately 2.2° C. This form of shading compared to other configurations gave the best outcomes in terms of impacts on sugar accumulation and berry cell vitality. Overhead shade also increased relative humidity. There was no difference in minimum temperature between treatments.
- Overhead shaded vines were under less stress as indicated by higher chlorophyll content and higher ψ.
- Leaf gas exchange revealed that shaded vines were more functional under heat stress (>40° C) where A_{sat} at any value of g_s was higher in overhead shaded vines than in the control.
- Berry ripening was affected by overhead shade. TSS and pH were lower in overhead shaded vines while fresh berry mass, berry water content, TA and berry cell vitality were all higher than unshaded controls. No consistent differences were

detected in phenolic composition, although there was a trend of lower values in Shaded vines.

- Wine composition showed a lower level of alcohol with no changes in anthocyanin concentration. However, chemical age and colour density were lower in shaded vines.

Inside canopy misting to reduce heat stress during Cabernet Sauvignon berry ripening.

- Micro-spray sprinklers successfully decreased maximum temperature in the canopy by around 3° C when air temperature was near 45° C.
- Misting spray inside the canopy used less water than overhead sprinklers (determined from other published results) and coefficients were developed to quantify the cooling efficiency and water use efficiency of hydro-cooling.
- Fresh berry mass was higher in misted vines. No effect on sugar accumulation was observed, but sugar per berry tended to be larger in misted vines.
- No effect on anthocyanin levels was found in this study
- There was no apparent effect of cooling on berry cell vitality as determined from impedance spectroscopy.

Wrapping arms in cordon establishment

- Wrapping arms restricted arm growth during the first season.
- The arm transversal area decreased less in non-wrapped vines with distance from the trunk.

- Pruning weight was around 20% higher in unwrapped vines with higher pruning weight occurring at the distal portion of the arm.
- At harvest berry assessment suggested less water stress in berries from non-wrapped vines.

Conclusions

Changes in berry cell vitality (defined by vital stains) can be objectively determined by impedance spectroscopy. The models presented had good coefficients of determination (r^2 >0.83) and were able to efficiently predict the data from the shade treatment (not included in the initial model) presented in Chapter 3. The fact that, no significant changes were observed in membrane capacitance in berries with low cell vitality, suggests that membranes were not degraded but became leaky.

Overhead shade treatments at or before veraison (8° Brix) to harvest has a beneficial impact in berry quality, vine performance and more importantly could assist in reducing the level of alcohol in wine. Inside canopy misting could ameliorate heat stress, using a fraction of the water used in overhead sprinkler systems. The main effect of misting in berry development was increasing berry mass.

The preliminary data from the viticultural trial with wrapped arms and non-wrapped arms has shown that this practice restricted arm growth during the first season, the arm transversal area (ATA) decreased more in wrapped vines, which also had 20 % less pruning mass. The berry composition at harvest also suggested less water stress in non-wrapped vines. These results support the hypothesis that wrapping arms onto a cordon wire comprised the arm vasculature restricting water and nutrient flow. Therefore, wrapping arms onto a cordon wire in global warming context should be

avoided, since this practice would compound water stress due to a less efficient vasculature in the cordon.

Future research

Berry cell death is a varietal specific process. So, it is highly likely that the model presented here needs to be calibrated for different cultivars. The equivalent electrical circuit can be approximated from readings at a few key frequencies. Therefore, a portable devise reading the impedance at certain frequencies could be developed for field measurements. Whether or not the cell membranes are disrupted in berries with low cell vitality based on vital stain will require examination of other indicators of cell death such as DNA fragmentation and activation of certain signalling pathways associated with senescence or programmed cell death. The fact that berry impedance was closely related to sugar accumulation implies that there is potentially role for this technique in berry composition assessment.

Overhead shade was the most effective vine protection treatment for heat-waves tested, although, some positive trends were also observed in the canopy shade treatment. Overhead shade will require a significant investment for a field application. Therefore, more shade layouts need to be tested in order to find one design with the same heat control capacity and that is also easier to install in the vineyard at veraison.

Inside canopy misting had a main effect on fresh berry mass, which is remarkable since the system only operated during a small fraction of days during berry ripening. The lack of effects on berry composition might be related to intermittency (compared with the significant effects caused by shading on berry composition). Therefore,

further research is needed to fully understand this tool to maximize the benefits, using short cycles to minimize water use and, the use of a variable settings related to a differential target temperature for imposing cooling more permanently during ripening.

The preliminary results from the wrapping arm trial have to be confirmed with more data and more experimental sites. The results demand an understanding of the changes in hydraulic conductivity across cordon arm sections, as well as xylem morphological changes in wrapped and non-wrapped arms. Future research should also examine carbohydrate dynamics in different cordon portions. To reveal differences in trunk diseases sensitivity (*Eutypa lat*a and *Botryosphaerea lutea*) and dead arm severity will be the final target assessment of this research.

The research presented provides quantitative evidence that heat stress under normal conditions (ie without shading or hydrocooling) during berry ripening alters vine physiology and berry composition. A significant body of new information about two possible ameliorating solutions for heat stress is provided. The evidence that wrapping arms is causing arm constriction affecting the vascular system has not been considered in the literature and should be investigated further. In addition, the increasing incidence of dead arms observed in the last decades could also be caused by chronic stress and increased susceptibility to infection induced by wrapping cordon arms on to the wire.

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