Late pruning to delay maturity and preserve wine identity in Barossa Shiraz

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Abstract

Warming is shifting vine phenology, compressing harvests, and altering the balance of fruit traits relevant to wine. The aim of this thesis was to test late pruning as a tool to delay maturity of Shiraz in the Barossa Valley of Australia, and its impact on vine yield, and wine chemical and sensory attributes. Pruning at three phenological stages were compared: winter (control), budburst and 2-3 leaves emerged. Two trials were established. First, three pruning treatments were carried out during four consecutive seasons on the same vines, to evaluate carry-over effects. Second, two thermal regimes (heating with open-top chambers vs unheated control) was combined with three pruning times during three seasons. In general, late pruning treatments delayed maturity with neutral or positive effects for yield and berry traits without carry-over effects on phenology, yield, leaf area and berry traits. Further, late pruning shifted the onset of berry anthocyanin in relation to sugars and increased the anthocyanin to sugar ratio, improved wine phenolics and altered sensory attributes. In a context of warming, delaying pruning until *2-3 leaves* have emerged can effectively spread the harvest and partially restore anthocyanin : sugar ratio, improve wine phenolics and preserve wine sensory attributes with no yield penalties.

Thesis Declaration

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

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Publications

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Chapter 4:
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Chapter 1:

Introduction and literature review

1.1 Overview

Warming is shifting vine phenology, advancing grape maturity, impacting on fruit balance and wine sensory attributes, and contributing to the compression of the harvest between cultivars in many regions worldwide (Jones et al. 2005, Petrie and Sadras 2008, Mira de Orduña 2010, Sadras and Petrie 2011, Webb et al. 2011, Sadras et al. 2013b, Sadras et al. 2014). The potential loss of regional wine styles could be exacerbated under future warmer scenarios (Jones et al. 2005, Webb et al. 2008a). This thesis tested the hypothesis that late pruning is a useful tool to mitigate negative warming impact on Shiraz fruit and wine, provided this practice: a) delays maturity with neutral or positive impact on yield, b) it has neutral or minimal carry-over effects on vine phenology, yield components, and fruit composition, c) maintains or improves wine chemical and sensory attributes, d) mitigates heating effects on vine phenology and fruit composition, and e) mitigates heating effects on wine composition and sensory attributes. Two field experiments were set up using Shiraz vines to test this hypothesis in the Barossa Valley. Experiment 1 consisted in three pruning times (winter, budburst and 2-3 leaves) repeated in three consecutive seasons in a commercial vineyard at Marananga, in the Barossa Valley. Experiment 2 consisted in a fully factorial trial with two thermal regimes (unheated and heated) combined with three pruning times (winter, budburst and 2-3 leaves) at the Nuriootpa Research Station. In Experiment 1 (Chapters 2 and 3), late pruning treatments were repeated on the same vines during four consecutive seasons to explore the compounding effects on canopy leaf area, yield components and fruit composition, whereas in Experiment 2 (Chapter 4 and 5) late pruning was implemented in a rotational basis (late pruning-winter-late pruning) for three seasons.

1.2 Warming impact on viticulture

Temperature modulates plant vegetative and reproductive development. The globe surface and air temperatures are rising as result of the increase in greenhouse gases (carbon dioxide, methane, nitrous oxide) which are released into the atmosphere primarily as a result of human activities (Stocker et al. 2013). Higher current and projected temperatures are likely to advance phenology and to alter the synchrony between plant and animal interrelationships such as pollination and herbivory (Cleland et al. 2007). The advancement in phenology for commercial summer crops is bringing the ripening period and the harvest to an earlier and warmer scenario. For instance this can change the synchronicity of total soluble solids from other key fruit compositional and physical traits (e.g. flesh firmness, organic acids, flavour, antioxidant activity and flavonoids content) and therefore may negatively impact the balance of traits in many cultivated fruit and vegetable species (Warrington et al. 1999, Moretti et al. 2010).

Elevated temperature advances fruit maturity and impacts on wine quality, as shown in studies comparing regions or vintages (Jones and Davis 2000, Jones et al. 2005, Petrie and Sadras 2008, Webb et al. 2011), and studies using controlled temperature conditions in greenhouses or heating systems in field experiments (Tarara et al. 2000, Mori et al. 2005, Sadras and Soar 2009). Warmer vintages are also compressing the harvest among varieties (Sadras et al. 2014) and creating a bottleneck for the winery logistics. While some cultivars may benefit from current warming, others may reach the upper thermal limits to maintain wine styles that are specific to a location (Jones et al. 2012, Sadras et al. 2013b). For instance, warmer temperatures disrupted anthocyanin and sugar ratios in the Barossa Valley Shiraz and Cabernet Franc from field experiments (Sadras and Moran 2012) and reduced biosynthesis of skin anthocyanin in three year old potted Darkridge vines, in Tsukuba, Japan (Mori et al. 2007).

1.3 Methods used to measure thermal effects on grapevine berry composition

The selection of a grape cultivar to suit a specific location is determined by the match between the thermal requirements for the cultivar to ripen its fruit and temperatures achieved at the site. Temperature is the major factor used to formulate climatic indices that define a wine region or macroclimate, from cold to very hot (Winkler et al. 1974a, Dry and Smart 1988, Gladstones 1992).

To understand thermal effects on grape composition, indirect comparisons have been widely used that compare vintages or locations (Barnuud et al. 2014a). With this approach temperature is confounded with other factors that also affect fruit composition, e.g. solar radiation, vapour pressure deficit, rainfall, soil composition and temperature, management practices (Kliewer 1977, Dokoozlian and Kliewer 1996, Bergqvist et al. 2001, Wolf et al. 2003, Downey et al. 2006b, Field et al. 2009). Direct methods are designed in controlled environments or heating systems in the field (e.g. greenhouses, open-top chamber and open systems) where thermal effects can be separated with a higher degree of confidence. Bonada and Sadras (2015) thoroughly reviewed the methods that have been used to investigate temperature effects on fruit composition. They described and compared both indirect and direct methods and their limitations. They concluded that indirect methods are an easy and inexpensive approach to understand and infer thermal effects on fruit compositional traits but these methods are inconclusive and prone to confound other factors that interact with temperature. Hence, direct methods are more likely to separate thermal effects from other factors but are not necessary free of error (artefacts). Nevertheless, the use of indirect methods can be a valuable tool on analysing plausible scenarios of temperature effects on fruit composition, and it helps to identify some gaps of knowledge. For example, Petrie and Sadras (2008) time series demonstrated that between 1993 and 2006 there was an advancement on designated maturity (12.1 Baume) about half and three days per year in Chardonnay, Cabernet Sauvignon and Shiraz. Sadras and Moran (2013c) investigated direct thermal effects on vine phenology in field experiments of Chardonnay, Semillon, Shiraz and Cabernet Sauvignon in the Barossa Valley. They concluded that time series from the earlier report overestimated the thermal effects on phenology that need to be assessed in field experimentation to account for cause-and-effect relationships and to avoid oversimplification of indirect methods.

1.4 Warming impact on yield

The first step in the development of the inflorescence primordia is the initiation of the uncommitted primordia. This takes place after budburst about a few or more weeks depending on variety, node position and environmental conditions. For example, in Chardonnay uncommitted primordia initiated after 4 weeks in a hot region compared with 6 weeks of a cool region at the fourth node (Watt et al. 2008). The uncommitted primordia then can differentiate into inflorescence primordia, tendril or a combination of both (Vasconcelos et al. 2009). This process was observed in Chardonnay from 6 to 9 weeks after budburst in both hot and cool regions in Australia (Watt et al. 2008). The inflorescence primordia continue to develop until the buds enter dormancy and inflorescence branching and number of flowers is not defined until just before budburst (Petrie and Clingeleffer 2005). Here flowers are differentiated and continue to grow and expand until anthesis. Consequently, inflorescences start to develop from early spring of preceding season until fully development occurs in the spring of the following season. Temperature influences all of these stages that will determine final yield.

The major concern of warming is its effect on fruit composition and also bunch components because these factors are closely correlated (e.g. berry size, skin to flesh ratio, seed size). Sadras and Moran (2013a) found that elevated temperature in field experiments reduced, increased or maintained yields of Barossa Shiraz depending on the spring temperatures of preceding season where initiation of inflorescence primordia takes place (May 2000). In the long term, from the pooled data of 7 seasons, heating (0.9 to 2° C) did not affect yield of Shiraz grown in the Barossa Valley (Sadras et al. 2017). Therefore, it is expected that yields will be maintained under future warming (in warm regions), and this needs to be assessed in consideration of the seasonal variation on background thermal distribution for each particular location.

1.5 Elevated temperature impact on fruit, wine composition and sensory traits

The temperature and light effect on fruit composition have been reported widely (Ewart and Kliewer 1977, Kliewer 1977, Dokoozlian and Kliewer 1996, Mori et al. 2007, Cohen et al. 2012, Sadras and Moran 2012, Bonada et al. 2013a, Sadras et al. 2013a). These effects are difficult to separate and they are closely related. For instance, the exclusion of sunlight from the canopy increases the relative humidity and this reduces transpiration (Downey et al. 2006b). Therefore, to separate light and temperature effects, Spayd et al. (2002) cooled Merlot bunches from the west-exposed side and heated bunches from the south-shaded side. They found that total skin monomeric anthocyanin increased when west-exposed bunches were cooled and it decreased when shaded bunches were heated. Mori et al. (2007) found that growing potted Cabernet Sauvignon at 35° C daytime temperature reduced by half the amount of total anthocyanin when compared to a control grown at 25° C (night time temperature was maintained at 20° C for both treatments). They concluded that reduction of the pigment was due to both degradation and inhibition of mRNA involved in the transcription for its biosynthesis. In Shiraz and Cabernet Franc field experiments, Sadras and Moran (2012) found that higher temperature disrupted anthocyanin and sugar that were decoupled at the onset of ripening (lower anthocyanin to sugar ratio for elevated temperature) and this discrepancy was maintained constant up to harvest. Sadras et al. (2013a) demonstrated that heating ~2° C above mean ambient temperature disrupted berry sensorial traits of Cabernet Franc, Shiraz, Semillon and Chardonnay. Furthermore, elevated temperature impacted on grape and wine composition, and sensory traits of Shiraz grown in the Barossa Valley (Bonada et al. 2015). Wines made from heated vines had less colour and phenolic substances, and less intense fruit flavours. In a similar experiment, elevated temperature disrupted sensory traits in Shiraz wines (Sadras et al. 2013b). Wines made from heated vines had more cooked fruit compared to controls that had more berry fruit.

Recent and projected warming has been demonstrated to impact on grape and wine composition in field grown Shiraz. The advancement of maturity and higher total soluble solids as result of the relatively slower tannin and flavour maturity rate (under warmer conditions) at harvest, has led to higher alcohol wines. Wine fermentation may become sluggish or stuck due to a higher content of ethanol (Bisson 1999). This can increase the risk of wine spoilage from other microorganisms such us bacteria or undesirable yeast species. Furthermore, the earlier onset of ripening under higher temperature might also degrade or partially inhibit the development of aromatic volatile compounds that might alter the sensorial balance in the wine. For example, Shiraz wines

1.6 Canopy management that alters the course of ripening

Palliotti et al. (2014) reviewed management practices that can assist to maintain wine regional identity under current and future warming, including:

a. Altering the source to sink balance (Palliotti et al. 2013c, Parker et al. 2015),
b. using exogenous auxin-like hormones (Böttcher et al. 2011),
c. light exclusion (Ristic et al. 2007, Chorti et al. 2010),
d. antitranspirants (Palliotti et al. 2013a, Gatti et al. 2016)
e. double pruning (Gu et al. 2012b), and
f. late pruning to delay phenology (Coombe 1964, Martin and Dunn 2000, Friend and Trought 2007b, Palliotti et al. 2017, Petrie et al. 2017a, Wei et al. 2017).

Most of these studies, except for Ristic et al. 2007, did not include a formal sensory assessment to identify difference on wine styles. Therefore in this thesis sensory analysis is included to further explore insights of practical management (late pruning) to delay maturity that might impact on wine styles made under current and projected warmer climates.

1.6.1 Altering plant source-to-sink balance to change the course of ripening

Source is defined as any organ that can translocate solutes to other organs to be used in metabolic reactions or be temporally stored as reserves (e.g. leaves, canes, cordons, trunk and roots). Sink is any organ that demand solutes that are used to generate growth or maintain vitality (Osorio et al. 2014). In *Vitis vinifera*, bunches are the principal sinks and require large amounts of carbohydrates and nutrients from flowering to maturity. Other sinks like active growing shoots, young leaves and growing roots also depend on photo-assimilates from adult leaves and reserves (Dry and Loveys 1998). In practice and in plant physiology studies, vine balance can be determined by either the yield to pruning weight ratio or leaf area to yield ratio (Iland et al. 2011a). Often, in viticulture, yield to pruning ratio weight is refer as the Ravaz index (Ravaz and Sicard 1903).

Canopy management affects the source-to-sink balance, by either reducing the source (i.e. leaf or shoot trimming) or the sink (i.e. bunch thinning) and it is a common practice to improve fruit and wine composition. The timing of source-to-sink manipulation will determine the impact on yield components, fruit and wine composition. For instance, pulling leavings from the bunch zone (node 1 to 6) at pre-bloom improved must composition (higher total soluble solids and berry anthocyanin concentration) in Barbera and Lambrusco in Piacenza, Italy (Poni et al. 2009). Manipulating source-to-sink ratio can be used to advance or delay maturity which is likely to impact on fruit and wine composition, and therefore on wine styles. The timing and the kind of source to be removed (e.g. upper to bunch leaves) are key to hasten or delay maturity.

1.6.1.1 Leaf removal at or prior to anthesis

Overall, leaf removal before flowering is reported to reduce yield and increase source to sink ratio that is likely to enhance ripening compared to untreated or control vines. On the other hand, tipping or topping during full bloom reduced source to sink ratio by both reducing the canopy and improving yield. This is more likely to delay maturity.

The leaf removal around the inflorescences zone may restrict photo-assimilates that can reduce fruit set depending on the intensity. This practice is beneficial to regulate yield on highly productive vines that require bunch thinning to improve fruit and wine composition. For example, Poni et al. (2006) removed 6 basal leaves before anthesis to control yield and improve fruit composition of both potted Sangiovese and field grown Trebbiano, in a study conducted near Piacenza in Italy. This was attributed to a reduction on fruit set and an improvement on berry skin-to-pulp ratio that increased phenolic compounds and sugars at harvest. Other researchers showed similar results in Sangiovese when they removed leaves prior to flowering (Palliotti et al. 2011, Gatti et al. 2012). In a recent study, pre-bloom leaf removal on Tempranillo (Badajoz, Spain) improved wine colour due to higher synthesis of phenolic compounds that can be bounded to co-pigments and form stable complexes (Moreno et al. 2015). This practice is likely to advance maturity by enhancing TSS at harvest. On the other hand, tipping or topping at flowering can enhance yield by improving fruit set and hence delay maturity. For instance, tipping or topping shoots (removal of 8 cm or less and 15 cm or more of main shoot respectively) before or at 50% cap fall of flowers increased fruit set, due to a likely higher partitioning to inflorescence, and hence vine yield of Cabernet Sauvignon, Chardonnay and Tempranillo, in cool climates (Collins and Dry 2009). Total soluble solids were not reported, however, higher fruitset and the manipulation to a lower leaf area to fruit mass ratio can delay veraison and ripening (Parker et al. 2015).

1.6.1.2 Leaf removal around fruit set

Leaf removal and shoot thinning at or shortly after fruit set is used to alter the microclimate in the zone which can improve fruit composition and reduce the risk of fungal diseases. In a study where three different leaf removal times (from fruit set to after 6 weeks) were combined with three levels of leaf thinning on Sauvignon Blanc, removing leaves did not affect yield components. Although a slight increase in total soluble solids was observed in the earliest treatment, this also reduced pH and malic acid (Bledsoe et al. 1988). Furthermore, defoliation at fruit set and veraison was used to investigate the effects on fruit composition and wine sensory of Grenache (Tardaguila et al. 2008). The early treatment reduced malic acid and increased the colour of the wines. Early defoliation was more effective at improving wine sensory and composition compared with leaf removal at veraison. Both treatments did not affect yield components. Parker et al. (2016b) showed that reducing canopy size at fruitset up to 30% of the control, improved fruit set and yield on Pinot noir and Sauvignon Blanc in New Zealand. This significantly delayed the date of 4 days at the onset of ripening or a TSS of 4.4°Be and up to 15 days to reach a TSS of 11.1°Be. The yield improvement was attributed to a redistribution of resources from active growing tips into berry development after trimming that increased number of berries per bunch and bunch weight.

1.6.1.3 Shoot or leaf trimming at after veraison

Leaf thinning of the bunch zone has been a common practice to improve light microclimate that might assist to increase basal buds fruitfulness and improve fruit composition (Jackson and Lombard 1993, Dry 2000). More recently, leaf removal has been aimed to delay maturity by targeting the younger leaves in the upper third of the shoot after veraison. Palliotti et al. (2013c) found that trimming leaves or shoots between TSS of 8.3° to 9.4°Be in the last third of growing shoots, reduced total net photosynthesis and thus slowed the rate of accumulation of total soluble solids. This was attributed to a temporary reduction in sugar accumulation without affecting other berry traits. At harvest, berry juice total soluble solids were 0.67° Be less in trimmed vines and the wine had 0.6% v/v less alcohol. Similarly, Poni et al. (2013) demonstrated that removing 6 to 7 leaves and laterals from the medial-apical shoot zone of Sangiovese potted vines was able to delay ripening (up to 1.3°Be), in a single season in Piacenza Italy.

1.6.2 Using exogenous auxin-like hormones

The understanding of the role of hormones in fruit maturity is vital for the management and manipulation of ripening of both climacteric and non-climacteric fruit. Climacteric fruits have a distinctive ripening mechanism that consists in a peak of respiration by a coincidental timing of endogenous ethylene burst that triggers the onset of ripening (Alexander and Grierson 2002). Non-climacteric fruits, like grapes, are not responsive to ethylene during ripening.

Although grapes are considered to be ethylene independent, hormones still play a role in cell berry expansion and may have some implications on ripening when interacting with abscisic acid (ABA) even at low concentrations (Chervin et al. 2004, Sun et al. 2010). Nonetheless, ABA and sucrose are the main regulators to trigger the onset of ripening. Gambetta et al. (2010) studied the hormonal ripening induction by using ABA and sucrose in cultured berries collected from Cabernet Sauvignon in California. The initiation of ripening is explained by the change of colour, and the softening of cells wall that allows berry to expand and increase in size, metabolize acids and accumulate soluble solids, that occurs between stage II and III of the double sigmoid berry growth curve (Coombe and Hale 1973).

Synthetic auxin-like compounds can delay the onset of ripening if applied before veraison. Davis et al. (1997) suggested that auxins in conjunction with ABA may regulate the expression of genes involved in ripening. They found that application of exogenous auxin 'BTOA' (benzothiazole 2-oxyacetic acid) to Shiraz bunches delayed the onset of ripening about 2 weeks. In a more recent study, Böttcher et al. (2011) found that exogenous auxin-like compounds applied before veraison delayed the onset of Shiraz ripening and increased the synchronicity of total soluble solids within a bunch. On the other hand, exogenous ABA hastened the onset of ripening, increased total soluble solids, total anthocyanin and phenolic compounds, all measured at harvest in Cabernet Sauvignon (Balint and Reynolds 2013).

1.6.3 Light exclusion

Shading is a practice that may assist to reduce heat stress and delay maturity in grape vines. There is a cost in the carbon budget that needs to be considered as exclusion of light reduces the net photosynthesis and hence carbon input to reproductive allocation (Greer et al. 2011). Klenert (1975) found that shading vine canopies by 40-50%, from fruit set to harvest, delayed the onset of grape ripening and reduced the rate of organic acid degradation. At harvest berry total soluble solids were about 0.9-1.6°Be less in the shaded treatments and malic acid was higher in comparison with controls. Morrison et al. (1990) similarly found that shading some leaves or the whole canopy retarded the rate of accumulation of sugars, reducing soluble solids at harvest by about 1.1°Be compared with non-shaded controls. In contrast, shading only clusters did not delay grape maturity and did not change berry anthocyanin, but wines made from these bunches had lower colour, total anthocyanin and tannin content (Ristic et al. 2007). Wines made from shaded vines had less fruit flavour intensity and were less astringent. Furthermore, a significant reduction of sunlight can be detrimental on the development of bud fruitfulness. This can be attributed to primary bud necrosis or loss of inflorescence primordia formation that occurs under shaded environments inside of crowded canopies (Perez and Kliewer 1990, Dry 2000). Overall, the exclusion of light has been found to delayed maturity but with a detrimental impact on grape colour, tannin and aroma profiles, and carbon economy.

1.6.4 Antitranspirant

The use and effect of plant exogenous antitranspirant has been studied by several authors (Gale and Poljakoff-Mayber 1965, Gale and Hagan 1966, Davenport et al. 1972). Briefly, gas interchange through the stomata is partially impeded by a film formed in the abaxial side of the leaf. The film increases the resistance of carbon dioxide diffusion into the leaves and the outflow of water vapour into the atmosphere. As a result the photosynthesis declines and the leaf water potential is increased. Despite the reduction in photosynthesis, it has been reported that the improvement in water use efficiency can enlarge fruit and shoot size, on the other hand it might decrease the ability of plants to cool their canopies down during extreme heat events (Gale and Poljakoff-Mayber 1965, Davenport et al. 1972).

The use of di-1-p-menthene antitranspirant slowed the rate of total soluble solids accumulation of Sangiovese berries. Treated vines had less sugars at harvest (0.7° Be) and less alcohol (1%) in wines in comparison with controls (Palliotti et al. 2013b). Antitranspirant effect on fruit and wine phenolic, pH and organic acids were negligible but berry and wine anthocyanin were reduced 19% and 15% respectively.

1.6.5 Double pruning

The term ‘double pruning’ was first from a research trial conducted by Peter Dry and Richard Smart in 1977-78 at Roseworthy (Dry 1987). The trial consisted on pruning vines to 6 node canes in summer, removing bunches and laterals to force N+2 latent compound buds to break before entering into organic dormancy. Forcing latent buds to grow in summer shifted the phenology to a warmer period of initial growth from budburst to fruit set and to a cooler window from berry development to harvest during the growing season. In Fresno, California, Gu et al. (2012b) forced growth of latent buds in four treatment dates using Cabernet Sauvignon. They found that shifting the harvest from hot (1st September) into a cooler (October-November) part of the year improved berry composition; interestingly, berry flavonoids (anthocyanin, tannin and phenolic) increased linearly until harvest in forced vines, while flavonoids decreased in controls. Total soluble solids were similar at harvest time ranging from 12.8 to 13.6°Be.

1.6.6 Late pruning to delay maturity

Pruning is an ancient practice used in woody perennial species to adjust the balance between vegetative and reproductive growth. In grapevines, winter pruning defines the potential yield that is a function of total number of nodes and their fruitfulness. Traditionally, pruning is carried out during the winter time when vines are under organic dormancy.

Delaying spur-pruning to during or just after budburst retards either the budburst or the growth and elongation of basal nodes from unpruned canes. This phenomenon can be explained by the apical dominance and correlative inhibition that distal nodes exert on proximal nodes in canes. A hypothesis states that buds from distal nodes may break first due to the ability to leach auxin hormone (budburst inhibitor) toward the basal nodes where it is accumulated and inhibit bud growth (Cline 1994). Antcliff and May (1961) found that this dominance was set at least a month prior budburst in Sultana vines, grown at Merbein, Victoria.

Bangerth (1989) hypothesis of 'primigenic' dominance will be adopted to explain the physiological mechanism of dominance that exists between nodes arising from different positions along matured canes. He proposed this as a more general hypothesis over the apical dominance concept, and it is defined as the sequential 'correlative dominance' that is exerted by the first developed sinks onto the later developed ones. The level of dominance may occur simultaneously with low to severe strength. For example, nodes that do not burst are regulated by a dominant signal (i.e. by hormonal response) and those that developed later, growing at lower growth rate are controlled by resource cues for nutrients and carbohydrates. Delaying winter pruning in grapevines is likely to delay the budburst of nodes arising from basal part of canes while more distal commence to grow.

Martin and Dunn (2000) found that pruning in early July vs late August delayed budburst about 5 days. Friend et al. (2007b) reviewed the impact of delayed pruning on bud break and found a range from 3 to 32 days. These studies also recorded impact on yield and yield components.

Late pruning has been widely researched as a tool to prevent frost damage in cool wine regions. Howell and Wolpert (1978) investigated freeze injury of Concord basal nodes on bearers pruned with a range of 2 to 20 nodes. After the freeze event, 10 or more node canes treatments had less damage to the shoots on the basal nodes (<10%) in comparison with the 2 or 5 node cane (50 to 35%). This was due to shoot growth from the apical nodes on the longer canes inhibiting growth of basal nodes, thus delaying their development and susceptibility to freeze injury. In New Zealand, Friend et al. (2011b) found that late pruning delayed Chardonnay budburst and decreased frost damage from 33% to 3% when a frost event was recorded between bud-swell and woolly bud stage in controls.

Friend et al. (2007b) described the impact of delaying winter pruning on Merlot yield components in Blenheim, New Zealand. They observed an improvement on fruit set that was due to both better fertilisation (more seeded berries) and increased in number of total berries per bunch. They suggested that fruit set was possibly enhanced due higher temperatures, as flowering occurred later in the late pruned vines. Coombe (1964) also found that delaying time of pruning increased yield on Grenache grapes during 3 seasons, in the Barossa Valley. Late pruning delayed budburst between 2-3 weeks and flowering between 1-2 weeks.

More recently, late pruning have been used as a tool to spread maturity in Barossa Shiraz in Australia and Sangiovese in Italy (Frioni et al. 2016, Petrie et al. 2017a, Gatti et al. 2018). These studies agreed on delaying winter pruning up to phenological stage of 2-3 leaves in order to avoid loss of yield. Palliotti et al. (2017) delayed winter pruning up to 9 to 10 unfolded leaves to reduced yield (-43%) and improve fruit composition in Sangiovesse. There was also a delay on ripening associated with the very late pruning treatment (BCCH 19). This may also offer an interesting tool in order to control yield in high yielding cultivars (such as Sangiovesse, Tannat and Corbeau), with the potential to reduce bunch weight and compactness, improving air movement in both canopy and inside bunches, with the resultant likely reduction of botrytis incidence.

1.7 Sensory assessment to define wine styles affected by practical management to delay maturity

Wine is a beverage that evokes large number of odours and produces complex sensations in a human palate (Thorngate 1997). Descriptive analyses (DA) provides a methodology to quantify the intensity of a given aroma, flavour, taste or tactile sensation (Bastian et al. 2010). Sensory assessment can be typically separated by visual (colour), olfactory (aromas and flavours), taste (sweetness, bitterness and saltiness), mouthfeel (oral sensations) and aftertaste There are no wine sensory reports in the literature from practical management studies that were aimed to delay maturity. For instance, Ristic et al. (2007) shaded bunches to study the impact on fruit and wine composition, and sensory traits. Although there was no effect on delaying ripening, wines made from shaded fruit had less fruit intensity flavours and less astringent mouthfeel. This indicates that practical management to delay maturity is likely to alter wine styles in regions that are experiencing warmer than average vintages. Henceforth the importance to conduct sensory assessment to understand the likelihood to preserve wine styles from iconic wine regions.

1.8 Summary and objectives of research

Warming is affecting the wine industry by advancing phenology, impacting on fruit and wine composition, compressing the harvest window and increasing pressure on winery logistics. To explore practical management to mitigate the issues associated to warming, late pruning is proposed as a beneficial tool.

The aims of this thesis were to assess:

(i) Late pruning as a tool to delay maturity, with neutral or positive impact on yield.

(ii) For carry-over effects on vine phenology, yield components, and fruit composition by repeating late pruning in the same vines over consecutive seasons.

(iii) Late pruning impact on wine and sensory attributes

(iv) Late pruning, higher daytime temperature and their interaction impact on vine phenology, yield components and fruit composition.

(v) Late pruning, higher daytime temperature and their interaction impact on wine composition and sensory attributes

Two experiments were established in Shiraz vineyards in the Barossa Valley. In Experiment 1, we established 3 pruning times (winter, budburst and 2-3 leaves) repeated in 3 consecutive seasons, to address aims (i), (ii) and (iii). In Experiment 2, we established 3 pruning times (winter, budburst and 2-3 leaves) combined with two thermal regime (unheated vs heated) using open top chambers, targeting aims (i), (iii), (iv) and (v).

Chapter 2

Late pruning and carry-over effects on phenology, yield components and berry traits in Shiraz

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Author Contribution

Martin Moran conducted the literature review, set up and managed the experiment, drafted and constructed the manuscript.

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.

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By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Victor O. Sadras contributed to the experimental design, research ideas, supervision of research, and editing of manuscript.

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Abstract

Background and Aims: Global warming is shifting vine phenology, compressing harvests, and altering the balance of fruit traits relevant to wine. Our aim was to test late pruning as a tool to delay maturity and to assess carry-over effects from repeated late pruning on phenology, yield components, dynamics of leaf area and berry traits of Shiraz grown in the Barossa Valley of Australia.

Methods and Results: A trial was established in a commercial vineyard comparing three pruning times during four consecutive seasons: (i) winter (Control), (ii) budburst and (iii) 2–3 leaves emerged. Compared to the Control, TSS in berries of vines pruned at 2–3 leaves reached 12°Be 7 days later in the first three seasons, and 14 days later in the last season; the budburst treatment was intermediate between that of winter and of 2–3 leaves. Yield was unchanged by late pruning in three seasons and increased in one. Leaf area index at harvest in 2–3 leaves was greater or similar than in the Control. Late pruning shifted the onset of anthocyanin accumulation against TSS, increasing the anthocyanin concentration and the anthocyanin to sugar ratio in two seasons.

Conclusion: Late pruning delayed maturity with neutral or positive effects for yield and berry traits. Carry-over effects on phenology, yield, leaf area and berry traits were negligible.

Significance of the Study: In a context of global warming, delaying pruning to 2–3 leaves can effectively spread the harvest and partially restore the anthocyanin : sugar ratio with no penalty for yield in Barossa Valley Shiraz.

Keywords: anthocyanin, harvest, leaf area index, total acidity, TSS, veraison.

Introduction

Recent increase in temperature has advanced vine phenology (Petrie and Sadras 2008, Clark and Thompson 2010, Webb et al. 2011, Sadras and Moran 2013c), and compressed the harvest window, thus stressing fruit processing capacity in wineries (Sadras et al. 2014). Elevated temperature can also disrupt berry and wine balance, reducing the acid : sugar and anthocyanin : sugar ratios, causing overripe flavours and higher alcohol (Mori et al. 2007, Tarara et al. 2008, de Orduña 2010, Sadras and Moran 2012, Bonada et al. 2013a, Sadras et al. 2013a). Berry response to elevated temperature is partially associated with early onset of mesocarp cell death and shrivelling in Shiraz (Bonada et al. 2013b).

There is a need to develop practical, cost-effective management tools to decompress harvest, re-establish the fruit balance and improve wine composition (Palliotti et al. 2014). Physiologically, there are two putative, non-mutually exclusive paths to delay maturity: (i) delaying the onset of veraison; and (ii) reducing the rate of sugar accumulation. In the Adelaide Hills (January mean temperature, 19.1°C), the onset of ripening was delayed by about 2 weeks and harvest (TSS ~ 13.5°Be) by around 3 weeks when exogenous auxins were applied to Shiraz vines (Davies et al. 1997, Davies et al. 2015). Poni et al. (2013) delayed ripening (TSS ~ 10°Be) by about 1 week by removing apical leaves during the lag phase and post-veraison of potted Sangiovese in Piacenza (July mean temperature, 22°C). In a field experiment in New Zealand, reduced source : sink ratio by mechanical trimming around fruitset delayed maturity (TSS = 11.1°Be) by about 11 days in Pinot noir (Parker et al. 2016a). Late pruning has been used: (i) to delay budburst in spur-pruned vines to improve fruitset (Friend and Trought 2007c); and (ii) to reduce the risk of frost damage (Howell and Wolpert 1978, Friend et al. 2011a). Further, it was demonstrated that late pruning shifted phenology and spread maturity at harvest by 3 weeks in Shiraz and 2 weeks Cabernet Sauvignon in a single season in the Barossa Valley (Petrie et al. 2017). Yield was unchanged in late-pruned vines but delaying pruning beyond 2–3 leaves reduced yield. Pruning after budburst might have a cost in terms of vine carbohydrate reserves, hence repeatedly pruning the vines over successive seasons may have carry-over effects. For example, defoliation after flowering in Chardonnay vines reduced carbohydrate reserves, inflorescences and flowers per inflorescence in the following season in Canterbury, New Zealand (Bennett et al. 2005). Shoot growth and pruning mass were also decreased by defoliation in the previous season. Repeated late pruning could thus exacerbate the depletion of reserves associated with elevated temperature (Sadras and Moran 2013a).

The aim of this study was to assess the effect of late pruning on ripening and on carry-over effects on vine phenology, leaf area, yield components and berry traits in a Shiraz vineyard in the Barossa Valley.

Methods

Vineyard

A trial was established in a commercial Shiraz vineyard (clone 1654, own roots) in the Marananga region (-34.50 S, 138.89 E) in the Barossa Valley Geographical Indicator (www.wineaustralia.com). Vines were planted on sandy loam soils in 2006 at 3 m between and 1.8 m within rows, and trained to a bilateral cordon with a single fixed foliage wire located 0.4 m above the cordon. Vines were spur-pruned with an average of nine, two-node spurs per metre. The vineyard was managed according to local practice, including supplementary drip irrigation providing 0.8 to 1.0 ML/ha per growing season.

Experimental design and pruning treatments

During four consecutive seasons starting in 2012/13, three times of pruning were established using the protocol in Table 1a. Treatments were laid out in a randomised block design with six or four replicates; each replicate comprised nine vines. Three pruning times were compared: winter (WP), budburst (BB) and 2–3 leaves (2–3 L) (core treatments), and new late-pruning treatments were introduced each season to account for carry-over effects on phenology, canopy growth, yield components and selected berry traits (Table 1a). The timing of pruning was linked to phenology of the Control (WP), rather than date, to account for seasonal variation. Late pruning treatments, BB and 2–3 L, were undertaken when at least 50% of developing shoots in nodes one and two reached these phenostages in the winter Control. The treatments were undertaken as follows: WP in mid-July, BB on the 13 September 2012, 2 September 2013, 1 September 2014, and 9 September 2015; and 2–3 L on the 3 October 2012, 11 September 2013, 19 September 2014 and 17 September 2015. In the last season, vines allocated to first season of late pruning treatments were pruned inadvertently in winter hence these treatments were not represented.

Measurements

Phenological development was monitored using the modified E-L system (Coombe 1995) until veraison, and with berry TSS afterwards. We assumed onset of veraison corresponded to 1% of coloured berries within a bunch. Owing to the qualitative nature of the E-L scale in contrast to the quantitative characterisation of berry sugar concentration during ripening, we used the approach of Sadras and Moran (2013b) to calculate and analyse the date when a given E-L stage was reached. Phenological stages up to veraison were assessed in two vines per replicate, where we randomly selected six to eight spurs per plant to record phenology in lower (N1) and upper (N2) nodes at about weekly periods. In the 2–3 L treatment, the lower and upper nodes were phenologically different, thus E-L stage was analysed separately for each node (Table 2).

Digital photos were taken from underneath the canopy of two vines per replicate to measure leaf area index (LAI) in 2013/14, 2014/15 and 2015/16 (Fuentes et al. 2012). A wooden frame with a bubble level was used to standardise the distance and the level from the camera to the canopy, and the photos were taken using the forward facing camera of an iPhone 4S (Apple, Cuppertino, CA, USA). The leaf area index was calculated using Image J software (https://imagej.nih.gov/ij/index.html) based on the method described by (Macfarlane et al. 2007).

Between veraison and harvest, a weekly sample of 150 berries per replicate was obtained from approximately 17 bunches per vine in each of the nine plants per replicate. At each sampling time, single berries from each bunch were sampled from either bottom, middle or top bunch positions. Both bunches and berries within the bunch were randomly chosen. A subsample of 50 berries was frozen at -20 °C for the analysis of anthocyanin (Iland et al. 2004), and condensed tannin by the methyl cellulose precipitable (MCP) assay (Mercurio et al. 2007). The remaining berries were crushed with a manual press, and the free-run juice was decanted into a 50 mL centrifuge tube and spun at 1800 g x for 5 min. Total acidity (tartaric acid equivalent at end point pH 8.2) (TA), pH (autotitrator; CRISON, Barcelona, Spain) and TSS (digital refractometer; HI 96801, Hanna Instruments, Woonsocket, RI, USA) were measured shortly after sampling. Technological harvest was targeted at a TSS of approximately 15°Be. At harvest, bunch number and mass were recorded for all vines in each replicate.

Statistical analysis

Phenology, yield and its components, leaf area index and berry traits were assessed with ANOVA using Statview (SAS Institute, Cary, NC, USA). Carry-over effects were assessed between treatments that were pruned at the same time but repeated in successive seasons. Where ANOVA showed significant effects of treatments, we used post-hoc multiple comparison tests (Fisher LSD, Tukey or Dunnett) for separation of means according to the criteria in SAS Institute (1999).

Results

Growing conditions

Monthly mean temperature in spring was similar to or warmer than the long-term median over the four seasons (Figure 1). In 2014/15 and 2015/16, mean temperature exceeded the 90th percentile in September and October. Likewise, the summer was warmer than the median in all seasons. Winter rainfall (April to August), ranged from 200 to 300 mm and summer rainfall (September to February) from 73 to 132 mm. In most seasons, rainfall was closer to or below the long term median but in January 2014 and February 2015 rainfall exceeded the 90th percentile due to large rain events.

Phenology from budburst to veraison

Late pruning delayed vine phenology ($P < 0.001$) by between 2 and 4 weeks at budburst, and between 1 and 2 weeks at flowering, pea size and veraison (Table 2). The Dunnett test showed significant ($P < 0.05$) differences between WP and late pruned vines for all phenology stages. Pruning treatments had no effect on the thermal time between budburst and flowering. From budburst to pea size and from budburst to veraison, however, there was a consistent difference of ~42 °Cdays between WP and the average of late pruning treatments (Table 2). There were no carry-over effects of late pruning on phenological development in successive years ($P > 0.05$).

The phenology of individual shoots from node one (N1) was consistently delayed relative to node two (N2) in vines pruned at 2–3 L. In fact, the phenology of node 2 in 2–3 L was similar to the phenology of BB throughout the various phenostages.

Phenology from veraison to harvest: dynamics of TSS

Compared to vines pruned during winter, TSS in berries of vines pruned at 2–3 L reached 12°Be 7 days later in the first three seasons, and 14 days later in 2015/16 (Figure 2). The trajectory of sugar accumulation of the BB treatment was between that of WP and 2–3 L. At a TSS of 13°Be, the separation between pruning treatments was similar as found at 12°Be with an exception in 2015/16 where all treatments converged at this point. At a TSS of 14°Be, there was a separation of about 6 days between 2–3 leaves and WP in 2012/13 and 2014/15, and about 20 days in 2013/14 due to a significant rainfall event that slowed TSS accumulation.

Canopy growth

The leaf area index (LAI) varied across seasons and pruning times (Figure 3a-c); it peaked between pea size and veraison in 2013/14 and 2014/15, and a few days after flowering in 2015/16. The highest LAI peak was for vines pruned at 2–3 L in 2014/15, then for WP in 2015/16 and was unaffected by pruning time in 2013/14. Around harvest time, LAI was greater in vines pruned at 2–3 L and BB compared with WP in 2013/14 and 2014/15. In 2015/16, LAI remained similar in vines pruned at 2–3 L and WP, but LAI from BB was lower than 2–3 L.

Late pruning increased the number of shoots by 15% in BB and 30% in 2–3 L in comparison to winter in two seasons (Figure 3d-e). Around harvest LAI was correlated with pruning mass (Figure 3f).

Yield components

Late pruning maintained yield in three out of four seasons and vines pruned at 2–3 L outyielded WP in 2014/15 due to an increase in bunch number (Table 3). Yield was unaffected by repeated late pruning for several seasons (Table 3). Lack of yield response to treatments was occasionally related to compensation between components, for example between bunch number and bunch mass in the second season (Table 3).

Berry traits

Total soluble solids, pH and total acidity (TA) at harvest responded to pruning time, season and their interaction (Table 4). In 2014/15, TSS in juice of 2–3 leaves was 1.1°Be lower than that of WP in spite of 2–3 leaves being harvested 1 week later. In the rest of the vintages, 2–3 leaves were harvested at higher TSS than WP, between 0.5 to 1°Be. Likewise, BB TSS was higher in comparison with WP in 2012/13 and 2013/14; however, it remained unchanged in the following seasons. Averaging the four seasons, BB was harvested between 3 to 6 days later than WP with an average of TSS increase of 0.6°Be, and 2–3 leaves was harvested between 7 to 12 days later with an average increment of 0.8°Be.

In 2013/14 and 2014/15, juice pH increased by about 0.14 and TA decreased by about 0.56 g/L in vines pruned at 2–3 L in comparison with that of WP. In 2014/15 and 2015/16, however, TA was higher in 2–3 L than in WP, but pH was similar among treatments. Juice pH and TA in BB was similar to WP except in 2013/14 when it was higher.

Anthocyanin concentration and anthocyanin-to-sugar ratio were higher in late pruning treatments. A linear relationship between anthocyanin and sugars is illustrated in Figure 4 and the rate and onset coefficients from this regression are shown in Table 5. These traits also responded to vintage and interaction effects in two seasons. Plotting the linear phase of anthocyanin dynamics on a TSS scale (Sadras and Moran 2012), we found that late pruning shifted the onset of anthocyanin accumulation of 2–3 L in comparison to that of WP in 2013/14 and 2014/15 (Figure 4, Table 5); the onset was similar between WP and BB. The rate of anthocyanin accumulation on a TSS scale did not respond to late pruning, and neither the onset nor the rate responded to interaction effects between late pruning and season. Berry tannin and the tannin : sugar ratio at harvest were unaffected by pruning time or season.

Discussion

Carry-over effects

This study investigated the feasibility of late pruning to counteract warming effects on maturity and berry traits, with an emphasis on the carry-over effects of repeatedly late pruning the same vines over several seasons. In grapevine, carbohydrates and nitrogen are mobilised after budburst from root and trunk to the shoots, thus supporting the burst of spring growth (Zapata et al. 2004). Delaying pruning after budburst could deplete carbohydrates and if this practice is repeated over several seasons, it may compromise reserves, vigour and yield. For instance, a ~2.5-fold reduction of pruning mass and yield was observed when pruning was delayed up to eight unfolded leaves in Barossa Valley Shiraz (Petrie et al. 2017). In this study we did not quantify the dynamics of carbohydrates; instead, we focused on the viticulturally relevant impacts on yield and pruning mass. Petrie et al. (2017) suggested a boundary of 2–3 leaves as the latest pruning time without a negative impact on vine yield, and this was further explored in our study over several seasons. If depletion of reserves was important, repeating late pruning at budburst and 2–3 leaves in successive years could reduce yield and canopy size in the long term. After four consecutive seasons of pruning at BB or 2–3 L, we found no evidence of negative carry-over effects on yield, canopy size and phenology. The only statistically significant carry-over effect was an increase in bunch number in 2014/15 after repeated pruning at 2–3 L (Table 3). The reasons for this effect are unknown, but a conservative conclusion is that for Shiraz vines grown under Barossa Valley conditions, with a yield range of 0.7–1 kg/m2, a single late pruning event is unlikely to reduce yield and pruning mass.

Phenology to veraison

Apical dominance describes the suppression of outgrowth from lateral buds that is exerted by the apical meristems in the apex of the shoot (Cline 1997). More broadly, correlative inhibition or 'primigenic dominance' explains how the earlier developed sinks inhibit later developed organs (Bangerth 1989). In grapevines, nodes from the upper part of canes develop first during spring and the activation of basal buds is temporally inhibited when spur pruning is delayed. Then, once it is pruned back to a two-node spur, the correlative dominance is released. Delaying the pruning to BB and 2–3 L in our study effectively delayed the budburst of most buds that were at earlier stages of budswell and woody bud (E-L 2 and 3). Interestingly, delaying the pruning later than budburst, the phenology in node one of 2–3 L was delayed in comparison with node two by about 2 weeks at the BB stage. This may be explained by the hierarchy of dominances described in Bangerth (1989).

The maximum difference in phenology between treatments was at budburst, from 10 to 27 days, and treatments tend to converge afterwards; differences were narrower at flowering and even narrower at veraison (Table 2). Martin and Dunn (2000) found that delaying pruning from July to mid-August delayed veraison of Cabernet Sauvignon in central Victoria by about 4 days.

When assessing phenology in a thermal time scale from budburst, no difference was found between winter and late-pruned vines at flowering. In contrast, winter required an extra 40 °Cdays to reach both pea size and veraison in comparison with late-pruned vines, indicating the narrowing difference in phenology between winter and late pruning at this stage. Further, in 2015/16, the average thermal time from the pooled treatments was an extra ~120 °Cdays to veraison compared to that of previous seasons. This suggests that thermal summation is not the only factor influencing phenology and the need to account for both non-resource driven development, and resource-driven growth (Sadras and Moran 2013c, Petrie et al. 2017b).

Fruit maturity

Under our experimental conditions, late pruning delayed the time to TSS 12°Be from 1 to 2 weeks in four consecutive seasons, and ripening was significantly delayed in three out of four seasons for TSS between 12 and 14°Be. The offset in maturity with late pruning in our study was similar or slightly smaller than in a single-season study in the same region with Shiraz and Cabernet Sauvignon (Petrie et al. 2017b) and with Merlot in cooler New Zealand environment (Friend and Trought 2007c). In the latter study, however: (i) fruit was harvested at the same date amongst pruning treatments and the dynamics of TSS was not characterised; and (ii) TSS at harvest ranged from 10.0 to 12.7ºBe amongst treatments, in comparison to higher TSS of Shiraz in warmer regions such as the Barossa Valley (Table 4).

The actual effects of delayed pruning have to be considered against the background environment; for example, temperature around ripening was above the long term median for all the seasons in this study. Rainfall close to harvest influenced the trajectory of TSS. A significant rain event (76 mm) about mid-February shifted the target time of harvest (15°Be) by diluting sugars in the berry by about 3 weeks in 2013/14 (Figure 2). Similar effects of rainfall have been described for Shiraz in the Murrumbidgee Irrigation Areas region of Australia (Rogiers and Holzapfel 2015a).

Vine growth and yield

The LAI peaked about 2 weeks before veraison in two seasons and just after flowering in one season, partially reflecting seasonal differences in temperature and rainfall (Figure 1). Late pruning shifted the dynamics of canopy growth (Figure 3a). To accommodate these changes, some practices including irrigation, summer pruning and fungicide spraying may need some adjustment. The smaller reduction in canopy size from its peak to harvest in 2–3 leaves might be beneficial to protect bunches during heat waves, though this requires further investigation to deal with the trade-off between sunlight exposure required for synthesis of anthocyanin (Cortell and Kennedy 2006, Guan et al. 2014) and greater exposure increasing risk of heat damage (Bergqvist et al. 2001).

Pruning around budburst may improve yield in cultivars that crop erratically due to poor fruitset (Coombe 1964, Friend and Trought 2007c). In our study, yield was largely unresponsive to pruning time, partially because we relied on previous work that determined the latest phenological stage where yield is compromised for Shiraz under Barossa conditions (Petrie et al. 2017b). Variation in yield was only registered in 2014/15 where 2–3 leaves increased yield due to higher bunch number (Table 3). This 30% increase in bunch number corresponded to a proportional 27% increase in cane number, suggesting that fruitfulness per shoot remained similar. Nevertheless, in 2013/14 late pruning increased shoot number in 2–3 leaves by about 30% but yields were unchanged.

Berry pH, TA and anthocyanin

We targeted harvest at a similar grape juice TSS among treatments, but logistical imperatives in the commercial vineyard and rainfall events caused some deviation from these targets. In general, TSS was higher in late-pruned vines at harvest, except for 2–3 leaves in 2015 (Table 4). The effect of late pruning on pH and TA at harvest varied with the season. In the two first seasons pH increased and TA decreased in 2–3 leaves. This was related to higher TSS in late-pruned vines, and no difference in pH and TA was found for data interpolated at the same TSS (data not shown). In the following two seasons, pH remained the same; however, TA in the 2–3 leaves treatment increased by about 0.5 g/L.

Sadras and Moran (2012) showed that high temperature delays the onset of anthocyanin accumulation on a TSS scale in Shiraz and Cabernet Franc berries. In this study, late pruning advanced the onset of anthocyanin against TSS in comparison with WP thus favouring the anthocyanin : sugar ratio (Figure 4, Table 5). These results are consistent with a previous study where late pruning increased the berry anthocyanin in Shiraz and Cabernet Sauvignon in the Barossa Valley (Petrie et al. 2017b). Similarly, spraying an antitranspirant at pre-flowering and pre-veraison advanced the onset of anthocyanin against sugars in Piacenza Barbera (Gatti et al. 2016). The rate of anthocyanin accumulation on a TSS scale appears to be a more conserved trait as it did not respond to pruning time (Figure 4, Table 5) or temperature (Sadras and Moran 2012).

Extreme temperature impacts negatively in the development and concentration of anthocyanin in red grapes cultivars (Kliewer 1977, Mori et al. 2005). In our study, the maximum temperature in the week after veraison was 9ºC cooler in 2–3 leaves compared to that of WP in both 2013/14 and 2014/15. The lower temperature was observed as veraison occurred later in the season for the late pruned treatments. This can partially explain the improved anthocyanin : sugar relation in 2–3 leaves berries. Further work is needed to determine to what extent this improvement in berries is reflected in wines.

Conclusion

Late pruning can be used as a practical, cost-effective tool to spread harvest without penalty on yield or carry-over effects for Shiraz under Barossa Valley conditions. Late pruning could improve or maintain the anthocyanin:sugar ratio in berries. In this trial vine canopy management and irrigation were set to the requirements of WP vines. Fine-tuning management to late-pruned vines could return further benefits. Extrapolation of these results to other cultivars and regions is not warranted.

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Table 1. (a) Layout of late pruning treatments in a Shiraz vineyard in the Barossa Valley in South Australia and (b) measurements made during the four seasons.

(a)

Season	Treatment
2012/13	**WP, 1st BB, 1st 2–3 L**
2013/14	**WP, 2nd BB, 2nd 2–3 L**; 1st BB, 1st 2–3 L
2014/15	**WP, 3rd BB, 3rd 2–3 L,** 2nd BB, 2nd 2–3 L; 1st BB, 1st 2–3 L
2015/16	**WP, 4th BB, 4th 2–3 L,** 3rd BB, 3rd 2–3 L; 2nd BB, 2nd 2–3 L, 1st BB, 1st 2–3 L

(b)

Season	Yield	Berry traits			Phenology	LAI	Grape phenolic substances		Pruning mass
		TSS	pH	Total acidity			Anthocyanin	Tannin	
2012/13	✓	✓	✓	✓					
2013/14	✓	✓	✓	✓	✓	✓	✓	✓	✓
2014/15	✓	✓	✓	✓	✓	✓	✓	✓	✓
2015/16	✓	✓	✓	✓	✓	✓			

A set of vines was used as the winter pruning Control (WP) over the four seasons. Late pruning treatments included budburst (BB) and 2–3 leaves (2–3 L). Arrows indicate the time sequence of each set of vines and 1st, 2nd, 3rd and 4th indicate vines that were late pruned in a single season, or where late pruning was repeated in the same vines during 2, 3 and 4 seasons, respectively. Treatments in red highlight the inclusion of a new set of vines to be late-pruned each season, hence the increase in the number of treatments from three in 2012/13, to five in 2013/14 to seven in 2014/15. In the fourth season, the 1st BB and 1st 2–3 L treatments (blue) were not established because the vines allocated to this treatments were inadvertently pruned in winter. Bold font indicates six and plane font indicates four replicates. LAI, leaf area index.

Table 2. Effect of pruning in winter, at budburst and at 2–3 leaves on Shiraz phenology during three consecutive seasons.

			Budburst	Flowering		Pea size		Veraison	
Season	**Pruning time**	**Node**	**Days**	**Days**	**°Cdays**	**Days**	**°Cdays**	**Days**	**°Cdays**
2013/14	WP (6†)	N1, N2	31 ± 0.4	94 ± 0.7	318	121 ± 1.0	485	163 ± 0.4	885
	2nd‡ BB *(*6)	N1, N2	39 ± 0.5	97 ± 0.4	286	130 ± 1.1	515	168 ± 0.6	924
	2nd 2–3 L (6)	N1	58 ± 0.6	111 ± 0.6	293	145 ± 2.4	584	176 ± 0.6	967
	2nd 2–3 L *(6)*	N2	42 ± 0.7	101 ± 1.1	296	130 ± 1.3	499	169 ± 0.7	929
	1st BB (4)	N1, N2	41 ± 0.6	98 ± 0.8	284	130 ± 1.2	500	167 ± 0.8	885
	1st 2–3 L (4)	N1	57 ± 0.8	111 ± 0.9	294	143 ± 0.9	570	176 ± 0.5	968
	1st 2–3 L (4)	N2	43 ± 1.2	103 ± 2.6	300	135 ± 1.1	517	170 ± 0.5	950
2014/15	WP (6)	N1, N2	36 ± 0.5	90 ± 0.5	309	111 ± 0.6	491	151 ± 0.3	873
	3rd BB (6)	N1, N2	49 ± 0.7	97 ± 0.9	316	118 ± 1.0	514	155 ± 0.5	887
	3rd 2–3 L (6)	N1	61 ± 1.3	101 ± 0.6	301	125 ± 1.2	544	160 ± 0.9	924
	3rd 2–3 L (6)	N2	52 ± 1.5	96 ± 0.7	306	120 ± 0.9	528	156 ± 0.3	900
	1st BB (4)	N1, N2	51 ± 0.4	98 ± 0.9	322	118 ± 0.4	512	155 ± 0.5	884
	2nd BB (4)	N1, N2	50 ± 1.0	98 ± 0.9	325	117 ± 0.5	508	155 ± 0.5	887
	1st 2–3 L (4)	N1	61 ± 0.8	101 ± 0.6	301	126 ± 0.6	555	160 ± 0.9	924
	1st 2–3 L (4*)*	N2	53 ± 0.0	96 ± 0.7	302	122 ± 0.6	552	156 ± 0.3	896
	2nd 2–3 L (4*)*	N1	63 ± 0.3	101 ± 0.6	295	125 ± 0.6	539	160 ± 0.9	919
	2nd 2–3 L (4)	N2	51 ± 0.5	96 ± 0.7	311	118 ± 1.1	512	156 ± 0.3	905
2015/16	WP (6)	N1, N2	39 ± 0.9	90 ± 0.5	329	108 ± 0.7	494	152 ± 0.7	997
	4th BB (6)	N1, N2	47 ± 0.9	92 ± 0.4	316	115 ± 1.5	529	159 ± 0.9	1054
	4th 2–3 L (6)	N1	62 ± 0.4	97 ± 0.4	336	121 ± 0.9	547	159 ± 0.6	1032
	4th 2–3 L (6)	N2	48 ± 0.8	95 ± 0.3	331	117 ± 0.7	542	158 ± 0.9	1032
	2nd BB (4)	N1, N2	49 ± 1.3	92 ± 0.4	311	115 ± 1.1	525	157 ± 0.8	1025
	3rd BB (4)	N1, N2	48 ± 1.2	92 ± 0.4	315	115 ± 0.8	528	157 ± 1.2	1029
	2nd 2–3 L (4)	N1	59 ± 0.6	97 ± 0.6	336	119 ± 1.3	534	161 ± 0.5	1056
	2nd 2–3 L (4*)*	N2	49 ± 1.3	95 ± 0.3	331	116 ± 1.7	527	158 ± 0.0	1032
	3rd 2–3 L (4)	N1	58 ± 0.9	97 ± 0.6	327	119 ± 1.7	525	160 ± 1.0	1036
	3rd 2–3 L (4)	N2	47 ± 0.3	95 ± 0.3	337	116 ± 1.4	533	158 ± 1.5	1037

Values are mean ± standard error for days after 1 August; °Cdays from budburst are also shown [base temperature = 10ºC (Winkler et al. (1974b)]. †Number of replicates. ‡1st, 2nd, 3rd, and 4th, late-pruning treatments in a single season, two, three and four successive seasons, respectively. BB, budburst;2–3 L, 2–3 leaves; WP, late pruning.

Table 3. Effect of pruning in winter, at budburst and at 2–3 leaves on yield and its components in Shiraz vines during four seasons.

Season	**Treatment**	**Yield (kg/vine)**	**Bunch No./ vine**	**Bunch mass (g/bunch)**	**Berry No./bunch**	**Berry mass (g)**
2012/13	WP	3.4 ± 0.222	48 ± 1.7	72.1 ± 3.86 a	86 ± 4.7 a	0.84 ± 0.032
	1st BB	3.5 ± 0.221	51 ± 2.0	67.9 ± 5.16 a	79 ± 4.1 a	0.87 ± 0.072
	1st 2–3 L	2.8 ± 0.220	52 ± 1.5	54.2 ± 2.41 b	64 ± 3.6 b	0.85 ± 0.041
	P-value	0.0743	0.1799	0.0137	0.0094	0.9278
2013/14	WP	3.4 ± 0.42	63 ± 4.1 a	53.1 ± 3.55 b	63 ± 4.4 b	0.84 ± 0.025 bc
	2nd BB	3.5 ± 0.24	48 ± 1.4 b	70.4 ± 2.54 ab	83 ± 4.9 a	0.86 ± 0.026 bc
	2nd 2–3 L	2.9 ± 0.30	45 ± 1.7 b	63.7 ± 4.65 ab	58 ± 3.4 b	1.10 ± 0.024 a
	1st BB	3.8 ± 0.87	52 ± 6.4 ab	71.1 ± 6.84 a	89 ± 8.8 a	0.80 ± 0.005 c
	1st 2–3 L	3.6 ± 0.37	55 ± 5.2 ab	65.5 ± 3.31 ab	71 ± 2.0 ab	0.92 ± 0.020 b
	P-value	0.7211	0.0144	0.0327	0.0009	<0.0001
2014/15	WP	3.7 ± 0.20 a	46 ± 2.1 c	78.8 ± 1.42	77 ± 1.7 a	1.02 ± 0.010
	3rd BB	3.7 ± 0.10 a	51 ± 0.6 abc	72.9 ± 2.28	72 ± 2.1 ab	1.01 ± 0.010
	3rd 2–3 L	4.9 ± 0.31 a	60 ± 2.1 a	80.6 ± 3.60	78 ± 2.9 a	1.03 ± 0.028
	2nd BB	3.5 ± 0.25 a	49 ± 0.9 bc	71.4 ± 4.04	67 ± 3.1 ab	1.04 ± 0.038
	2nd 2–3 L	3.5 ± 0.29 a	52 ± 3.8 abc	67.7 ± 1.45	65 ± 3.6 b	1.06 ± 0.032
	1st BB	4.1 ± 0.45 a	51 ± 3.9 abc	78.9 ± 5.52	74 ± 2.8 ab	1.07 ± 0.054
	1st 2–3 L	4.7 ± 0.60 a	57 ± 2.5 ab	80.7 ± 7.71	78 ± 4.4 a	1.03 ± 0.049
	P-value	0.0135	0.0026	0.1632	0.0106	0.8119
2015/16	WP	4.3 ± 0.27	83 ± 3.7 a	51.3 ± 1.72	72 ± 2.9 a	0.71 ± 0.02
	4th BB	3.3 ± 0.27	71 ± 2.4 ab	45.8 ± 2.59	61 ± 3.7 a	0.75 ± 0.02
	4th 2–3 L	3.7 ± 0.32	60 ± 1.9 b	60.7 ± 3.73	77 ± 3.2 a	0.78 ± 0.02
	3rd BB	3.4 ± 0.58	72 ± 3.8 ab	47.8 ± 7.02	62 ± 7.2 a	0.77 ± 0.03
	3rd 2–3 L	3.4 ± 0.25	59 ± 1.4 b	58.1 ± 3.93	76 ± 4.3 a	0.77 ± 0.03
	2nd BB	4.2 ± 0.59	80 ± 5.1 a	52.0 ± 5.85	65 ± 2.4 a	0.80 ± 0.02
	2nd 2–3 L	3.4 ± 0.64	57 ± 3.2 b	58.6 ± 7.76	76 ± 4.1 a	0.76 ± 0.04
	P-Value	0.3983	<0.0001	0.1462	<0.0001	0.1581

Mean values were separated using post hoc Tukey HSD. Different letters indicate a significant difference (*P*=0.05) between means for each season. Three pruning times were compared: winter (WP), budburst (BB) and 2–3 leaves (2–3 L) where 1st, 2nd, 3rd, and 4th indicate late-pruning treatments in a single season, two, three and four successive seasons, respectively. Values are means ± standard error and *P* values are from ANOVA.

Table 4. Effect of pruning time on Shiraz berry traits.

Season	Pruning time	Harvest date	TSS (°Be)	pH	TA (g/L)	Anthocyanin (mg/g)	Tannin (mg/g)	Anthocyanin:TSS [(mg/g) /°Be]	Tannin:TSS [(mg/g) /°Be]
2012/13	WP	13 Feb	14.5 ± 0.10 b	3.46 ± 0.026 b	6.7 ± 0.16 a				
	BB	19 Feb	15.9 ± 0.17 a	3.60 ± 0.036 a	5.8 ± 0.21 b				
	2–3 L	25 Feb	15.6 ± 0.02 a	3.59 ± 0.027 a	6.0 ± 0.15 b				
2013/14	WP	5 Mar	14.1 ± 0.15 b	3.91 ± 0.025 b	4.4 ± 0.04 a	1.5 ± 0.01 b	7.3 ± 0.27	0.108 ± 0.002 b	0.52 ± 0.022
	BB	11 Mar	14.6 ± 0.08 a	3.95 ± 0.018 b	4.2 ± 0.04 a	1.8 ± 0.04 a	6.9 ± 0.33	0.125 ± 0.002 a	0.47 ± 0.014
	2–3 L	17 Mar	14.6 ± 0.02 a	4.05 ± 0.030 a	4.0 ± 0.04 b	1.8 ± 0.03 a	6.8 ± 0.18	0.123 ± 0.002 a	0.48 ± 0.011
2014/15	WP	16 Feb	16.9 ± 0.13 a	3.70 ± 0.017 ab	5.1 ± 0.04 a	1.8 ± 0.03 a	7.3 ± 0.05	0.107 ± 0.002 a	0.43 ± 0.005
	BB	18 Feb	16.5 ± 0.15 a	3.66 ± 0.018 b	5.1 ± 0.02 a	1.8 ± 0.04 a	6.8 ± 0.02	0.112 ± 0.002 a	0.42 ± 0.013
	2–3 L	23 Feb	15.8 ± 0.15 b	3.75 ± 0.029 a	5.5 ± 0.06 b	1.7 ± 0.04 a	6.5 ± 0.27	0.107 ± 0.002 a	0.40 ± 0.018
2015/16	WP	15 Feb	14.8 ± 0.12 b	3.63 ± 0.020 a	4.8 ± 0.06 a				
	BB	18 Feb	14.7 ± 0.09 b	3.66 ± 0.014 a	4.7 ± 0.09 a				
	2–3 L	22 Feb	15.5 ± 0.15 a	3.66 ± 0.019 a	5.3 ± 0.04 b				
	P-Pruning		0.0003	<0.0001	0.0006	0.027	0.5604	0.0005	0.1589
	P-Season		<0.0001	<0.0001	<0.0001	0.0064	0.1199	<0.0001	<0.0001
	P-Interaction		<0.0001	0.0081	<0.0001	0.0001	0.8566	0.0182	0.4782

Values are means ± standard error from six replicates, each including nine vines, and *P* values are from ANOVA. Mean values were separated using post hoc Fisher LSD; different letters indicate a significant difference between means in each season (*P*=0.05). BB, budburst; 2–3 L, 2–3 leaves; WP, winter pruning.

Table 5. Rate and onset of anthocyanin accumulation from linear relationship between anthocyanin and TSS (Figure 4).

Season	Rate [mg/g)/ °Be] Pruning time WP	BB	2–3 L	Difference (%)† BB-WP	Difference (%) 2–3 L-WP	Onset (°Be) Pruning time WP	BB	2–3 L	Difference (%) BB-WP	Difference (%) 2–3 L-WP
2013/14	0.19 ± 0.005	0.19 ± 0.005	0.18 ± 0.005	-1.9	-6.5	5.74 ± 0.398 a‡	4.93 ± 0.382 a	4.63 ± 0.377 b	-14	-19
2014/15	0.15 ± 0.005	0.15 ± 0.004	0.14 ± 0.004	3.8	-1.3	3.66 ± 0.486 a	3.26 ± 0.351 a	3.25 ± 0.286 b	-11	-11
P-Pruning				0.64					0.019	
P-Season				<0.001					<0.001	
P-Interaction				0.31					0.39	

† Percentage difference between winter Control (WP) and late pruning treatments at budburst (BB) and 2–3 leaves (2–3 L). ‡ Mean values were separated using post hoc Fisher LSD (*P*=0.05). Dissimilar letters indicate a significant difference.

Caption to figures

Figure 1. Monthly (a) mean temperature and (b) mean rainfall for four consecutive seasons, 2012/13 (○), 2013/14 (▽), 2014/15 (□) and 2015/16 (◇), in contrast to the the 90th (——), 50th (——), and 10th (——) percentile long term records from the period 1957–2016 at Nuriootpa, South Australia meteorological station. Inset shows the accumulated rainfall of winter (April–August) (■) and summer (September–February) (□).

Figure 2. Dynamics of TSS in Shiraz berries affected by pruning in winter (●), at budburst (●) and at 2–3 leaves stage (●) during the (a) 2012/13, (b) 2013/14, (c) 2014/15 and (d) 2015/16 seasons. Values are mean ± standard error and the significance difference between treatments is indicated by **, $P<0.01$ and ***, $P< 0.001$. Arrow heads indicate rainfall events over 5 mm. The area between the horizontal dashed lines indicates the maturity harvest band based on the alcohol concentration for red wines from 1984 to 2014 in Australia (Godden et al. 2015).

Figure 3. Dynamics of leaf area index in vines pruned in winter (●), at budburst (●) and at 2–3 leaves (●) in the(a) 2013/14, (b) 2014/15 and (c) 2015/16 seasons; significance difference between treatments is indicated by *, $P<0.05$, **, $P<0.01$ and ***, $P<0.001$. Number of shoots per vine at pruning as altered by pruning time in the (d) 2013/14, (e) 2014/15 seasons; treatment effects were significant at $P<0.001$. (f) Correlation between leaf area index and pruning mass (R2=0.94) for winter (●), budburst (■) and 2–3 leaves (▲) in 2013/14, and winter (○), budburst (□) and 2–3 leaves (△) in 2014/15. Values are means ± standard error per treatment (a–f). (a–c) The phenology stages of (F) flowering, (V) veraison and (H) harvest are indicated. DOY, days of the year.

Figure 4. Effect of pruning in winter (●), at budburst (●) and at 2–3 leaves (●) on the relationship between concentration of anthocyanin and TSS (from onset of anthocyanin onwards), in seasons (a) 2013/14 and (b) 2014/15. Lines are least square regressions (0.92≤ r ≤0.99). The rate (slope) and onset (x-intercept) of the regression are in Table 5.

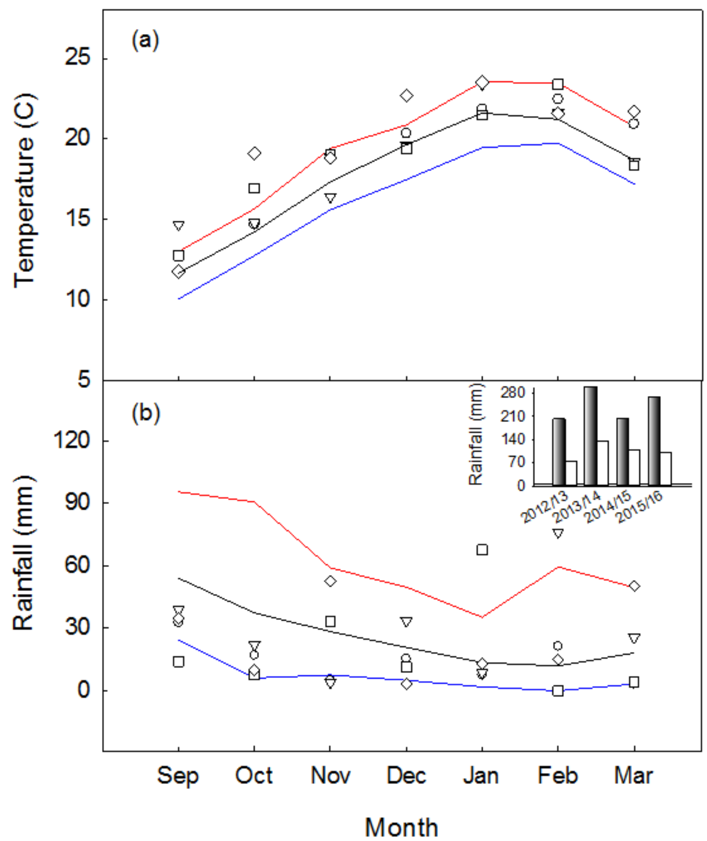


Figure 1

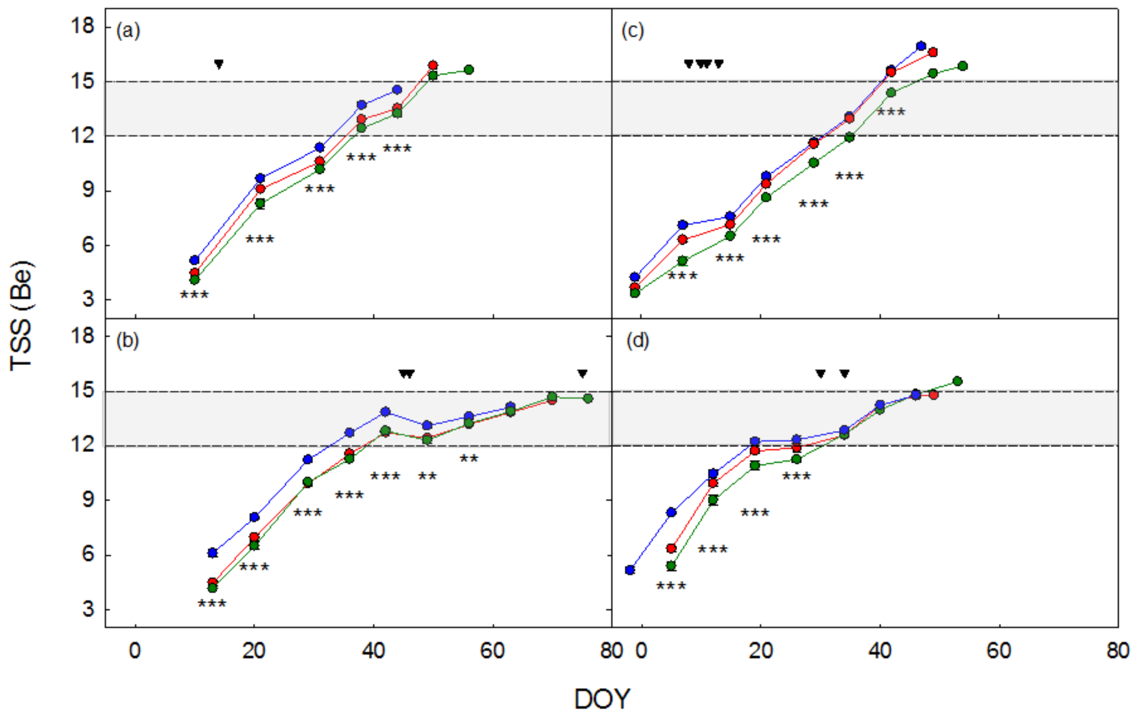
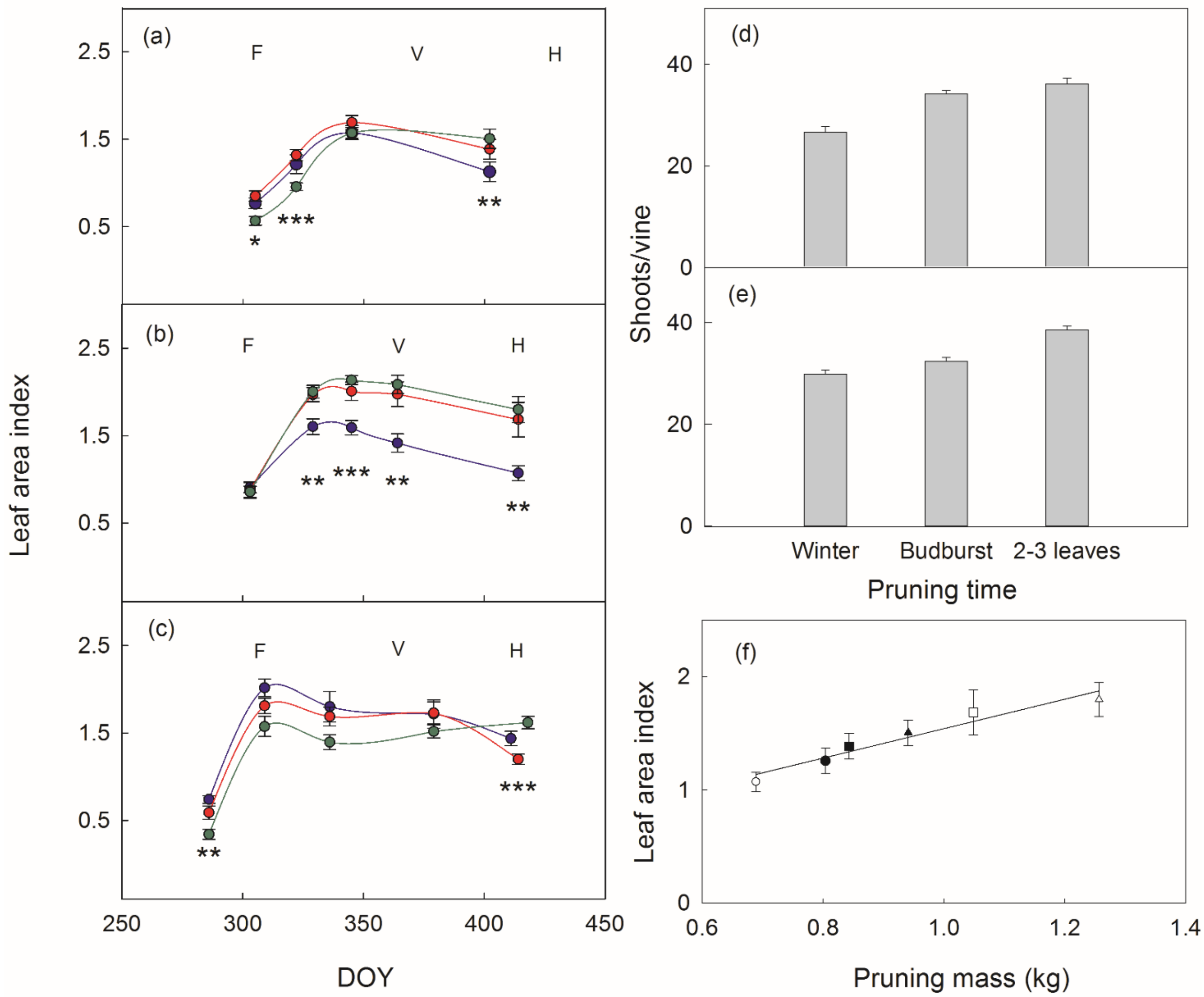


Figure 2



(a)
F
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2.5
1.5
0.5
*
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**
(b)
F
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2.5
1.5
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(c)
F
V
H
2.5
1.5
0.5
**
***
250
300
350
400
450
DOY
Leaf area index
(d)
40
20
0
(e)
40
20
0
Shoots/vine
Winter
Budburst
2-3 leaves
Pruning time
(f)
2
1
0
0.6
0.8
1.0
1.2
1.4
Leaf area index
Pruning mass (kg)

Figure 3

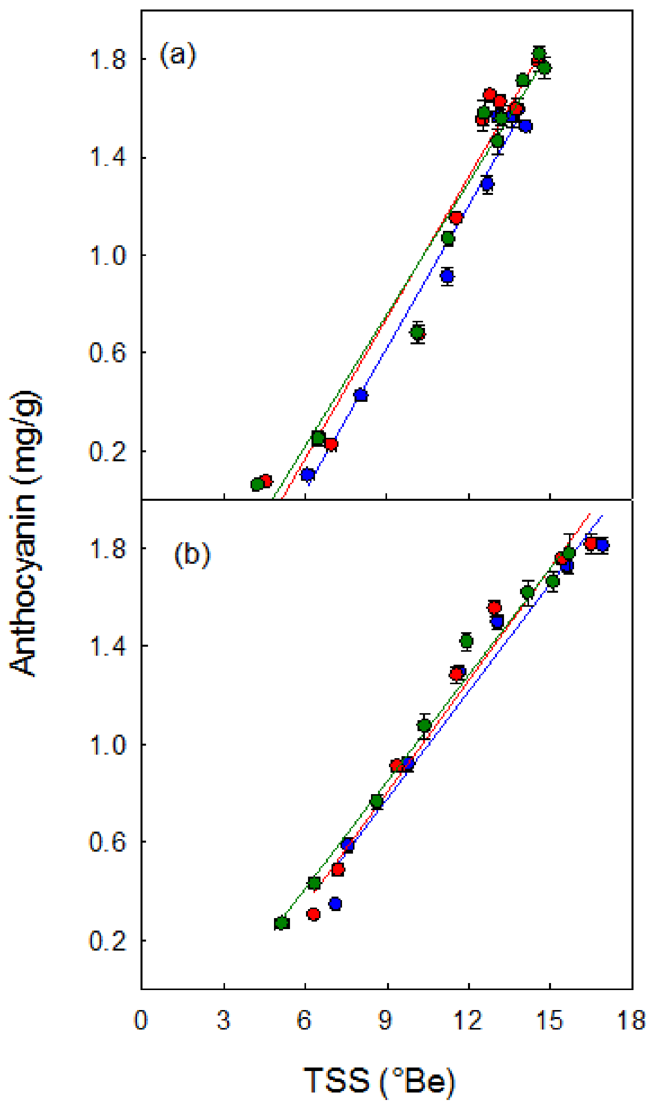

Figure 4

Chapter 3

Late pruning impacts on chemical and sensory attributes of Shiraz wine

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Statement of Authorship

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Author Contribution

Martin Moran conducted the literature review, set up and managed the experiment, drafted and constructed the manuscript.

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.

Overall Percentage: 75% ________________ Signature   Date 9/8/19

Co-Authors Contribution

By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Victor O. Sadras contributed to the experimental design, research ideas, supervision of research, and editing of manuscript.

Signature   Date 9/8/19

Paul R. Petrie contributed to research ideas, experimental design and editing of manuscript.

Signature   Date 9/8/19

Sue E. Bastian contributed to the supervision of the sensory panel and editing of manuscript.

Signature   Date 9.8.19

Abstract

Background and Aims: Warming has two major effects on the wine industry: compressing harvest duration, thus stressing the current capacity of wineries to process more fruit in a shorter time; and compromising fruit composition and wine style. Late pruning can effectively delay vine development and contribute to decompressing harvest, but its impact on wine is unknown. Our aim was to measure the effects of late pruning on wine chemical and sensory attributes.

Methods and Results: We compared wines made from Shiraz vines pruned in winter (Control), and in two late pruning stages, when Controls reached budburst and 2–3 leaves in two vintages. Late pruning consistently increased wine anthocyanin, tannin, pigmented tannin and colour density and altered the wine's sensory profiles over two vintages. In 2014, colour intensity, fruit aroma, fruit flavours and body were more intense in wine made from late pruning treatments. In 2015, wine made from late pruning treatments showed more intense savoury flavours with a dryer palate and a smoother texture tannin (roughing sub-quality). The colour improvement was associated with cooler temperature one week after veraison in the late pruned vines.

Conclusions: Late pruning consistently improved wine chemical and altered sensory profiles of Shiraz under Barossa Valley conditions.

Significance of the Study: Late pruning is a cost-effective tool to decompress harvest, with neutral effects on yield and positive effects on wine chemical attributes with enhancement of fruit and colour intensity perception in an extended vintage (2014), and smoother tannin texture with dryer perception in a short and compressed vintage (2015).

Keywords: *anthocyanin, Barossa Valley, climate change, tannin, temperature, veraison*

Introduction

Growing grapes and making wine in warming environments has brought new challenges to vignerons and winemakers. Warming has been associated with earlier maturity in time series studies and field experiments in Australia (Petrie and Sadras 2008, Sadras and Petrie 2011, Webb et al. 2011, Sadras and Moran 2013b) and other wine regions worldwide (Duchene and Schneider 2005, Jones et al. 2005). Furthermore, warming has increased pressure on winery logistics as a result of harvest compression (Sadras et al. 2014). Field experiments showed that elevated temperature can disrupt ratios between sugars and berry compositional traits, and alter the sensory properties of wines (Spayd et al. 2002, Tarara et al. 2008, Sadras and Moran 2012, Sadras et al. 2013b, Bonada et al. 2015). Thus, there is an increased risk of harvesting overripe fruit, leading to higher potential alcohol, astringent phenolic substances and loss of fresh fruit flavours. This is likely to impact on wine regional styles. For instance, high concentration of sugars during fermentation might add stress to yeast metabolism and it can lead to stuck fermentations and high alcohol wines (Bisson 1999, de Orduña 2010). High alcohol in wines can alter the sensory perception by masking fruit aromas or flavours and increasing bitterness and hotness (Vidal et al. 2004, Goldner et al. 2009, Meillon et al. 2009). Wine alcohol can be reduced in the winery (Aguera et al. 2010), but even this could be difficult in a compressed vintage. Consequently, management to delay ripening and assist in scheduling the harvest according to grape maturity targets would provide multiple benefits to mitigate fruit downgrading and cost of production.

Mechanical leaf removal (apical-to-bunches) and the application of anti-transpirant after veraison have been investigated as vineyard techniques that can delay fruit maturity. Both methods reduced berry TSS by approximately 0.7°Be in Sangiovese (Perugia, Italy) when the

Control was harvested at 13.3°Be, producing wines with 1% less alcohol but with a 15% decrease in anthocyanin when anti-transpirant was applied (Palliotti et al. 2013a, Poni et al. 2013, Palliotti et al. 2014). Synthetic auxins (NAA) delayed maturity of Shiraz by about 10 days at a harvest TSS target of 13.7°Be at Hahndorf, in the Adelaide Hills, South Australia (Böttcher et al. 2011). Analysis of 128 volatile compounds of the wine headspace (SPME/GC/MS) showed higher concentration of 14 compounds in wines from NAA treated vines, and more aliphatic alcohols and linalool (monoterpene alcohol) in Control wines. Informal sensory analysis, however, showed no difference between wines. Forcing primary buds to burst in the same season delayed maturity and increased fruit quality of Cabernet Sauvignon in Fresno, California (Gu et al. 2012a). In all these studies, cultural practices to delay maturity involved higher costs, reliance on chemicals or both when compared with late pruning.

Late pruning is a cost effective approach to shift development when seeking to improve adaptation to elevated temperature (Moran et al. 2017, Petrie et al. 2017a) and reduce frost risk (Friend and Trought 2007). For this practice to be effectively used to decompress harvest, late-pruning must be neutral or positive for wines. This paper reports chemical and sensory profiles of Shiraz wines in response to late pruning under Barossa Valley conditions during two contrasting vintages.

Methods

Site and experimental design

The site, vineyard, growing conditions, treatments and experimental design have been described elsewhere (Moran et al. 2017). Briefly, a pruning trial was set up in the winter of

2011 in a commercial vineyard at Marananga (-34.50 S, 138.89 E) in the Barossa Valley. Own-rooted Shiraz vines were established at 1.8 m within and 3 m between rows and trained to a single cordon (as two arms from the trunk), spur-pruned at 18 nodes per metre, with a sprawling canopy supported by a single wire about 0.4 m above the cordon.

To assess phenology, the modified E-L system was used (Coombe 1995). Three treatments were compared: vines were pruned in winter (Control, WP), or when Controls reached budburst (BB) in E-L 4 or 2 to 3 leaves (2–3 L) in E-L 9 phenology score system. Each treatment included six replicates in a randomised design, and each replicate included nine vines. Pruning treatments were repeated in 3 consecutive years over the same vines, and wines were made in 2014 and 2015 vintages. In 2014, pruning dates were on 1 September in BB and 19 September in 2–3 L. In 2015, pruning dates were on 9 September in BB and 17 September in 2–3 L. Pruning dates in WP were in mid-July in 2014 and 2015 (Moran et al. 2017).

Winemaking

Harvest was aimed at a TSS of ~15°Be in conjunction with berry sensory assessment done by the winemaker, but there was seasonal variation around this target. In 2014, fruit was harvested at TSS of approximately 14.5°Be. In 2015, fruit was harvested at TSS of 16.9°Be in WP , 16.5°Be in BB, and 15.8°Be in 2–3 L. In 2015, acidified distilled water (5 g/L tartaric acid) was added to must to achieve dilution of approximately 12% in WP, 10% in BB and 5% in 2–3 L to standardise across treatments and ensure complete fermentation. The occurred high TSS was due to berry shrivelling as a result of warm and dry conditions around harvest (Moran et al. 2017).

Wines were made on a small scale for each field replicate. Fruit parcels of 25–30 kg were crushed, destemmed and fermented in 75 L open top containers. Yeast (EC 1118 Lalvin, Blagnac, France) was added at 20 mg/L and during active fermentation the cap was plunged twice a day. After a week, ferments were basket-pressed and the wine was transferred into 10 L demijohns with an immediate initial addition of 1 g/L of tartaric acid. Then, pH was adjusted to 3.50 prior to MLF. Malolactic bacteria (Lalvin VP41, *Oenococus oeni*, Lallemand) were inoculated once the wines had completed primary fermentation and the residual sugar was below 3 g/L, except for winter Control in 2015 (Table 1). Malolactic fermentation was assumed to be completed when wines were below 100 mg/L of malic acid as measured with Accuvin colour strip test (Accuvin, Napa, CA, USA). After completion of MLF, the wine was racked off from gross lees, corrected to about 40 mg/L of free sulfur dioxide with potassium metabisulfite, and refrigerated at 4°C for approximately 2 weeks before bottling. The wines were bottled in 375 mL Burgundy bottles using screw cap lids, packed in cartons and aged for 4 to 5 months at ~18°C prior to chemical and sensory analysis.

Analysis of wine composition

Wines were analysed in duplicate and included: (i) TA at pH end point 8.2; (ii) pH; (iii) volatile acidity; (iv) alcohol; and (v) free and total sulfur dioxide (SO_2) (aspiration method) as described in Iland et al. (2004). Residual sugar was measured with a colorimeter (Bayer Clinitest copper sulfate tablets) (Vintessential, Orange, NSW, Australia) in 2014 and infrared method, (OenoFoss™, Foss electric, Hilleroed, Denmark) in 2015. Wine colour was assessed by two methods; the tristimulus CIELAB (Ohno 2000) including L* (lightness), a* (from green to red), b* (from blue to yellow) and C* (chroma or saturation) parameters, and modified Somers (Mercurio et al. 2007). The wine colour spectra including (i) chemical age 1 and 2, (ii) colour density and (iii) hue (SO_2 corrected), (iv) pigmented tannin (denotes to SO_2

resistant pigments), (v) anthocyanin and (vi) phenolic substances; and tannin by methylcellulose precipitable technique were measured as described in (Mercurio et al. 2007). The co-pigmentation factor was measured to capture the pigmented tannnin that accounts for 40–70% of the total colour of young red wines (Gutiérrez 2003). Parameters measured were co-pigmentation (%), polymerisation (%) and delta colour (%).

Wine sensory analysis

A panel comprised of 12 graduates from The University of Adelaide, School of Agriculture Food and Wine, was trained in ten 2 h sessions to undertake descriptive analysis following the protocol described in Bastian et al. (2010). Supplementary Table S1 shows the reference standards used in the training sessions. During the formal tasting, each wine was assessed in triplicate for each field replicate in clear glasses. There was a total of three formal sessions comprising 18 wines with breaks of 5 min between six samples assessed. Each attribute was scored on a line scale from zero (low) to 100 (high) under fluorescent light and in individual computerised booths. The panel decided to score the attributes in the following order: 1, colour; 2, aroma; 3, taste; 4, flavours; 5, mouthfeel; and 6 aftertaste. There were 33 attributes assessed in 2014 and 36 in 2015. For mouthfeel attributes, body was anchored as medium to full bodied, tannin quantity from non-drying to very drying and tannin quality from smooth (silky) to harsh (sandpaper), with three intervening categories, velvet, suede, and chalky. The aftertaste attributes, fruit, non-fruit and alcohol were described after expectoration; fruit as any fruit perceived, non-fruit as anything that was non-fruit, and alcohol as heat perception from low to high.

Statistical analysis

Wine colour spectrum, % co-pigmentation and tannin were assessed with a two-way ANOVA with pruning time and vintage as fixed effects (Statview, SAS Institute, Cary, NC, USA). The sensory descriptors were analysed with a mixed model two-way ANOVA with pruning time as a fixed factor and assessor and assessor-by-pruning interaction as random effects (XLSTAT Version 2015.5.01.23654, Addinsoft, Paris, France). Fisher LSD test was used to account for mean difference between treatments when ANOVA was significant. Linear regression was fitted to correlate mean temperature (averaged for the week after veraison) with anthocyanin concentration in wines. Principal component analysis (PCA) was used to explore associations amongst climate variables, such as temperature (min, max and mean), radiation, vapour pressure deficit (VPD) and anthocyanin, and among chemical and sensory traits using chemical as active and sensory as supplementary variables; the analysis was constrained to those variables that responded to pruning treatment according to ANOVA. The responsive variables changed with vintage, hence a separate PCA was performed for 2014 and 2015.

Results

Growing conditions during fruit ripening

General growing conditions, phenology and yield have been described in a previous paper (Moran et al. 2017). In 2014, late pruning delayed the onset of veraison by 5 (BB) to 13 days (2–3 L) in relation to WP. In 2015, late pruning delayed the onset of veraison by 4 (BB) to 9 days (2–3 L) compared to WP. In 2014 late pruning did not affect yield, which averaged 3.3 kg per vine amongst treatments. In 2015, yield increased from 3.7 kg in WP to 4.9 kg per vine in 2–3 L.

Here we focus on the period from veraison to harvest for its relevance to wine (Soar et al. 2008). In both seasons, fruit of late-pruned vines developed under cooler conditions immediately after veraison in comparison to winter-pruned controls (Figure 1).

In 2014, from veraison (E–L 35) to 1 week after veraison, average maximum temperature was 4.8°C lower in BB and 8.6°C lower in 2–3 L treatment than in Controls (Figure 1a). The average minimum temperature for the same period was 0.4°C lower in BB and 3.7°C lower in 2–3 L in comparison with WP. The vapour pressure deficit (VPD) was 0.4 kPa lower in BB and 0.8 kPa in 2–3 L in comparison with WP. Radiation was 0.7 MJ/m^2 lower in 2–3 L in comparison with that in WP and BB. From veraison to harvest, the mean temperature was 0.5°C lower in BB and 1.5°C lower in 2–3 L than in WP. Radiation was 1.8 MJ/m^2 lower in 2–3 leaves in comparison with WP.

In 2015, from veraison to 1 week after veraison, average maximum temperature was 7.2°C lower in BB and 9.3°C lower in 2–3 L treatment than in Controls (Figure 1a). The average minimum temperature for the same period was practically unchanged among treatments. Radiation and VPD in the week following veraison were about half in late pruning in comparison with WP, about 1.1 kPa and 11 MJ/m^2, respectively. From veraison to harvest, the difference in temperature and VPD was negligible amongst treatments, however, radiation was 2.3 MJ/m^2 lower in 2–3 leaves in comparison to that in WP.

Wine composition

In 2015, residual sugars were significantly higher in WP (8 g/L) wines in comparison to those from late pruning (2.2 g/L) (Table 1). Likewise, pH was lower in WP (3.56) in comparison to that in late pruning (3.70).

Wine sensory attributes

Nine out of 33 wine sensory attributes in 2014 were significantly different amongst pruning treatments (Table 2). Relative to WP, BB increased colour intensity (opacity) and mouthfeel (body). Floral and dark fruit aromas, and dark and ripe fruit flavours were more intense in both BB and 2–3 L; red fruit flavour was more intense in 2–3 L. Overall, fruit aroma and flavour, colour intensity, and body were more intense in wine made from late pruning treatments.

In 2015, 17 out of 36 wine sensory attributes were significantly different amongst pruning treatments (Table 2). Savoury and miscellaneous aromas were more intense in 2–3 L wines. Sweet taste was more intense in WP wines, but wines made from late-pruned vines were more acidic and bitter, while BB wines were saltier. On the palate, higher intensity of pepper flavour was found in both BB and 2–3 L wines. Red fruit, vanilla and confectionery flavours were more intense in WP, whereas vegetal green and mint menthol were more intense in 2–3 L. The mouthfeel was higher in tannin quality in both BB and 2–3 L. For aftertaste, alcohol and non-fruit were more intense in late pruning and fruit flavour was more intense in WP wines. Overall, WP wines were more intense in sweet taste, fruit flavour, confected flavour sweetness, and fruit after taste; in contrast, wines made from late-pruned vines were dryer, with more intense savoury and peppery aromas and flavours, and coarser quality tannin mouthfeel.

Tristimulus CIELab, wine anthocyanin equilibria, and tannin

The tristimulus CIElab components ($a^*>1$), blue ($b^*<0$) and yellow ($b^*>1$) define the chroma (C^*); higher C^* indicates a deeper colour. Late pruning decreased lightness (L^*) and increased blue tones (a^*) in 2015 but these attributes were unaffected by the pruning treatments in 2014 (Figure 2). Late pruning increased yellow tones (b^*) in BB in 2015 (Figure 2).

Late pruning increased anthocyanin, phenolic substances, tannin, colour density and total pigments in both BB and 2–3 L wines (Figure 3). Late pruning did not affect the hue and chemical age 2, however, late pruning impacted on chemical age 1 and it was lower for 2–3 L in both vintages. Late pruning increased co-pigmentation of anthocyanin by about 20% in 2015, and pigmented tannin in BB in 2014. There was an interaction effect between pruning time and vintage on delta colour, with late pruning enhancing delta colour by about 28% in 2015 but not in 2014.

Associations between wine composition and sensory attributes

Associations between wine composition and sensory attributes are visualised in Figure 4; Tables S2 and S3 show the correlation matrix. Principal components PC1 and PC2 explained approximately 75% of the variance in wine composition for the two seasons. Therefore, wines from WP, BB and 2–3 L treatments separated clearly in 2014 and 2015 (Figure 4). Anthocyanin concentration in the wines correlated positively with fruit aroma and flavours in 2014. Phenolic substances correlated positively with colour intensity (opacity) and body. In 2015, anthocyanin correlated positively with tannin quality and non-fruit flavour aftertaste, and tannin with pepper flavour. Residual sugars correlated positively with fruit, vanilla and confectionery flavour, sweet taste and flavour aftertaste, and negatively with vegetable and pepper flavour, acid and bitter taste, and alcohol aftertaste.

Correlation between temperature and wine anthocyanin concentration

We used PCA to further explore the environmental conditions shortly after veraison linked to anthocyanin in wine. Anthocyanin correlated negatively with mean and maximum temperature, and VPD (Figure 1b). Linear regression showed a reduction in the concentration of the wine anthocyanin (standardized anthocyanin) at a rate of -0.0285 (unitless) per °C for the data pooled for both vintages (Figure 1c).

Discussion

Warming has advanced the harvest and hastened ripening in recent decades, thus higher TSS in grapes are attained earlier and ahead of other relevant fruit attributes related to colour, flavour and aroma (Jones et al. 2005, Sadras and Moran 2013b, Sadras et al. 2013b). Late pruning has been used to reduce the risk of frost and improve fruitset in cool climate regions such as New Zealand (Friend and Trought 2007b, Friend et al. 2011a). More recently, we have tested late pruning as a practice to shift development and improve adaptation to warming in the Barossa Valley of Australia (Moran et al. 2017, Petrie et al. 2017a) . This practice is also being tested in other systems, e.g. for Sangiovese in Italy (Frioni et al. 2016, Palliotti et al. 2017). Whilst these studies demonstrate the potential for late pruning to decompress harvest, the feasibility of this practice depends on its impact on wine attributes, the focus of this paper.

Impact of late pruning on wine sensory traits

Sensory analysis discriminated between wines made from different pruning times, however, quality rating or preference tests were not carried out. We found important differences in wine composition and sensory attributes in response to pruning time over two contrasting vintages. In 2014, a cooler and longer vintage with mild temperatures, late pruning delivered more intense wines in colour, fruit aroma and flavour. In 2015, a short and warmer vintage, late pruning delivered wines with a slightly more bitter taste, vegetal green palate and smoother tannin texture (Table 1). In 2015, WP wines had more residual sugar that enhanced flavours which are associated with sweetness perception such as vanilla and confectionery. In a study by Hjelmeland et al. (2013), residual sugar positively correlated to sweet taste, fruity aromas and low alcohol, and negatively correlated to bitter taste, astringency, vegetal aroma and pepper in Cabernet Sauvignon and Bordeaux blends in the USA. This suggests that residual sugar might mask bitter taste or enhance fruity and floral aromas in wines (Noble 1994). Despite the possible masking effect of residual sugar in 2015, pepper was more intense in wines made from late-pruned vines (Table 2). Pepper, a character often valued by Australian consumers (Williamson et al. 2012), is associated with rotundone, a sesquiterpene that is intrinsic to Shiraz and to a lesser extent other cultivars (Wood et al. 2008). In general, Shiraz wines made from cooler regions have a higher concentration of rotundone (Herderich et al. 2012). In a study of wines from 15 vintages from the same block in Mount Langhi, Victoria, the cooler and wetter conditions enhanced rotundone concentration in Shiraz wines (Zhang et al. 2015). The application of the plant regulator 1-Naphthaleaneacetic acid in Adelaide Hills Shiraz delayed veraison by about 2 weeks and increased rotundone concentration in comparison with that of Controls (Davies et al. 2015). In our study, in 2014 and 2015, late pruning delayed veraison between 4 to 13 days, shifting the initiation of ripening into a cooler window (Figure 1a). Therefore, temperature, VPD and radiation were lower and this might have enhanced pepper flavour in wines, however, rotundone was not measured in our study (Figure 4b).

The flavonoids, especially anthocyanin and tannin, form the matrix of wines that interacts with all sensory properties and contributes to the quality and stability of red wines (Downey et al. 2006a). Adding anthocyanin to Shiraz wines increased astringency and finer tannin grain (Oberholster et al. 2009). This agrees with our study, where late pruning increased anthocyanin in two seasons, impacting on the body intensity or tannin roughness subquality (smoother in wines made from late-pruned wines).

The Barossa Valley wine region is recognised for producing full-bodied Shiraz with ripe flavours (https://www.wineaustralia.com/discover-australian-wine/south-australia/barossa-valley). Since, late pruning produced wines with sensory attributes that are akin to regional styles, such as full-bodied, more intense colour and tannin, this offers a management tool to preserve wine attributes in warmer and compressed vintages.

Late pruning improved wine colour spectrum and concentration of phenolic substances

Late pruning altered the wine colour spectrum in both vintages. Higher anthocyanin, colour density, tannin and phenolic substances ranked 2–3 L > BB > WP. The consistent increase in wine colour and tannin by late pruning can be beneficial to mitigate potential loss of colour and tannin caused by warmer temperature, especially in warm regions (Kliewer and Torres 1972, Mori et al. 2007, Sadras and Moran 2012).

Shiraz wines made from the Barossa Valley are characterised by presenting intense colour and full-bodied mouthfeel (Iland et al. 2002). Anthocyanin binds to tannin to form more stable ‘pigmented tannins’ that prevent loss of colour by oxidation during the aging process (Versari et al. 2013). Owing to our winemaking protocol, pigmented tannins may not be formed in a bottle as well as it is under a micro-oxidative medium (Gómez-Plaza and Cano-López 2011). Co-pigmented anthocyanin, however, is known to enhance wine colour (Gutiérrez 2003). In 2015, the co-pigmentation factor was higher in wines produced from late-pruned vines enhancing wine colour by about 20% more than WP (Figure 3). It might be expected that wines with higher concentration of anthocyanin and tannin would form a higher proportion of pigmented tannin, though in our study wines were bottled after MLF was completed and assessed at about 6 months of age.

Temperature shortly after veraison impacts on wine anthocyanin

Correlation between temperature and indicators of vintage quality scores have been examined in Australia (Soar et al. 2008, Webb et al. 2008b) and elsewhere (Jones et al. 2005). For instance, lower maximum temperature around veraison or before harvest correlated positively to the quality of vintage in the Barossa Valley (Soar et al 2008). Furthermore, berry composition was correlated to climate in Shiraz, Cabernet Sauvignon and Chardonnay for all wine regions in Western Australia (Barnuud et al. 2014). The magnitude of thermal changes and other climatic variables were used to model the effect of climate on berry pH, TA and anthocyanin in a 700 km transect at a set maturity of 12.2°Be. Growing season, veraison to harvest and ripening periods (30 days preceding designated maturity) were included in the model in this study. The model predicted that in warm regions the likelihood of higher temperature is expected to reduce grape TA and anthocyanin in future climates but wine composition was not assessed (Barnuud et al. 2014b).

It has been well documented that elevated temperature reduces berry anthocyanin concentration in both field and controlled environments (Kliewer 1970, Mori et al. 2007, Tarara et al. 2008, Sadras and Moran 2012). In our study, late pruning shifted vine phenology including veraison. This placed post-veraison into cooler conditions in the late pruned vines (Figure 1), advancing the onset of anthocyanin relative to sugar accumulation in berries (Moran et al. 2017) and thus increasing the colour in the wines. There were, however, only slight or negligible difference in temperature between treatments from veraison to harvest. This indicates that a short spell of higher temperature around veraison might be enough to disrupt berry traits, and alter wine colour and sensory attributes.

The rate of change in anthocyanin concentration with mean temperature (Figure 1c) could be useful to model the effect of climate change on this important wine attribute. The link between temperature, however, shortly after veraison and wine anthocyanin is indirect (Bonada and Sadras 2015) and thus inconclusive; experiments are needed that directly manipulate temperature in this developmental window, with a similar level of light.

Conclusion

Late pruning improved wine composition and altered the sensory attributes of Shiraz in two thermally contrasting vintages. Late pruning consistently increased the anthocyanin and tannin concentration in the wines. It improved wine fruit profile and body intensity in 2014, and produced dryer wines with softer tannin in 2015, by mitigating fermentation issues and risks of residual sugar found in wines produced from winter-pruned vines. The increase in colour was likely associated with an advanced onset of anthocyanin synthesis in relation to sugars in berries, in turn associated with cooler conditions shortly after veraison. Late pruning is thus a practice that can be used to decompress harvest with neutral effects on yield, and positive effects on wine composition and sensory attributes.

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Table 1. Influence of pruning time on the composition of wine from the 2014 and 2015 vintages, measured after 5 months of storage.

Pruning time and vintage	**Harvest date**	**pH**	**TA (g/L)**	**Alcohol (% v/v)**	**Volatile acidity (g/L)**	**Free SO_2 (mg/L)**	**Residual sugar (g/L)**
2014							
WP	04 March	3.56 ± 0.019 a	7.0 ± 0.22 a	16.0 ± 0.20	0.41 ± 0.015 b	12 ± 0.4 a	<3
BB	11 March	3.75 ± 0.006 b	6.7 ± 7.13 b	16.4 ± 0.16	0.37 ± 0.010 a	17 ± 0.9 b	<3
2–3 L	17 March	3.70 ± 0.020 b	6.4 ± 4.04 b	16.1 ± 0.13	0.49 ± 0.012 c	16 ± 0.9 b	<3
P		*<0.001*	*0.024*	*0.281*	*<0.001*	*0.014*	
2015							
WP	16 February	3.68 ± 0.013	5.8 ± 8.04	14.7 ± 0.03	0.56 ± 0.004	27 ± 0.5	8.0 ± 0.9 a
BB	18 February	3.72 ± 0.012	5.8 ± 8.05	15.2 ± 0.11	0.54 ± 0.005	26 ± 0.4	2.9 ± 0.7 b
2–3 L	23 February	3.72 ± 0.009	5.7 ± 7.05	15.3 ± 0.22	0.51 ± 0.026	26 ± 1.9	1.4 ± 0.2 b
P		*0.0471*	*0.521*	*0.022*	*0.232*	*0.756*	*<0.001*

Values are means ± standard error of six replicates and *P* values are treatment effects from ANOVA. Mean values were separated using post-hoc Fisher's LSD (*P* = 0.05). Dissimilar letters indicate a significant difference. WP means vines pruned at winter, BB at budburst and 2–3 L at two to three leaves unfolded stage.

Table 2. Wine sensory attributes as affected by pruning time in two vintages.

		Vintage 2014				Vintage 2015			
Sense	**Trait**	**WP**	**BB**	**2–3 L**	***P*-Value**	**WP**	**BB**	**2–3 L**	**P-Values**
Colour	Opacity	91.3 ± 0.85 **a**	93.9 ± 0.73 **b**	91.8 ± 0.82 **a**	**0.0011**	NA	NA	NA	NA
Aroma	Red fruit	54.0 ± 2.91	55.0 ± 2.94	56.7 ± 2.89	0.1560	41.7 ± 1.81	42.4 ± 1.72	43.5 ± 1.75	0.6658
	Dark fruit	55.6 ± 2.74 **a**	58.9 ± 2.69 **b**	60.1 ± 2.87 **b**	**0.0015**	47.5 ± 1.78	46.8 ± 1.85	45.7± 1.84	0.4477
	Overripe fruit	45.9 ± 3.14	46.0 ± 3.12	46.1 ± 3.22	0.8085	40.6 ± 1.83	39.7 ± 1.97	41.2 ± 2.03	0.3614
	Black olive	34.8 ± 3.04	33.1 ± 2.77	35.8 ± 2.90	0.0682	14.3 ± 1.01	13.9 ± 1.06	15.3 ± 1.06	0.4529
	Floral	23.8 ± 2.01 **a**	27.6 ± 2.33 **b**	27.5 ± 2.24 **b**	**0.0119**	16.0 ± 1.15	17.1 ± 1.16	15.8 ± 1.11	0.3708
	Vegetal green	22.8 ± 2.11	23.1 ± 2.23	21.9 ± 2.13	0.4989	13.6 ± 1.10	11.8 ± 1.00	13.4 ± 1.09	0.1830
	Mint/menthol	30.1 ± 1.76	30.4 ± 1.92	28.2 ± 1.97	0.1569	15.0 ± 1.02	14.9 ± 1.06	15.6 ± 1.15	0.8115
	Pepper	29.9 ± 2.20	29.6 ± 2.53	29.3 ± 2.25	0.8865	14.9 ± 1.14	15.2 ± 1.12	16.4 ± 1.28	0.3079
	Spice	35.1 ± 2.35	34.4 ± 2.54	33.6 ± 2.33	0.4597	18.9 ± 1.20	19.1 ± 1.15	18.1 ± 1.19	0.5897
	Chocolate	32.7 ± 2.68 **a**	36.1 ± 2.87 **b**	33.0 ± 2.57 **a**	**0.0102**	10.5 ± 0.76	12.9 ± 0.86	11.9 ± 0.83	0.0515
	Confectionery	31.1 ± 3.04	33.1 ± 3.10	33.3 ± 3.18	0.2117	15.3 ± 1.24	17.2 ± 1.37	17.1± 1.29	0.3330
	Earthy	20.7 ± 2.02	19.6 ± 1.76	19.6 ± 1.58	0.5407	14.2 ± 1.17	14.5 ± 1.20	13.7 ± 1.11	0.8706
	Savoury	27.4 ± 2.16	25.5 ± 2.08	27.1 ± 2.08	0.3734	15.5 ± 1.12 **a**	14.6 ± 1.03 **a**	17.1 ± 1.14 **b**	**0.0111**
	Soya sauce	25.0 ± 1.85 **ab**	22.4 ± 1.76 **b**	26.4 ± 1.98 **a**	**0.0464**	NA	NA	NA	NA
	Leather	22.4 ± 2.16	22.4 ± 2.16	20.4 ± 1.62	0.0943	10.1 ± 0.77	11.6 ± 0.83	11.4 ± 0.83	0.1261
	Miscellaneous	NA	NA	NA	NA	12.1 ± 1.37 **a**	12.5 ± 1.50 **ab**	14.2 ± 1.79 **b**	**0.0229**
Taste	Acid	49.0 ± 2.19	49.2 ± 2.16	48.9 ± 2.19	0.9557	49.9 ± 1.35 **a**	54.7 ± 1.25 **b**	55.0 ± 1.37 **b**	**0.0006**
	Sweet	22.9 ± 2.46	22.1 ± 2.37	21.1 ± 2.12	0.3237	26.9 ± 1.63 **a**	17.9 ± 1.42 **b**	17.9 ± 1.44 **b**	**<.0001**
	Salty	NA	NA	NA	NA	8.1 ± 0.81 **a**	10.3 ± 1.15 **b**	9.8 ± 1.09 **ab**	**0.0377**
	Bitter	18.9 ± 2.14	19.2 ± 1.86	20.6 ± 2.20	0.3607	11.0 ± 0.93 **a**	12.9 ± 1.08 **b**	13.3 ± 1.07 **b**	**0.0228**
Palate	Red fruit	49.7 ± 2.97 **a**	52.2 ± 2.82 **ab**	53.1 ± 2.91 **b**	**0.0453**	43.0 ± 1.96 **a**	40.7 ± 1.88 **ab**	38.6 ± 1.91 **b**	**0.0085**
	Dark fruit	53.3 ± 3.05 **a**	57.7 ± 3.11 **b**	59.0 ± 3.23 **b**	**< .0001**	44.9 ± 1.90	45.2 ± 1.90	42.6 ± 1.83	0.1899
	Ripe fruit	37.4 ± 3.24 **a**	40.8 ± 3.19 **b**	40.8 ± 2.97 **b**	**0.0020**	39.1 ± 1.91	39.6 ± 1.89	38.6 ± 1.98	0.9935
	Fruit sweetness	NA	NA	NA	NA	39.4 ± 1.83 **a**	32.5 ± 1.91 **b**	30.4 ± 1.84 **b**	**<.0001**
	Vegetal green	26.3 ± 2.33	27.4 ± 2.42	27.1 ± 2.32	0.5766	11.0 ± 0.84 **a**	11.6 ± 0.87 **a**	14.7 ± 1.07 **b**	**0.0014**
	Mint/menthol	29.3 ± 2.07	31.7 ± 2.29	30.9 ± 2.33	0.1099	12.3 ± 0.96 **a**	13.6 ± 0.97 **ab**	15.0 ± 1.11 **b**	**0.0401**
	Pepper	31.1 ± 2.53	31.9 ± 2.72	31.8 ± 297	0.7412	16.1 ± 1.17 **a**	18.6 ± 1.18 **b**	19.4 ± 1.22 **b**	**0.0058**
	Spice	34.1 ± 2.46	35.3 ± 2.61	33.8 ± 2.61	0.4179	19.4 ± 1.19	18.6 ± 1.08	20.0 ± 1.16	0.5067
	Savoury	27.9 ± 2.40	26.1 ± 2.36	27.0 ± 2.47	0.4739	13.1 ± 1.08	12.7 ± 1.03	14.4 ± 1.05	0.1871
	Vanilla	NA	NA	NA	NA	18.2 ± 0.98 **a**	15.2 ± 0.84 **b**	13.7 ± 0.90 **b**	**<.0001**
	Confectionery	NA	NA	NA	NA	18.1 ± 1.56 **a**	15.5 ± 1.36 **ab**	14.8 ± 1.39 **b**	**0.0250**
Mouthfeel	Wine Body	53.5 ± 1.36 **a**	57.8 ± 1.47 **b**	56.3 ± 1.78 **ab**	**0.0072**	48.2 ± 1.19	49.3 ± 1.21	48.6 ± 1.18	0.7842
	Tannin quantity	53.7 ± 1.16	55.7 ± 1.26	54.0 ± 1.26	0.2869	47.4 ± 1.47	49.9 ± 1.56	50.1 ± 1.37	0.1172
	Tannin quality	56.4 ± 1.46	58.3 ± 1.42	58.7 ± 1.34	0.2742	41.0 ± 1.63 **a**	45.2 ± 1.62 **b**	45.7 ± 1.53 **b**	**0.0068**
After taste	Fruit flavour	50.2 ± 2.84	51.1 ± 2.62	48.7 ± 2.56	0.0941	53.8 ± 2.07 **a**	48.0 ± 2.19 **b**	46.5 ± 2.15 **b**	**<.0001**
	Alcohol	61.2 ± 2.21	62.5 ± 1.91	60.9 ± 2.05	0.3545	46.5 ± 1.79 **a**	50.2 ± 1.79 **b**	50.4 ± 1.76 **b**	**0.0137**
	Non fruit	60.1 ± 2.15	61.3 ± 2.24	61.6 ± 2.14	0.4256	41.3 ± 2.51 **a**	44.6 ± 2.51 **b**	47.2 ± 2.62 **b**	**<.0001**

Values are the mean scores ± standard error in a 0-100 scale; P values are from ANOVA, and different letters indicate means difference according to Fisher LSD at P < 0.05. WP means vines pruned at winter, BB at budburst and 2–3 L at two to three leaves unfolded stage.

Supplementary Table 1. Reference Standards Recipes

Attribute	Definition
Red fruits	One each of frozen: 1 raspberry, 2 red currant, ½ strawberry, mashed
Dark fruit	1 blackberry, 2 blueberries, ¼ plum, 4 black currants, all frozen and mashed
Dried fruits	6 raisins + 1 prune
Ripe fruits	One teaspoon each of: plum jam, blackberry jam, raspberry jam.
Savoury	2 pieces each of: salami, pepperoni, bacon
Floral	One drop of rosewater
Vegetal Green	1cm piece of tomato stem + 1cm green capsicum
Mint/menthol	A pinch dried mint + 3 drop from a "fresh wine solution" consisted of 1 drop peppermint dissolved in 30ml of wine.
Spice	Pinch of mixed spice
Aniseed/liquorice	5 Fennel seeds + 1cm piece liquorice
Pepper	Pinch of both black and white pepper (or mixed pepper)
Olives	One black olive and drop of olive brine
Chocolate	Chocolate
Earthy	Teaspoon of earth
Vanilla	Drop of vanilla
Mocha	Tsp mocha (use 1/10 of teaspoon of ground coffee and half a piece of dark chocolate).
Leather	Leather (no wine)
Confectionery	Strawberry and cream lolly

Captions to Figures

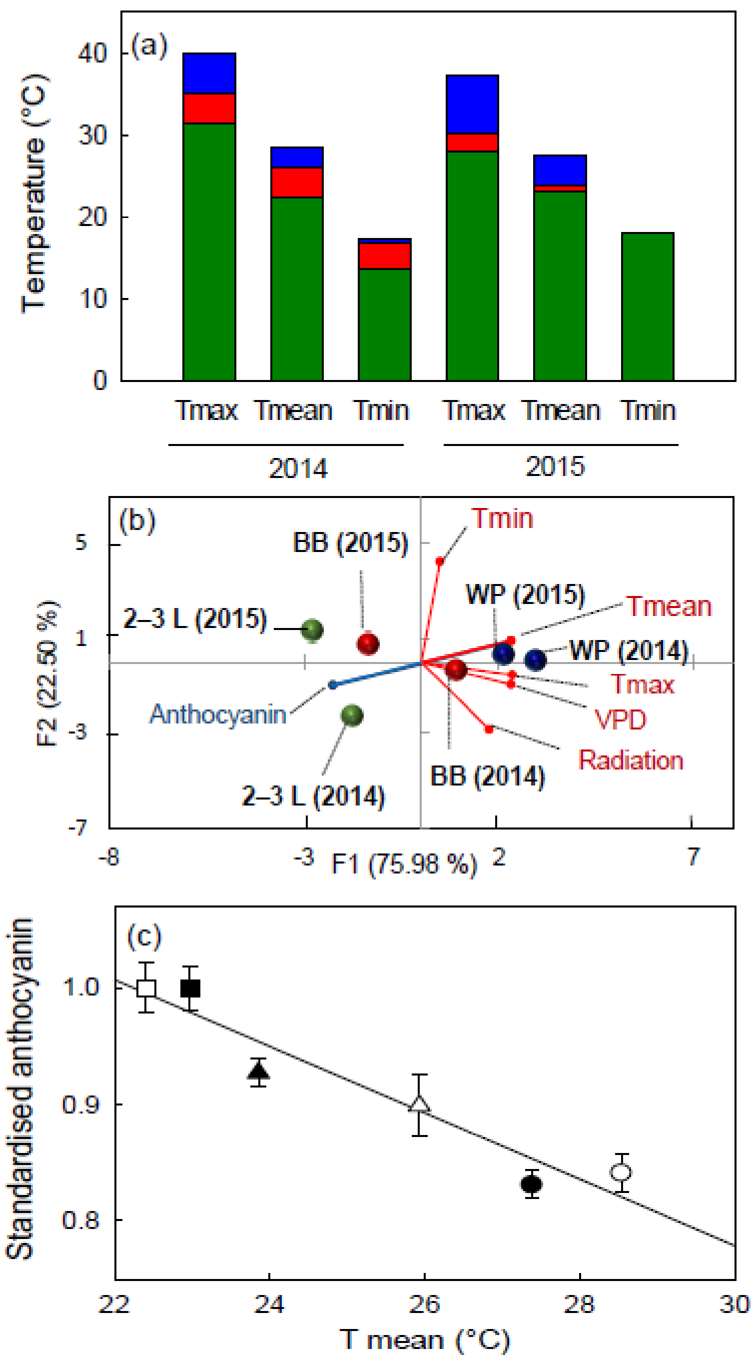
Figure 1. (a) Maximum (Tmax), mean (Tmean) and minimum temperature (Tmin) for the 7 day period after veraison in vines pruned in winter (—), at budburst (—) and at 2–3 leaves stage (—) in 2014 and 2015. (b) Principal component analysis relating standardised anthocyanin concentration and temperature, vapour pressure deficit (VPD) and radiation for the 7 day period after veraison as affected by pruning time and vintage. Standardised anthocyanin is relative to treatment 2–3 L. (c) Association between standardised anthocyanin as a function of mean temperature in the 7 day period after veraison as affected by pruning treatment and vintage. Pruning treatments are 2–3 L (□,■), budburst (△, ▲) and winter (○,●), in 2014 (□,Δ,○) and 2015 (■, ▲ ,●). Error bars are two standard errors of the mean. The line is least square regression, and the slope and its standard error are -0.0285*(°C) ± 0.004.

Figure 2. Effect of pruning time, vintage and their interaction on the tristimulus CIELab colour parameters of Shiraz wines of vines pruned in winter (●), at budburst (●) and at 2–3 leaves stage (●) in 2014 and 2015. Parameters are: (a) a* (red/green chromaticity); (b) L* lightness saturation; (c) b* (yellow/blue chromaticity); and (d) C* (chroma). Values are mean ± SE from six replicates. *P* values from ANOVA *(*P* < 0.05), ** (*P* < 0.01) and *** (*P* < 0.001); black asterisk indicates pruning effect, red asterisk interaction, and lack of asterisks indicates *P* > 0.05. Vintage had a significant effect on (a), (b) and (d) at *P* > 0.001 and on (c) at *P* > 0.01 level.

Figure 3. Effect of pruning time, vintage and their interaction on wine colour equilibria, phenolic substances, tannin and co-pigmentation factors in 2014 and 2015. From top left to right, (a) anthocyanin, (b) tannin, (c) phenolic substances, (d) colour density, (e) total pigments, (f) pigmented tannin, (g) hue, (h) chemical age 1, (i) chemical age 2, (j) %

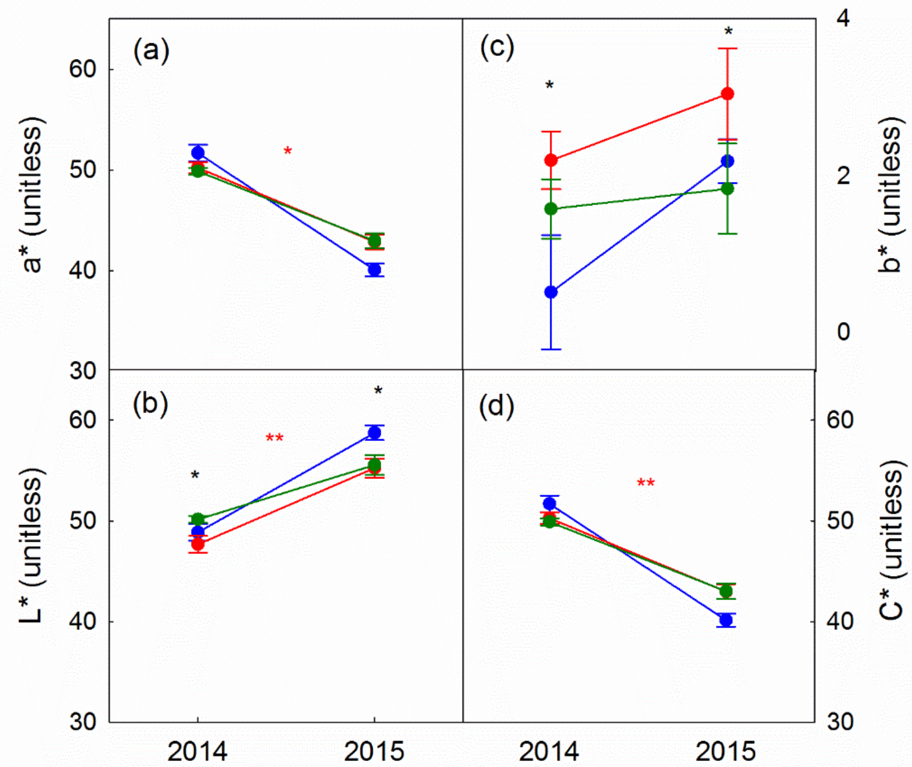
copigmentation, (k) % polymerisation, (l) % delta colour. Pruning times were winter (●), budburst (●) and 2–3 leaves (●). Values are means ± SE from six replicates. *P* values are from ANOVA with *(*P*< 0.05), ** (P < 0.01) and *** (P < 0.001); black asterisks indicate pruning effect, red asterisks interaction, and lack of asterisks indicate *P* > 0.05. Vintage effect was significant in all traits at *P* <0.001 except for tannin (*P* > 0.05).

Figure 4. Principal component analysis of the wine composition (red font, active variable) and sensory attributes (blue font, supplementary variable) that responded to pruning treatments during the (a) 2014 and (b) 2015 vintages. C, colour; A, aroma; P, palate; T, taste; MF, mouthfeel; AT, aftertaste, WP, winter; BB, budburst; and 2–3 L, 2–3 leaves.



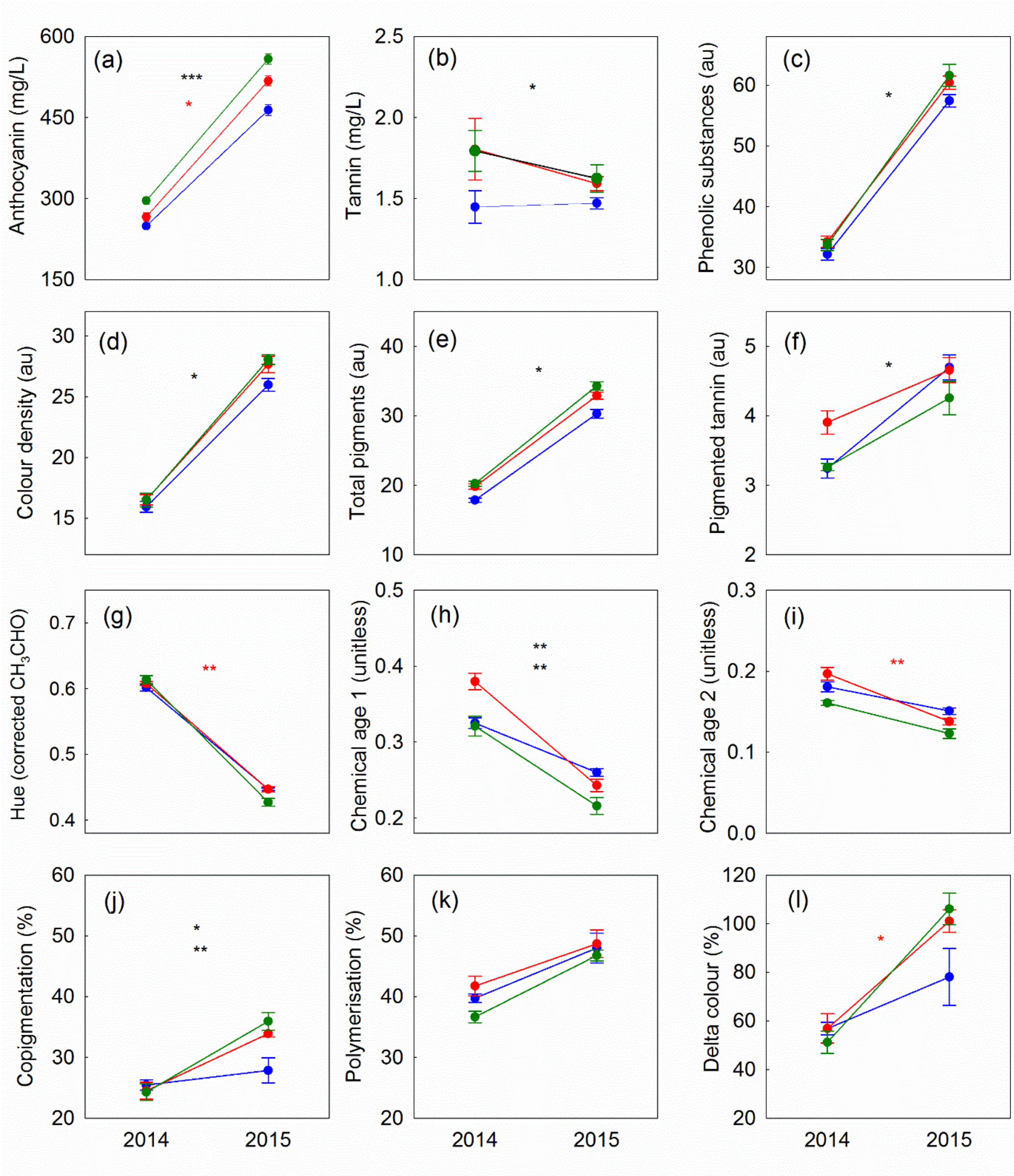
(a)
Temperature (°C)
40
30
20
10
0
Tmax
Tmean
Tmin
Tmax
Tmean
Tmin
2014
2015
(b)
F2 (22.50 %)
5
1
-3
-7
-8
-3
F1 (75.98 %)
2
7
Tmin
BB (2015)
WP (2015)
Tmean
2–3 L (2015)
WP (2014)
Tmax
VPD
Anthocyanin
Radiation
BB (2014)
2–3 L (2014)
(c)
Standardised anthocyanin
1.0
0.9
0.8
22
24
26
28
30
T mean (°C)

Figure 1



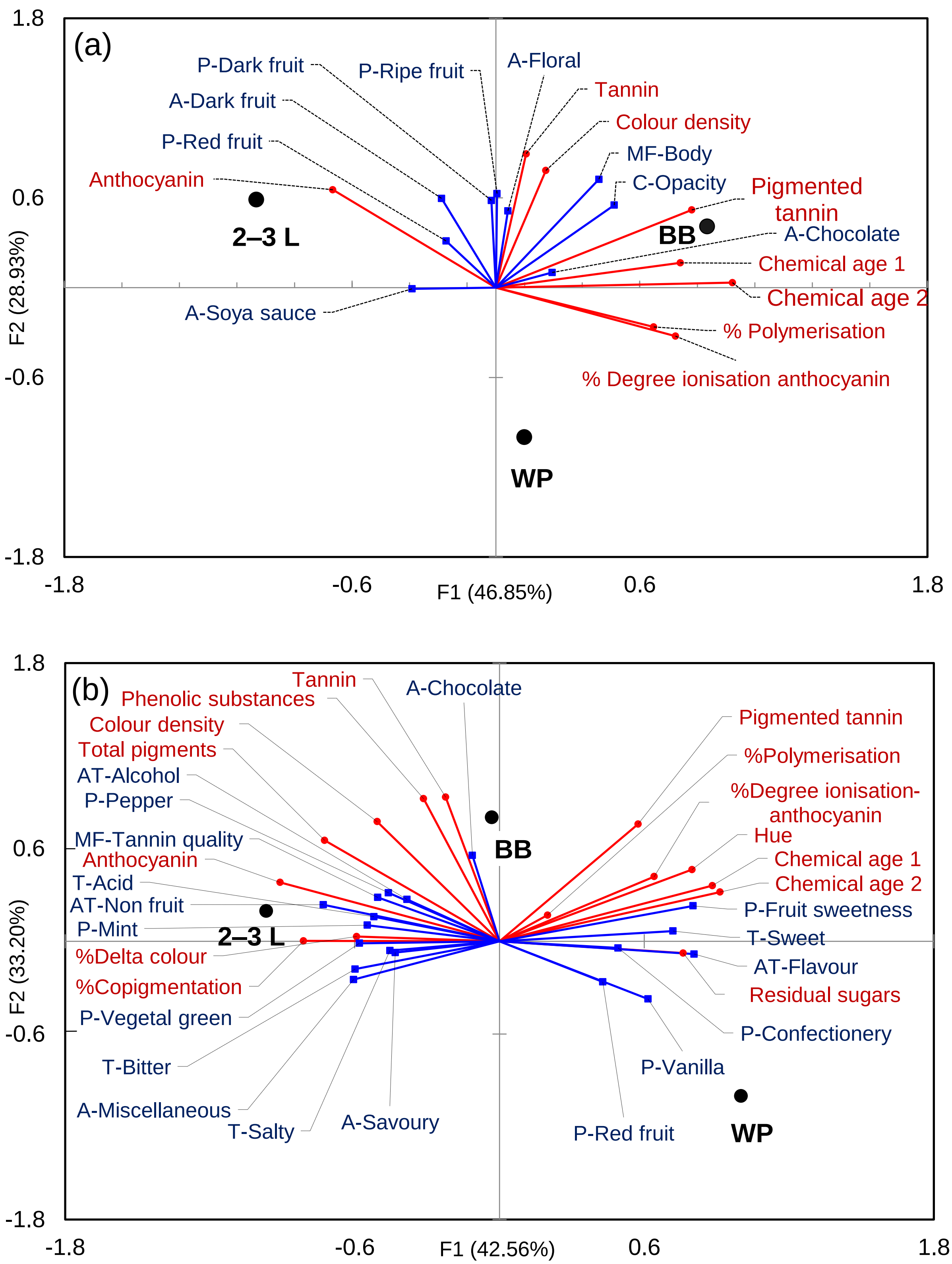
(a)
(b)
(c)
(d)
a* (unitless)
L* (unitless)
b* (unitless)
C* (unitless)
60
50
40
30
4
2
0
*
**
2014
2015

Figure 2



(a)
Anthocyanin (mg/L)
600
450
300
150
(b)
Tannin (mg/L)
2.5
2.0
1.5
1.0
(c)
Phenolic substances (au)
60
50
40
30
(d)
Colour density (au)
30
25
20
15
(e)
Total pigments (au)
40
30
20
10
(f)
Pigmented tannin (au)
5
4
3
2
(g)
Hue (corrected CH3CHO)
0.7
0.6
0.5
0.4
(h)
Chemical age 1 (unitless)
0.5
0.4
0.3
0.2
(i)
Chemical age 2 (unitless)
0.3
0.2
0.1
0.0
(j)
Copigmentation (%)
60
50
40
30
20
(k)
Polymerisation (%)
60
50
40
30
20
(l)
Delta colour (%)
120
100
80
60
40
20
2014
2015

Figure 3



(a)
P-Dark fruit
P-Ripe fruit
A-Floral
A-Dark fruit
Tannin
P-Red fruit
Colour density
MF-Body
Anthocyanin
C-Opacity
Pigmented tannin
2–3 L
BB
A-Chocolate
Chemical age 1
Chemical age 2
A-Soya sauce
% Polymerisation
% Degree ionisation anthocyanin
WP
F2 (28.93%)
F1 (46.85%)
1.8
0.6
-0.6
-1.8
-1.8
-0.6
0.6
1.8
(b)
Tannin
Phenolic substances
A-Chocolate
Colour density
Total pigments
Pigmented tannin
%Polymerisation
AT-Alcohol
P-Pepper
%Degree ionisation-anthocyanin
BB
MF-Tannin quality
Hue
Anthocyanin
Chemical age 1
Chemical age 2
T-Acid
AT-Non fruit
P-Fruit sweetness
P-Mint
2–3 L
T-Sweet
%Delta colour
AT-Flavour
%Copigmentation
Residual sugars
P-Vegetal green
P-Confectionery
T-Bitter
P-Vanilla
A-Miscellaneous
T-Salty
A-Savoury
P-Red fruit
WP
F2 (33.20%)
F1 (42.56%)
1.8
0.6
-0.6
-1.8
-1.8
-0.6
0.6
1.8

Figure 4

Chapter 4

Effects of late pruning and elevated temperature on phenology, yield components and berry traits in Shiraz

Published article – American Journal of Enology and Viticulture

Statement of Authorship

Title of published paper: Moran, M.A., Petrie P.R., and Sadras V.O. (2019) Effects of Late Pruning and Elevated Temperature on Phenology, Yield Components, and Berry Traits in Shiraz. American Journal of Enology and Viticulture, 70, 9-18.

Author Contribution

Martin Moran conducted the literature review, set up and managed the experiment, drafted and constructed the manuscript.

Overall percentage: 80% Date 9.8.19

Signature

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.

Co-authors contribution

By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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

Date 9. 8. 19

Signature

Paul R. Petrie contributed to research ideas, experimental design and editing of manuscript.

Date 9/8/19

Signature

Effects of late pruning and elevated temperature on phenology, yield components, and berry traits in Shiraz

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Abstract

Recent warming has shortened and compressed vintages and has altered enologically relevant berry traits. Late pruning can decompress harvest and preserve fruit quality. Here, we were interested in exploring the interactive effects of late pruning and heating on Shiraz development and composition. We established a factorial experiment that combined two thermal regimes (ambient versus heated) with open-top chambers, and three pruning times (late pruning at budbreak and at two to three leaves, a winter control) in Shiraz vines over three seasons in the Barossa Valley. Late pruning delayed budbreak by up to 23 days, flowering by up to 17 days, and veraison by up to 16 days. Heating advanced flowering in late-pruned vines by up to seven days, with a minor effect in winter-pruned controls. Heating advanced veraison by up to seven days, with greater advances in years with warmer springs. Pruning weights were unaffected by late pruning and were increased by heating. Yield was increased in a single season by late pruning and heating, but it remained unchanged for the pooled three-year data. Late pruning delayed maturity in four of six cases; the largest delay was 17 days in unheated vines pruned when two to three leaves had emerged. Late pruning maintained the anthocyanin-to-sugar ratio, which decreased with heating in two seasons. There was an interaction between the timing of pruning and heating, whereby late pruning enhanced the berry tannin-to-sugar ratio in heated but not in unheated control vines. Late pruning delayed the harvest by shifting the onset and rate of ripening, with a higher degree of response in the warmest season in both ambient and heated treatments.

Keywords: anthocyanin, berry, climate change, harvest, temperature, veraison, yield

Introduction

Impact of climate warming on the wine industry has become a focus of research in the last two decades (Schultz 2010, Webb et al. 2008). Modelling, time series analysis and direct measurement in field experiments have shown that warming is advancing maturity, compressing the harvest, and increasing pressure in winery logistics (Jones et al. 2005, Petrie and Sadras 2008, Sadras et al. 2014). In most regions, grape cultivars are mainly selected for their climatic suitability (Winkler et al. 1974). For example, 2 to 4° C increase in mean temperature may shift the climatic suitability for a given cultivar to an upper limit in intermediate to warm region and might favour its cultivation in cool regions (Duchene et al. 2010, White et al. 2006). Management interventions are required to maintain wine regional attributes (de Orduña 2010). Modelling and time-series analysis are useful for establishing putative relationships between warming and vine traits, but field experiments are required to prove cause and effect (Bonada and Sadras 2015).

Management practices that can delay ripening include manipulation of the source-to-sink ratio, and the use of antitranspirants, auxins, double pruning, and late pruning (Davies et al. 2015, Friend and Trought 2007, Gatti et al. 2016, Gu et al. 2012, Moreno Luna et al. 2017, Poni et al. 2013). Late pruning, the focus of this study, may be a cost-effective tool for delaying vine phenology and maturity and for reestablishing berry traits (Moran et al. 2017, Palliotti et al. 2017, Petrie et al. 2017). In the Barossa Valley of Australia, Shiraz yield was unchanged for up to four consecutive seasons when pruning was delayed until two to three leaves had developed (Moran et al. 2017). Howver, interactive effects of delayed pruning, heating, and seasonal temperature on vine phenology, yield components, and berry traits are unknown. Nevertheless, it is expected that late pruning may shift ripening into a relative cooler window during the summer. To investigate heating and late pruning interactions, an experiment including a factorial combination of three pruning times (winter, budburst and 2-3 leaves) and two temperatures (ambient and heating) was established with Shiraz vines over three seasons in the Barossa Valley.

Materials and Methods

Site and experimental design. The vines were established in 2004 in a red brown earth soil at the South Australian Research Institute in Nuriootpa, Barossa Valley (Dry and Coombe 2005). Barossa Valley is a warm region with a mean January temperature of 21.4 °C and 486 mm annual rainfall (Gladstones 1992). Shiraz clone 1654 vines were grafted on Schwarzmann rootstock and planted in 2004, at 2.25 m within and 3.0 m between rows in a northeast-southwest orientation, and trained to a single cordon (as two arms from the trunk), spur-pruned at 16 nodes per metre, with a sprawling canopy supported by a single wire about 0.4 m above the cordon. Supplementary drip irrigation (6.6 L/vine/hr, ~100 mm) was implemented weekly when berries were pea sized at the beginning of December until harvest. Fungicide was applied to prevent powdery mildew and downy mildew throughout the seasons as required.

In 2013, a field experiment was established that included a factorial combination of two thermal regimes and three pruning times over three successive growing seasons. The thermal regimes consisted of a heating treatment and a control; the pruning times included a winter control (WP), late pruning at budbreak (BB), and late pruning at 2 to 3 L leaves (2-3 L). Each treatment was replicated three times, with seven central vines and two buffers vines at each end. Open top chambers were used to increase air temperature as described in Sadras and Soar (2009). Briefly, polycarbonate sheet panels supported by ‘A’-shape steel frame were used to increase daytime temperature. The panels were installed in mid-January 2013 in each side of the vine row at the cordon level, immediately below the canopy, with an open gap of 50 cm on each side as shown in Figure 1. Pruning treatments were: early July for WP, and late pruning at phenostages E-L 4 (budbreak) and E-L 9 (2-3 leaves) when 50% of buds or shoots reached those stages in the winter control vines (Coombe 1995). Late pruning treatments (BB and 2–3 L) were allocated with a break (pruned back to winter time) in the second year. In the third year, late pruning was applied on the same vines that were late pruned in the first year.

Measurements. Ambient temperature at midcanopy was recorded at 15-min intervals using TinyTags Ultra 2 loggers, in three vines per treatment at 10 cm above the cordon (Hastings Dataloggers). Then, thermal time was calculated as the summation of the daily average temperature from 15 min readings minus a base temperature of 10°C (Winkler et al. 1974). Summation of temperatures started at the phenological stage of budbreak for each pruning treatment. Phenology was scored weekly using the modified E-L system (Coombe 1995) from budburst until veraison, and berry total soluble solids (TSS) was measured afterwards. We assumed onset of veraison corresponded to 50% of colored berries within a bunch. Owing to the qualitative nature of the E-L scale, we used the approach of Sadras and Moran (2013a) to calculate and analyse the date when a given E-L stage was reached. Phenological stages up to veraison were assessed in two vines per replicate, where we randomly selected six to eight spurs per plant to record phenology in lower (node 1) and upper (node 2) nodes. In the 2–3 L treatment, the lower and upper nodes were phenologically different; thus, E-L stage was analysed separately for each node from budburst up to veraison (Moran et al. 2017). Between veraison and harvest, a weekly sample of 150 berries per replicate was obtained from approximately 20 bunches per vine in each of the seven plants per replicate. Both bunches and berries within the bunch were randomly chosen. A subsample of 50 berries was frozen at -20°C for further analysis of anthocyanin (Iland et al. 2004) and condensed tannin by the methyl cellulose precipitable assay (Mercurio et al. 2007). The remaining berries were crushed by hand with an aluminium alloy press, and the free-run juice was poured into a 50-mL centrifuge tube and spun at 1800 × g for 5 min. Total acidity (TA) at endpoint pH 8.2, pH (autotitrator; CRISON, Barcelona, Spain) and TSS (digital refractometer; HI 96801, Hanna Instruments, Woonsocket, RI, USA) were measured soon after sampling. Technological harvest was targeted at a TSS of approximately 14 Baume, with a ± 3% error.

At harvest, yield per vine and bunch number were recorded for all vines in each replicate. In two seasons, 2013 to 2014 and 2014 to 2015, we measured pruning weight, counted canes and calculated yield-to-cane weight ratio as an approximation to sink-to-source ratio.

All treatments were managed with the region's standard irrigation regime (Iland et al. 2011). This irrigation regime suits the unheated, winter-pruned vines but does not account for shifts in canopy development with delayed pruning (Moran et al. 2017). To capture putatively different effects of a single water regime on the treatments, we measured stomatal conductance as described in Soar et al. (2009). Briefly, stomata conductance was measured in three leaves in a total of three vines per treatment with a leaf porometer (www.metergroup.com). The selected leaves were in a fully sun-exposed position from the northwest side of the canopy.

Statistical Analysis. A two-way analysis of variance (ANOVA) was used to account for the effects of pruning time, temperature treatments and their interaction on vine traits including phenology, yield, yield components, pruning weights, and yield to cane weight ratio. Post-hoc analysis (Tukey's honest significant difference) was used to discriminate between means when ANOVA was significant. Because of the interactions between vintage and pruning time, and of vintage and temperature on yield and berry traits, ANOVA is presented for individual years.

Results

Thermal regimes, seasonal conditions and vine water status. To put the unheated controls in context, Figure 2 shows actual and long-term monthly mean temperatures. Average temperatures during the three seasons were warmer than long-term temperatures, especially in the spring; monthly mean temperatures rose above the 90th percentile in 2014 to 2015 and 2015 to 2016 (Figure 2A). Higher than average rain in February 2014 (111 mm versus 21 mm historical mean) delayed harvest by about three to four weeks in 2013 to 2014, compared to other seasons.

Relative to the controls, mean air temperature in the heated treatments increased by 0.40°C (SD = 0.119) in 2013 to 2014, 0.68°C (SD = 0.252) in 2014 to 2015, and 0.67°C (SD = 0.109) in 2015 to 2016 between September and February. The heating chambers increased the mean maximum air temperature by 1.37°C (SD = 0.867) in 2013 to 2014, 1.70°C (SD = 0.084) in 2014 to 2015, and 1.99°C (SD = 0.079) in 2015 to 2016 (Figure 2B). Heating chambers increased the mean minimum air temperature by 0.23°C (SD = 0.38) in 2014 to 2015, 0.53°C (SD = 0.64) in 2013 to 2014, and 0.30°C (SD = 0.46) in 2015 to 2016.

In 2013 to 2014, mean temperatures in the unheated controls between veraison and harvest were as follows: 22.6°C in WP, 22.6°C for BB, and 22.4°C for 2-3 L. Under heated conditions, mean temperatures between veraison and harvest were 22.8°C for WP, 22.8°C for BB, and 22.6°C for 2-3 L. In 2014 to 2015, mean temperatures in the unheated controls between veraison and harvest were 19.9°C for WP, 21.4°C for BB, and 21.9°C for 2-3 L; mean temperatures under heated conditions were 21.3°C for WP, 20.8°C for BB, and 21.7°C for 2-3 L. In 2015 to 2016, mean temperatures in the unheated controls between veraison and harvest were 22.1°C for WP, 22.2°C for BB, and 21.3°C for 2-3 L; mean temperatures under heated conditions were 23.6°C for WP, 22.8°C for BB, and 22.1°C for 2-3 L.

Stomata Conductance. Irrigation commenced in early December, after fruit set, except in 2014 to 2015 when it started on the 14^{th} of November (Figure 3). In 2013 to 2014 and 2014 to 2015, stomata conductance around flowering and fruit set was above 100 mmol/m^2.s. In 2015-16 stomata conductance around flowering was ~200 mmol/m^2.s in winter-pruned vines and 54 mmol/m^2.s in late-pruning treatments.

Phenology from budburst to veraison. Compared to vines pruned in winter, late pruning delayed budbreak in all three seasons by ~23 days at node 1 (N1) and 14 days at node 2 (N2) in 2-3 L vines, and by 13 days in the BB treatment (Table 1). In relation to vines pruned in winter, flowering was delayed by ~15 days in N1 and 10 days in N2 in the 2-3 L treatment, and by seven days in BB treatment. Veraison was delayed by two to 15 days in N1 and N2 in 2-3 L and by two to seven days in BB, depending on the season. Heating had minor and inconsistent effects on budbreak.

When the data were pooled across pruning and temperature treatments, thermal time from budbreak was 361 ± 8.9°C to flowering, 633 ± 15.5°C to pea size, and 1132 ± 17.5°C to veraison. Relative to WP in unheated controls, thermal time was 15% to 25% higher in 2-3 L N1 and N2 at flowering, in BB 2-3 L N1 at pea size, and in BB 2-3 L N1 at veraison in the warmer seasons (2014 to 2015 and 2015 to 2016). Relative to WP in heated vines, thermal time was ~5% higher in 2-3 N2 and 10% higher in 2-3 L N1 at flowering and veraison. Differences between treatments in thermal time from flowering to veraison were not significant among treatments, but there was a significant effect of vintage ($p > 0.0001$).

Phenology from veraison to harvest: dynamics of TSS. Late pruning delayed harvest, targeted at ~14 Baume, in four out of six cases in the three seasons (Figure 4). In 2013 to 2014, BB and 2-3 L delayed maturity by about one week in unheated vines. Under heated conditions, there were negligible differences in harvest date between WP and late pruning. In

2014 to 2015, late pruning in unheated controls delayed harvest by eight days in BB and by 17 days in 2-3 L. In heated vines, late pruning delayed harvest by seven days in BB and by 12 days in 2-3 L. In 2015 to 2016, heated vines pruned at 2-3 L reached maturity nine days later than WP, and late pruning did not delay harvest day in unheated vines.

Yield components, pruning weight and fruit-to-shoot weight ratio. The effect of late pruning on yield depended on the season (Table 2). There were no effects of pruning time on yield in 2013 to 2014. In 2014 to 2015, BB increased yield by 50% (due to an increase in bunch number and berries per bunch) compared to the WP control under both ambient and heating. In 2015 to 2016, late pruning reduced yield by 55% as a result of decreased bunch number, bunch weight, and berries per bunch under ambient and heated conditions. Late pruning had no effect on pruning weight in 2013 to 2014 or 2014 to 2015. In 2014 to 2015, late pruning reduced the number of shoots per vine and increased the fruit-to-shoot weight ratio by ~45%.

In 2013 to 2014, heating increased yield by ~60% due to increased numbers of bunches per vine and berries per bunch. Elevated temperature did not affect yield in 2014 to 2015 or 2015 to 2016, but elevated temperature decreased the number of bunches by ~15% in 2015 to 2016. Elevated temperature increased pruning weight by 40% in 2013 to 2014 and by 20% in 2014 to 2015. Elevated temperature did not affect the yield-to-shoot weight ratio.

Berry traits at harvest. We used weekly assessments of TSS and a staggered harvest with the goal of harvesting all treatments at 14 Baume. This was largely achieved, but some differences were unavoidable and were reflected in significant treatment effects (Table 3). For example, winter-pruned unheated vines were harvested at 14.6 Baume in 2013 to 2014, whereas the remaining treatments were harvested at slightly below 14 Baume. Late pruning increased berry pH by ~0.12 in both heated and unheated vines in 2013 to 2014. In 2014 to

2015, interactions between pruning and heating were found; late pruning increased pH by 0.14 in unheated 2-3 L vines and decreased pH by 0.17 in heated BB vines. In 2013 to 2014, TA did not respond to heating or late pruning. In 2014 to 2015, late pruning decreased TA by 23% in unheated BB and 2-3 L vines and increased TA by 21% in BB vines. In 2015 to 2016, late pruning increased TA by 10% in unheated 2-3 L vines and decreased TA by ~15% in heated BB and 2-3 L vines.

To account for variation in TSS, anthocyanins and tannins were analyzed as ratios. Late pruning did not affect the anthocyanin-to-sugar ratio in 2013 to 2014 or 2014 to 2015. Elevated temperature decreased the anthocyanin-to-sugar ratio by 11% in 2013 to 2014, and by 9% in 2014 to 2015 (Table 3). Pruning time and temperature did not affect the tannin-to-sugar ratio, but the interaction was significant. Compared to winter pruning, late pruning increased the tannin-to-sugar ratio by ~25% in the unheated treatment and decreased tannins by ~11% in the heated treatment in 2013 to 2014. In 2014 to 2015, the tannin-to-sugar ratio was unaffected by pruning treatments in unheated vines, but the ratio increased in BB by 13% and decreased by 10% in 2-3 L in heated treatments.

The anthocyanin-to-TSS ratio was negatively correlated with yield for the two-season pooled data for two seasons (ratio = 0.132 – 0.004 × yield, r2 = 0.49, $p < 0.013$); this correlation was primarily driven by seasonal effects. In 2013 to 2014, when heating increased yield, the anthocyanin-to-TSS ratio declined with yield (ratio = 0.141 – 0.005 × yield, r2 = 0.72, $p < 0.032$). In 2014 to 2015, the anthocyanin-to-TSS ratio was unrelated to yield ($p > 0.34$).

Discussion

Three criteria have to be met in order for late pruning to be a useful viticultural practice. First, late pruning has to be able to delay maturity particularly in seasons with compressed harvest, as this will release pressure on winery logistics. Second, late pruning must have either neutral or positive effects on yield. Third, late pruning must have either neutral or positive effects on berry and wine attributes.

Phenology from budburst to veraison. For the pooled three-season data, late pruning delayed budbreak by two to three weeks compared to the winter-pruned control under both unheated and heated conditions. Late pruning consistently delayed the flowering of unheated controls by one to two weeks. This delay could help to minimize the risk of late frost or cooler conditions affecting flowering, which could improve fruit set (Friend and Trought 2007). Relative to controls pruned in winter, late pruning delayed veraison by an average of about one week. In general, the delays in phenology from budbreak to veraison were similar to those reported for Shiraz in Australia and Sangiovese in Italy (Frioni et al. 2016, Moran et al. 2017, Palliotti et al. 2017, Petrie et al. 2017).

Elevated temperature advanced flowering in late-pruned vines (Table 1). Heating had smaller effects on flowering time in winter-pruned vines. The magnitude of the thermal effects on phenology depended on the background temperature of each particular season, as discussed by Sadras and Moran 2013b. The elevated temperature treatment had less of an effect on flowering date in 2015 to 2016, under high temperatures in October and November, compared to cooler seasons (Figure 2). Trought et al. (2015) indicated that the modulation of phenology in response to temperature is cultivar-dependent, and they argued that some mitigation of climate warming may be possible with vineyard cultural practices. For instance, modifying the sink-to-source ratio by thinning leaves and bunches at fruit set delayed veraison in Sauvignon blanc and Pinot noir in New Zealand (Parker et al. 2014). Hence, management practices might mitigate climate change by delaying veraison and shifting ripening to a cooler part of the season. In our study, thermal time from flowering to veraison was similar in heated and late-pruned vines, but there was a strong vintage effect (Table 1). This reinforces the notion that temperature is the main modulator of phenology between flowering and veraison; heating advanced the date on which those stages were reached, while late pruning delayed it (Table 1). Earlier field experiments and time-series studies in Barossa Shiraz showed that the advancement of harvest due to elevated temperature was largely explained by earlier ripening (Sadras and Petrie 2011, Sadras and Moran 2013b).

Phenology from veraison to harvest: dynamics of TSS. During three seasons and across heating treatments, late pruning delayed maturity at harvest (TSS ~14 Baume) in four of six cases (Figure 4). In 2013 to 2014, a significant rain event (111 mm in two days) followed by slow ripening and late harvest dampened the differences in maturity in heated vines. A study in Australia showed that rain delayed harvest by diluting sugars in berries through absorption of water through the skin (Rogiers et al. 2006). However, in unheated vines, there was a delay of seven days in ripening between the WP and 2-3 L pruning treatments. The longest delay in harvest (up to 17 days) occurred in 2014 to 2015 under both unheated and heated conditions despite the warmer conditions preceding harvest, with mean February temperatures close to the 90th percentile (Figure 2). Furthermore, the mean air temperature from veraison to harvest was 2°C higher in vines pruned at 2-3 L compared to WP under unheated conditions; despite this, TSS of ~14 Baume was reached 17 days after WP. This could be due to a delay in the onset of ripening or a change in ripening rate; the latter may be a result of a delay in berry cell death linked to berry shriveling.

Shriveling of Shiraz berries is common under the conditions found in Barossa. The shriveling process is related to mesocarp cell death, water status, berry respiration, and hypoxia (Tyerman et al. 2004, Rogiers and Holzapfel 2015). Elevated temperature advanced the onset of cell death in Shiraz berries (Bonada et al. 2013, Xiao et al. 2018). Therefore, late pruning might have delayed cell death, more effectively reducing shriveling and delaying harvest under warmer conditions. This could partially explain the delay in ripening with late pruning, especially in vines pruned at 2-3 L (Figure 4). Manipulation of the sink-to-source ratio by shoot thinning at fruit set delayed maturity of Pinot noir in New Zealand (Parker et al. 2016). In the current study, however, vines pruned at WP and 2-3 L had similar yields and yield-to-cane weight ratio in 2014 to 2015, indicating that the difference in harvest date was related to the shift of development early in the season, and this delay was carried throughout the whole cycle. Nevertheless, there are other possible causes for delayed maturity that would need future research.

Yields. Shiraz yields in the Barossa Valley are moderate due to the scarcity of water for supplementary irrigation (100 to 150 mm/year), low-density plantations (1850 vines/ha), and intentional water stress to achieve high polyphenol concentrations in wine, a trademark of its style (Iland et al. 2002). Hence, the goal of late pruning in this study was to achieve neutral or positive effects on yield, unlike other studies in which the aim of late pruning was to reduce yield in fruitful cultivars such as Sangiovese (Frioni et al. 2016, Palliotti et al. 2017). Petrie et al. (2017) first established that pruning Shiraz after two to three leaves per shoot had unfolded would reduce yield under Barossa conditions. We thus worked with two to three leaves as the latest pruning treatment. Overall yields for the pooled data were maintained by late pruning (Table 2). This is comparable to the findings of Petrie et al. (2017) and Moran et al. (2017), where Shiraz yields were maintained in five seasons. In the environments sampled in this study, elevated temperature increased yield in one season, and had no effect in two others. This is consistent with the conservative conclusion from a larger sample that the magnitude of warming used in this study has neutral effects on Shiraz yield in the Barossa Valley (Sadras et al. 2017).

Late pruning did not affect pruning weight, although the yield-to-cane weight ratio improved in 2014 to 2015 due to higher yield. Heated vines had higher pruning weight in 2013 to 2014 and 2014 to 2015, which agreed with a previous study that showed that pruning weight in Shiraz grown under heating conditions may increase slightly (Sadras and Moran 2013a). In 2015 to 2016, late pruning reduced yield and the number of berries. In that season, irrigation started one month after fruit set, and late-pruned vines were more water stressed than their winter-pruned counterparts (Figure 3), as reflected in a four-fold difference in stomatal conductance. Therefore, irrigation needs to be adjusted to late pruning.

Berry traits. Berry traits were affected by late pruning and temperature, and the effects of treatments varied seasonally. In 2013 to 2014, above-average temperatures during ripening (January and February) likely depleted malic acid (Sweetman et al. 2014), regardless of temperature or pruning time, resulting in the lowest total acidity in the three seasons. However, late pruning increased pH despite similar total acidity among pruning treatments. Similarly, in 2014 to 2015, late pruning increased pH and reduced TA in unheated vines. However, late pruning decreased pH and increased TA in heated vines. There were lower mean temperatures during January of 2014 to 2015, which could have increased malate concentrations in berries compared to the previous season. Heating did not affect pH but decreased total acidity. In 2015 to 2016, pH and total acidity were not affected by temperature or late pruning. Total acidity responded to both late pruning and temperature; interaction effects resulted in reduced total acidity when late pruning was applied with heating and in increased total acidity when late pruning was applied without heating. Thus, pH did not respond to temperature treatment. On the other hand, total acidity could be increased by late pruning under warming scenarios.

Heating reduced the anthocyanin-to-sugar ratio in 2013 to 2014 and 2014 to 2015 (Table 3). Previous reports have shown that late pruning increased this ratio (Palliotti et al. 2017, Petrie et al. 2017). In our study, heating decreased the anthocyanin-to-sugar ratio by 10% to 12% in 2013 to 2014 and 2014 to 2015. This reduction in anthocyanins was similar in magnitude to that observed in previous field experiments with similar warming (Sadras and Moran 2012). Experiments in a controlled environment under extremely contrasting temperatures (15°C versus 35°C) showed that cooler temperatures increased anthocyanin concentrations by two to four times compared to grapes grown under warmer conditions in Cardinal, Pinot noir, and Tokay (Kliewer and Torres 1972). However, yield also influenced the anthocyanin-to- TSS ratio, with a strong seasonal effect. There was a negative correlation between yield and the anthocyanin-to-TSS ratio in 2013 to 2014, but the yield-to-cane weight ratio was not affected due to increased canopy mass in heated vines. In 2014 to 2015, yield did not impact the anthocyanin-to-sugar ratio, despite a 50% increase in yield in vines pruned at BB. Nonetheless, the yield-to-cane weight ratio also increased by 50% in BB vines.

The tannin-to-sugar ratio was not affected by either late pruning or heating but was affected by interaction effects of these variables. In heated vines, late pruning maintained a higher tannin-to-sugar ratio in 2013 to 2014 and 2014 to 2015. This could be beneficial for the wine style of the Barossa Valley under future warmer scenarios; wine chemistry and sensory analyses are needed to test this proposition.

Conclusion

Late pruning delayed phenology from budbreak to veraison and delayed harvest in four out of six cases. The delay in ripening (TSS) of late-pruned vines was greater when seasons were warmer close to harvest. Heating advanced phenology at flowering and veraison but did not hasten ripening from veraison to harvest. Heating did not affect pH over the three seasons. There were interactive effects of heating and pruning on pH and TA depending on whether late-pruned vines were unheated or heated. Late pruning enhanced the tannin-to-sugar ratio in heated vines, although the anthocyanin-to-sugar ratio was unchanged. Heating consistently lowered the anthocyanin-to-sugar ratio in two seasons. In addition to heating effects on reducing berry color, the anthocyanin-to-TSS ratio was negatively correlated with yield in 2013 to 2014, but not in 2014 to 2015. We conclude that late pruning can effectively delay development of Shiraz berries under conditions found in the Barossa Valley, while having neutral effects on yield. Therefore, late pruning may help to counteract some of the effects of warming on enologically important berry properties.

Captions to Figures

Figure 1. Open-top chambers used to increase daytime temperature in the field experiments.

Figure 2. (**A**) Monthly mean temperature during three growing seasons (scatter plot), and long-term records (lines) showing (top to bottom) the 90th, 50th, and 10th percentile for the period 1957 to 2016 at Nuriootpa, Australia. (**B**) Comparison of daily maximum temperature in heated and control treatments from September to February for three growing seasons.

Figure 3. (**A**) Daily rainfall and (**B**) stomatal conductance among pruning treatments in unheated vines during three growing seasons. Open symbols from left to right indicate flowering in WP (○), BB (▽), 2–3L N2 (□) and 2–3L N1 (◇), and close coloured symbols indicate stomata conductance measured on leaves from vines pruned at WP (●), BB (■) and 2–

3L (▲). Arrows indicate commencement of irrigation. Significant differences between treatments are indicated by asterisks ($p < 0.05$).

Figure 4. Dynamics of TSS in Shiraz berries affected by pruning in winter, at budburst, and at 2–3 leaves grown under ambient (A, B and C) and heating (D, E and F) during three seasons, in 2013-14 (A, D), 2014-15 (B, E) and 2015-16 (C, F). Values are mean ± standard error, and the difference between treatments is indicated by * (P <0 .05), ** (P < 0.01) and *** (P < 0.001). Arrow heads indicate rainfall events over 5 mm.

Table 1 Pruning time and heating effects on phenology from budburst up to veraison in three season. Pruning time, heating and season effects on phenology were significant (P>0.0001) with the exception of the effect of heating at budburst (P=0.0412) during three seasons.

Season	Pruning Time	Heating	Node	Budburst (d)	Flowering (d)	(°C d)	Pea size (d)	(°C d)	Veraison (d)	(°C d)	Veraison - flowering Difference (°C d)
2013-14	WP	Control	N1,N2	34[a] ± 0.6	99 ± 0.3	322	133 ± 3.8	572	170 ± 1.5	1052	730
		Heated	N1,N2	37 ± 0.3	99 ± 0.6	330	124 ± 0.9	522	170 ± 0.3	1082	752
	BB	Control	N1,N2	47 ± 1.0	107 ± 1.0	304	136 ± 1.5	543	172 ± 0.9	1033	729
		Heated	N1,N2	46 ± 1.0	100 ± 0.3	307	128 ± 1.5	522	171 ± 0.3	1080	773
	2–3L	Control	N1	52 ± 1.0	115 ± 1.5	347	143 ± 2.4	638	177 ± 0.3	1092	745
		Control	N2	43 ± 0.6	108 ± 0.9	321	139 ± 3.2	590	172 ± 0.3	1037	716
		Heated	N1	51 ± 0.3	108 ± 0.0	333	137 ± 1.7	583	173 ± 1.0	1095	762
		Heated	N2	47 ± 1.2	105 ± 2.0	324	128 ± 2.0	515	171 ± 0.0	1071	747
2014-15	WP	Control	N1,N2	34 ± 0.6	99 ± 0.3	365	129 ± 1.0	661	162 ± 1.0	1035	670
		Heated	N1,N2	38 ± 0.3	98 ± 0.3	407	123 ± 0.7	683	155 ± 0.7	1052	645
	BB	Control	N1,N2	47 ± 0.9	107 ± 1.0	415	135 ± 0.6	707	169 ± 0.7	1075	660
		Heated	N1,N2	46 ± 1.0	100 ± 0.3	379	128 ± 0.9	685	164 ± 1.2	1109	730
	2–3L	Control	N1	57 ± 1.0	114 ± 0.9	439	142 ± 3.1	731	177 ± 0.6	1118	679
		Control	N2	45 ± 2.1	107 ± 0.3	419	137 ± 2.0	732	169 ± 1.2	1069	650
		Heated	N1	57 ± 0.6	108 ± 0.3	423	136 ± 1.5	746	171 ± 0.7	1135	712
		Heated	N2	45 ± 3.0	103 ± 1.8	417	128 ± 1.0	695	164 ± 0.9	1116	699
2015-16	WP	Control	N1,N2	47 ± 1.7	93 ± 0.3	293	117 ± 0.7	520	165 ± 0.3	1164	871
		Heated	N1,N2	44 ± 0.3	92 ± 0.3	335	114 ± 1.2	563	162 ± 1.9	1223	888
	BB	Control	N1,N2	60 ± 0.9	98 ± 1.0	330	132 ± 0.3	681	170 ± 0.3	1223	893
		Heated	N1,N2	60 ± 0.3	96 ± 0.3	324	129 ± 0.6	696	167 ± 1.5	1243	919
	2–3L	Control	N1	63 ± 0.7	108 ± 0.6	401	131 ± 0.3	662	175 ± 0.9	1291	890
		Control	N2	62 ± 0.9	104 ± 0.9	378	128 ± 1.3	636	168 ± 0.9	1176	798
		Heated	N1	67 ± 2.5	107 ± 1.2	376	133 ± 1.2	704	177 ± 0.3	1345	969
		Heated	N2	65 ± 1.0	103 ± 1.2	368	126 ± 2.0	611	167 ± 1.5	1217	849

Table 2 Effect of pruning time in winter, at budburst and at 2–3 leaves combined with two temperatures, ambient (control) and heated, on yield, yield and bunch components, and pruning weight in Shiraz vines, during three seasons.

Season	Heating	Pruning time	Yield (kg/vine)	Bunch no/vine	Bunch wt (g/bunch)	Berry/bunch	Berry wt (g)	Cane no per vine	Cane wt (kg/vine)	Yield/cane wt (kg/kg)
2013-2014	Control	WP	3.6 ± 0.72	60 ± 10.6	60 ± 8.3	96 ± 11.7	0.73 ± 0.153	48 ± 3.1	1.9 ± 0.24	2.1 ± 0.68
		BB	3.7 ± 0.31	56 ± 0.8	68 ± 5.3	73 ± 3.4	0.92 ± 0.043	45 ± 1.8	1.4 ± 0.14	2.7 ± 0.07
		2–3L	4.9 ± 1.05	67 ± 2.3	75 ± 12.8	83 ± 8 .6	0.89 ± 0.059	44 ± 3.3	1.6 ± 0.30	3.1 ± 0.18
	Heated	WP	6.8 ± 0.88	84 ± 6.4	83 ± 9.7	105 ± 2.7	0.79 ± 0.093	48 ± 3.9	2.1 ± 0.23	2.5 ± 0.52
		BB	5.9 ± 1.47	73 ± 4.2	80 ± 15.0	105 ± 10.5	0.67 ± 0.114	47 ± 2.1	2.2 ± 0.33	2.6 ± 0.32
		2–3L	6.8 ± 0.77	82 ± 9.4	84 ± 6.6	105 ± 6.3	0.84 ± 0.024	53 ± 1.2	2.4 ± 0.15	3.0 ± 0.35
	P-Value	P-temp.	0.0001	0.0001	0.1846	0.0022	0.2064	0.1685	0.0187	0.7870
		P-pruning	0.5600	0.3643	0.8380	0.5151	0.4326	0.7137	0.7744	0.2227
		P-interaction	0.9100	0.8623	0.9686	0.0659	0.2068	0.4345	0.3627	0.7407
2014-2015	Control	WP	2.5 ± 0.37 a[b]	42 ± 3.1	55 ± 6.4	54 ± 6.4 b[a]	1.02 ± 0.013	50 ± 1.9	1.2 ± 0.08	1.8 ± 0.39
		BB	4.4 ± 0.39 b	61 ± 2.2	72 ± 5.0	70 ± 2.6 a	1.01 ± 0.040	50 ± 1.9	1.7 ± 0.10	2.6 ± 0.15
		2–3L	2.7 ± 0.34 a	47 ± 4.9	60 ± 7.4	63 ± 4.6 ab	0.93 ± 0.050	40 ± 3.1	1.3 ± 0.17	2.2 ± 0.24
	Heated	WP	3.3 ± 0.61 x	49 ± 7.0	63 ± 6.4	49 ± 4.5 b	1.29 ± 0.027	53 ± 2.6	1.8 ± 0.13	1.7 ± 0.26
		BB	4.3 ± 0.53 y	61 ± 5.1	71 ± 6.1	67 ± 4.8 a	1.06 ± 0.063	47 ± 3.3	1.7 ± 0.19	2.7 ± 0.28
		2–3L	3.8 ± 0.44 x	56 ± 2.8	66 ± 6.0	63 ± 3.3 ab	1.03 ± 0.057	45 ± 3.4	1.5 ± 0.22	2.7 ± 0.19
	P-Value	P-temp.	0.1345	0.1351	0.4159	0.5680	0.1368	0.4370	0.0350	0.3530
		P-pruning time	0.0040	0.0043	0.1627	0.0207	0.2888	0.136	0.1840	0.0030
		P-interaction	0.3861	0.5402	0.7601	0.9024	0.5636	0.4060	0.1500	0.4770
2015-2016	Control	WP	3.7 ± 0.15 a	82 ± 4.9 a	47 ± 2.0	82 ± 7.3 a	0.63 ± 0.027			
		BB	2.5 ± 0.14 b	63 ± 5.0 b	41 ± 1.8	71 ± 5.0 a	0.50 ± 0.076			
		2–3L	2.3 ± 0.20 b	51 ± 4.4 b	42 ± 1.9	57 ± 1.3 b	0.56 ± 0.099			
	Heated	WP	3.3 ± 0.22 x	67 ± 3.7 b	50 ± 2.8 a	75 ± 9.2 x	0.70 ± 0.100			
		BB	2.1 ± 0.21 y	62 ± 3.9 b	33 ± 2.7 b	48 ± 2.8 y	0.60 ± 0.062			
		2–3L	2.5 ± 0.21 y	51 ± 3.9 b	46 ± 2.8 a	60 ± 6.0 y	0.59 ± 0.099			
	P-Value	P-temp.	0.222	0.038	0.965	0.186	0.138			
		P-pruning time	<.0001	<.0001	<.0001	0.0141	0.329			
		P-interaction	0.2044	0.012	0.045	0.069	0.932			

[a]Values are mean ± standard error for days after 1 August; °C days from budburst are also shown [base temperature = 10°C (Winkler et al. 1974b)]

[a] Different letters, a, b and c, indicates mean differences when pruning time * heating interaction is significant at $p < 0.05$ by Tukey's HSD multiple range test. [b] If interaction is no significant, mean difference between pruning times is indicated with different letters in control vines (a, b, c) and in heated vines (x. y. z) at $p < 0.05$ by Tukey's HSD post-hoc test.

Table 3 Berry traits affected by pruning time and heating during three growing seasons.

Season	Heating	Pruning time	Harvest	TSS (^{0}Be)	pH	Total Acidity (g/l)	Anthocyanin :TSS [(mg/g)/ Baume]	Tannin :TSS [(mg/g)/ Baume]
2013-14	Control	WP	11-Mar	14.6 ± 0.30 a	4.03 ± 0.05 b[b]	4.7 ± 0.1	0.124 ± 0.011	0.588 ± 0.050 a[a]
		BB	12-Mar	13.8 ± 0.22 b	4.19 ± 0.05 a	4.7 ± 0.1	0.118 ± 0.002	0.471 ± 0.045 ab
		2–3L	12-Mar	13.7 ± 0.14 b	4.09 ± 0.08 a	4.5 ± 0.1	0.116 ± 0.009	0.421 ± 0.016 b
	Heated	WP	13-Mar	13.7 ± 0.08 b	3.97 ± 0.03 y	4.7 ± 0.1	0.110 ± 0.009	0.429 ± 0.021 b
		BB	13-Mar	13.9 ± 0.04 b	4.13 ± 0.02 x	4.4 ± 0.2	0.113 ± 0.005	0.466 ± 0.005 ab
		2–3L	13-Mar	13.6 ± 0.09 b	4.15 ± 0.03 x	4.5 ± 0.2	0.097 ± 0.003	0.503 ± 0.007 ab
	P-temp.			0.041	0.158	0.274	0.046	0.282
	P-pruning			0.035	0.039	0.244	0.319	0.271
	P-interaction			0.026	0.203	0.046	0.617	0.005
2014-15	Control	WP	10-Feb	13.9 ± 0.19	3.48 ± 0.012 bc	7.4 ± 0.1 a	0.129 ± 0.003	0.467 ± 0.009 ab
		BB	17-Feb	13.9 ± 0.13	3.53 ± 0.029 a	5.9 ± 0.2 b	0.120 ± 0.006	0.462 ± 0.011 ab
		2–3L	25-Feb	13.7 ± 0.31	3.62 ± 0.022 a	5.5 ± 0.3 b	0.116 ± 0.006	0.469 ± 0.032 ab
	Heated	WP	6-Feb	13.7 ± 0.16	3.65 ± 0.038 ab	6.0 ± 0.2 a	0.111 ± 0.002	0.462 ± 0.006 ab
		BB	12-Feb	13.8 ± 0.16	3.48 ± 0.054 c	7.3 ± 0.2 ab	0.111 ± 0.008	0.520 ± 0.019 a
		2–3L	19-Feb	14.5 ± 0.06	3.57 ± 0.027 bc	6.7 ± 0.1 ab	0.112 ± 0.005	0.421 ± 0.012 b
	P-temp.			0.274	0.001	0.006	0.033	0.911
	P-pruning			0.244	0.063	0.009	0.537	0.061
	P-interaction			0.046	<0.0001	0.002	0.437	0.031
2015-16	Control	WP	23-Feb	14.6 ± 0.21 a	3.88 ± 0.02	5.2 ± 0.1		
		BB	23-Feb	14.7 ± 0.05 a	3.88 ± 0.01	5.2 ± 0.3		
		2–3L	23-Feb	14.7 ± 0.07 a	3.76 ± 0.06	5.4 ± 0.1		
	Heated	WP	10-Feb	13.7 ± 0.16 b	3.82 ± 0.03	4.7 ± 0.3 y		
		BB	12-Feb	13.7 ± 0.05 b	3.83 ± 0.02	4.1 ± 0.1 y		
		2–3L	23-Feb	14.8 ± 0.13 a	3.84 ± 0.04	5.4 ± 0.1 x		
	P-temp.			0.0001	0.136	0.003		
	P-pruning			0.0005	0.145	0.007		
	P-interaction			0.0017	0.975	0.089		

[a] Different letters, a, b and c, indicates mean differences when pruning time * heating interaction is significant at $p < 0.05$ by Tukey's HSD multiple range test. [b] If interaction is no significant, mean difference between pruning times is indicated with different letters in control vines (a, b, c) and in heaed vines (x. y. z) at $p < 0.05$ by Tukey's HSD post-hoc test.

Figure 1

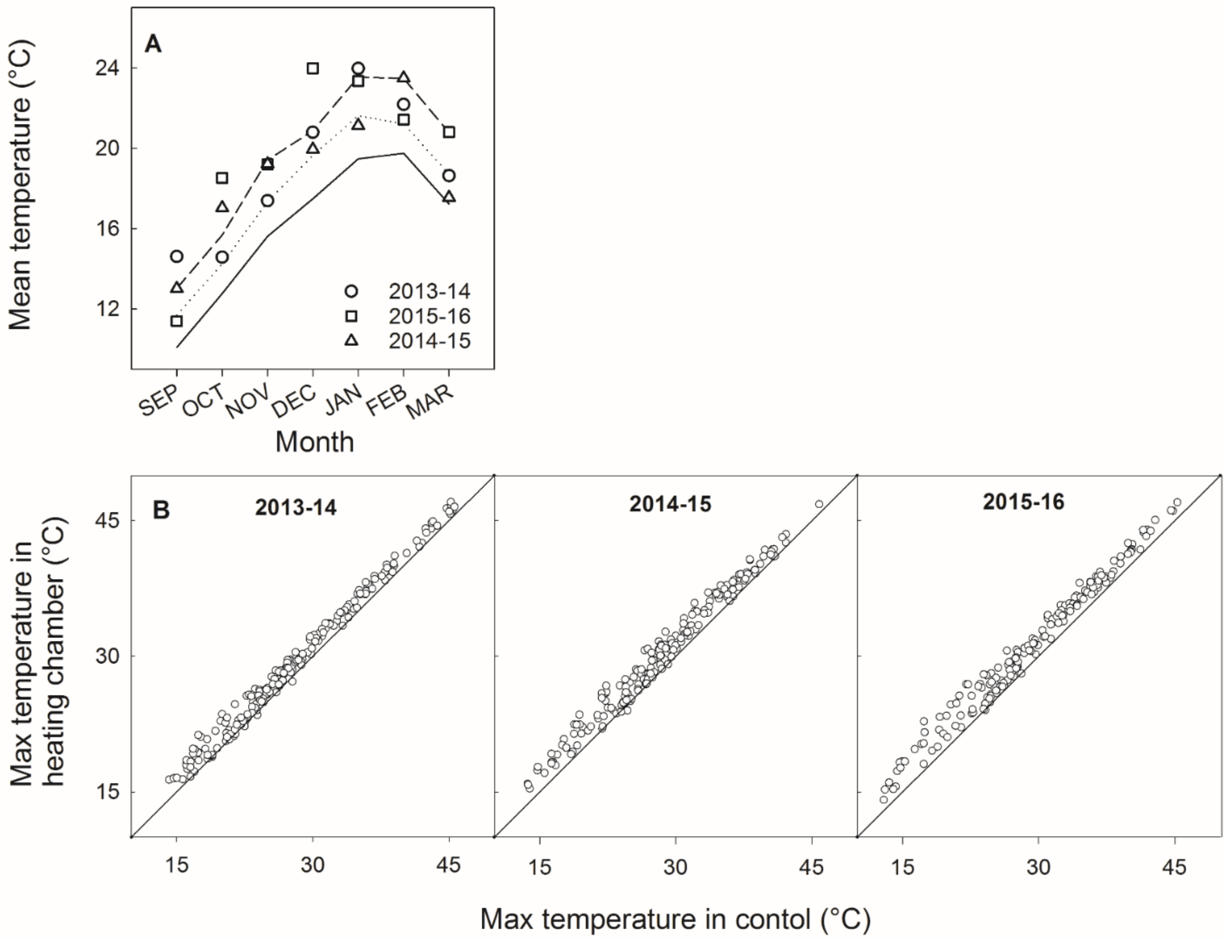
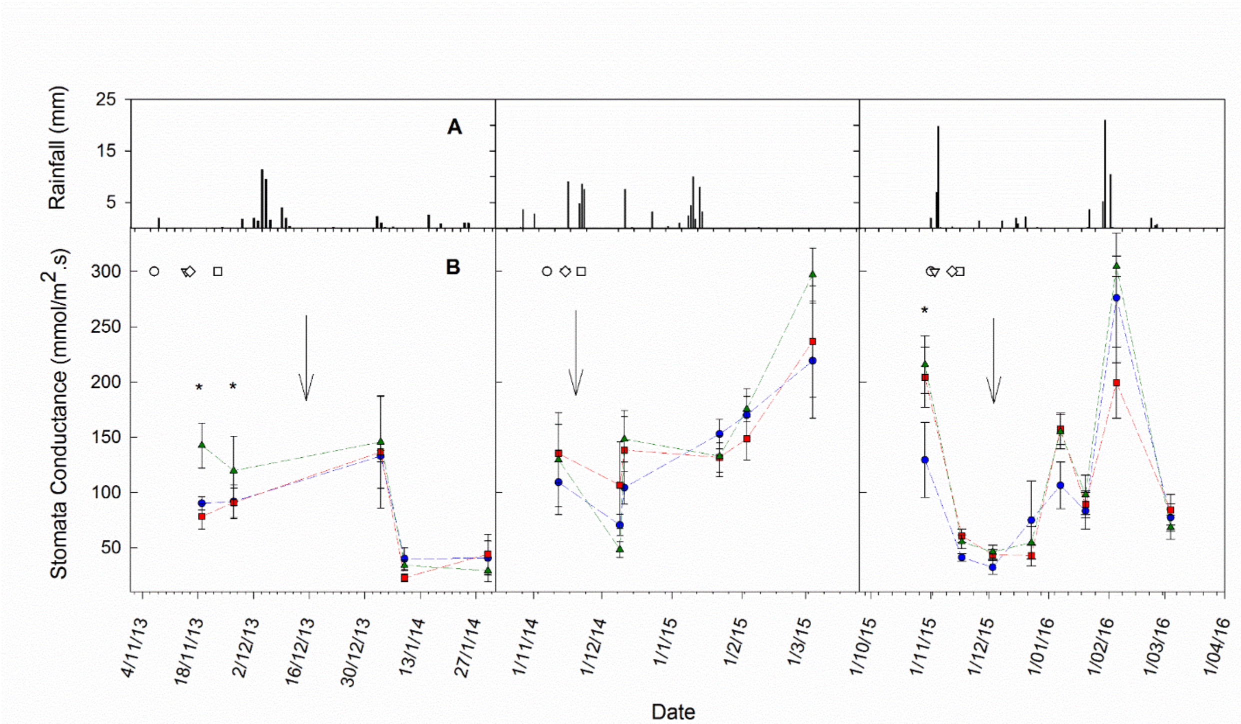

A
Mean temperature (°C)
24
20
16
12
2013-14
2015-16
2014-15
SEP
OCT
NOV
DEC
JAN
FEB
MAR
Month
B
2013-14
2014-15
2015-16
Max temperature in heating chamber (°C)
45
30
15
Max temperature in contol (°C)

Figure 2


Rainfall (mm)
25
15
5
A
B
Stomata Conductance (mmol/m^2.s)
300
250
200
150
100
50
*
4/11/13
18/11/13
2/12/13
16/12/13
30/12/13
13/1/14
27/1/14
1/11/14
1/12/14
1/1/15
1/2/15
1/3/15
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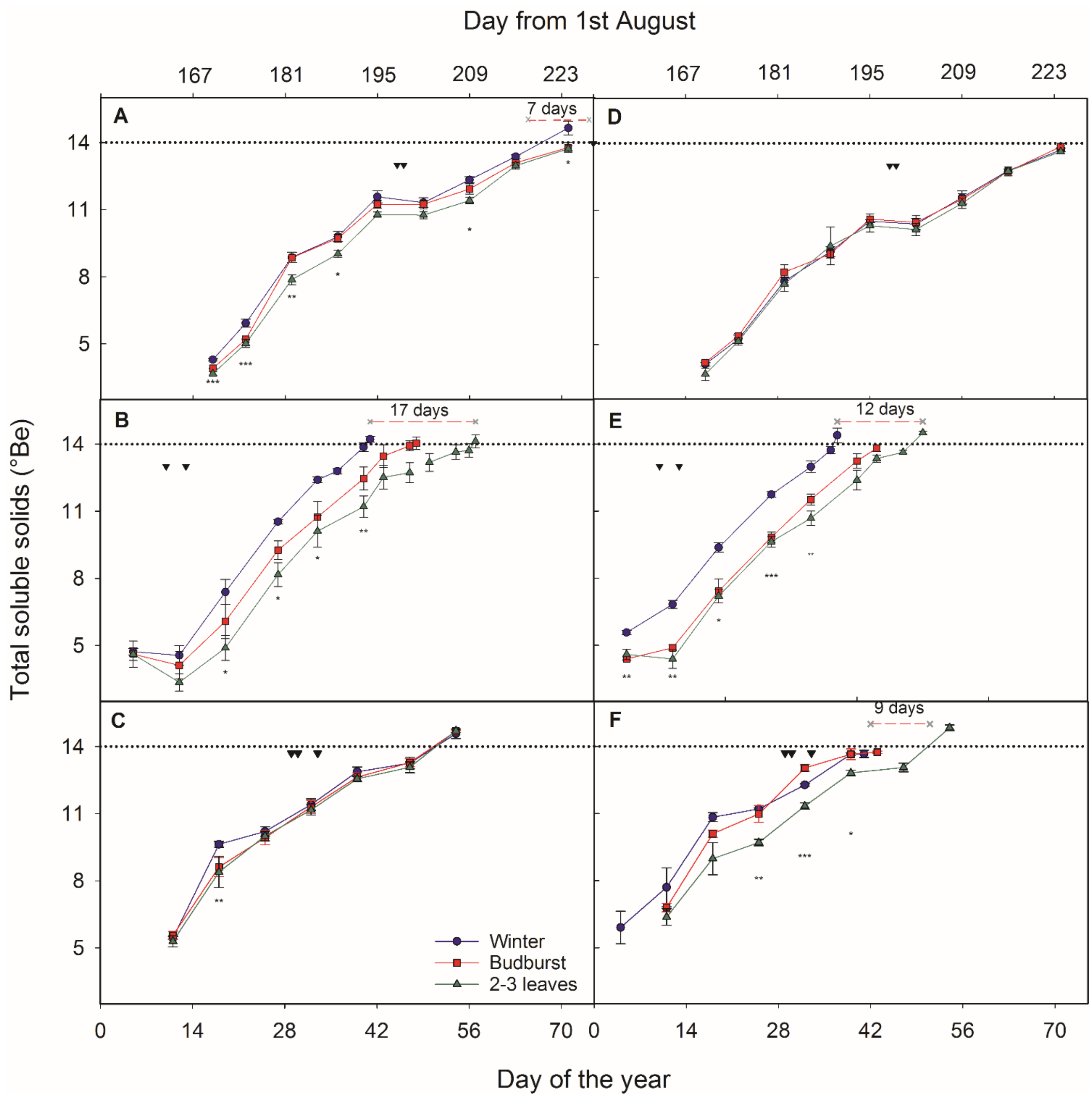
1/02/16
1/03/16
1/04/16
Date

Figure 3



Day from 1st August
167
181
195
209
223
A
D
B
E
C
F
7 days
17 days
12 days
9 days
14
11
8
5
Total soluble solids (°Be)
Winter
Budburst
2-3 leaves
0
14
28
42
56
70
Day of the year

Figure 4

Chapter 5

Impact of Late Pruning and Elevated Temperature on Shiraz Wine Chemical and Sensory Attributes

Submitted article – Australian Journal of Grape and Wine Research

Statement of Authorship

Title of submitted paper to the Austrlian Grape and Wine Research Journal:
Moran M. A., Bastian S.E., Petrie P.R. and Sadras V.O. Impact of Late Pruning and Elevated Temperature on Shiraz Wine Chemical and Sensory Attributes

Author Contribution

Martin Moran conducted the literature review, drafted and constructed the manuscript.

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.

Overall Percentage: 80%

Signature Date 9/8/19

Co-Authors Contribution

By signing the Statement of Authorship, each author certifies that:
i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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

Signature Date 9/8/19

Paul R. Petrie contributed to research ideas, experimental design and editing of manuscript.

Signature Date 9/8/19

Sue E. Bastian contributed to the supervision of the sensory panel and editing of manuscript.

Signature Date 9.8.19

Impact of Late Pruning and Elevated Ambient Temperature on Shiraz Wine Chemical and Sensory Attributes

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Keywords: anthocyanin, climate change, tannin, temperature, veraison

Abstract

Background and Aims: Viticultural practices are needed to counteract widespread, deleterious effects of warming on fruit and wine attributes. Late pruning is an effective tool to delay fruit ripening and improve berry and wine attributes, but the interaction between late pruning and elevated ambient temperature on wine chemical and sensory properties are unknown.

Methods and Results: A factorial combination of three pruning times (winter pruning control, and two late pruning treatments when controls reached budbreak or 2–3 leaves) and two thermal regimes (ambient temperature, heated) were established to investigate the interaction between pruning time and temperature on wine chemical composition and sensory traits during two seasons in Barossa Valley Shiraz. Average daily mean temperature at canopy level of heated treatment was 0.40 °C above control in 2013-14, and 0.68 °C in 2014-15. Sensory and chemical assessments showed a reduced colour concentration in wines made from heated vines. Wines made from heated fruit were lighter in body, and lacked palate length in comparison to unheated controls. Wine colour density, concentration of anthocyanin and total polyphenols correlated negatively with daily mean temperature in a short window (2 weeks) immediately after veraison.

Conclusion: Interactions between pruning and temperature treatments indicated that pruning at 2–3 leaves has the potential to partially mitigate heating effects by increasing wine colour and fruit flavour intensity.

Significance of the study: Delayed pruning can help to partially counteract realised and projected warming effects, with neutral or positive effects for vine phenology, yield and wine properties.

Key words: late pruning, temperature, heating, anthocyanin, sensory, chemical composition

Introduction

The realised and projected increase of ambient temperature has consequences for vine phenology, physiology, and berry and wine attributes. Warmer mean temperature during spring and summer often advance flowering, veraison and harvest (Petrie and Sadras 2008, Sadras and Petrie 2011, Tomasi et al. 2011, Webb et al. 2007). Elevated temperature can disrupt the balance of berry traits (Sadras and Moran 2012, Tarara et al. 2008) and wine sensory attributes (Bonada et al. 2015, Sadras et al. 2013). Harvest compression partially associated with warming trends can lead to delayed harvest and fruit processing, with implications for wine chemical and sensory attributes (Jones et al. 2005, Palliotti et al. 2014, Petrie 2016, Webb et al. 2012). Therefore, adaptive management practices are required to preserve or improve desirable wine attributes and to decompress harvest under warmer conditions.

A long-term adaptive strategy includes shifts to cultivars more suitable to warmer climates, and relocation of vineyards to cooler regions (Anderson et al. 2008). Short- to medium-term solutions in established vineyards have been reviewed by Palliotti et al. (2014), and include late irrigation, antitranspirants, manipulating source:sink ratio, and shifting the phenological development of vines with growth regulators or late pruning.

Early work on late pruning aimed at delaying flowering to reduce frost risk in Australia, USA and New Zealand vineyards (Coombe 1964, Friend and Trought 2007, Friend et al. 2011, Howell and Wolpert 1978). More recently, attention has shifted to late pruning to delay ripening in a context of warming, and this practice has been tested for Maturana in Spain, Sangiovese in Italy, and Shiraz and Cabernet Sauvignon in Australia (Moran et al. 2018a, Palliotti et al. 2017, Petrie et al. 2017, Wei et al. 2017). The interaction between delayed pruning and temperature has received less attention, except for a previous study focusing on vine phenology, yield and berry attributes (Moran et al. 2019). Here we report Shiraz wine composition and sensory attributes in response to the interactive effects of timing of pruning and elevated ambient temperature during two seasons in the Barossa Valley.

Methods

Site, vineyard and experimental design

The growing conditions, site, treatments, experimental design, phenology, yield and berry traits have been reported elsewhere (Moran et al. 2019). The experiment was established in a Shiraz vineyard (clone 1654 grafted on Schwartzman rootstock) at Nuriootpa (34 ^{o}S, 139 ^{o}E), Barossa Valley in mid-January 2013. The vineyard was planted in 2004, at 2.25 m within and 3.0 m between rows in a northeast-southwest orientation, and trained to a single cordon (as two arms from the trunk), spur-pruned at 16 nodes 69 per metre, with a sprawling canopy supported by a single wire about 0.4 m above the cordon. Supplementary drip irrigation (6.6 l vine^{-1} h^{-1}), approx.100 mm, was applied from berry pea size (beginning of December) until harvest.

The trial involved a factorial combination of two thermal regimes, heating vs control, and three pruning times, winter control (WP), and two late pruning, when winter-pruned controls reached 50% budbreak (BB) and 50% 2–3 leaves (2–3 L) (Coombe 1995). The experiment was carried out over two seasons, 2013-14 and 2014-15. To avoid carry-over effects, late pruning treatments in 2014-15 were applied to vines pruned in winter in 2013-14. The treatments were laid out in a split-plot design with three replicates, with thermal treatments applied to main plots and pruning time to subplots. Each replicate included 11 vines; measurements were taken in the seven central vines, with two vines used as buffers at each end.

Heating system

To increase the daytime temperature at the canopy level, passive open-top chambers were used as described in Sadras and Soar (2009). The system consists of modular rectangular units (158 cm high × 151cm wide) each supported by a pair of fold-out legs (870 mm tall) hinged 125 mm below the top of the panel face. The frame was made from 25 mm square tube steel (Stratco, Australia) and the unit face was made from solid Standard-Clear-Greca polycarbonate sheeting fastened to the steel frame (Suntuf, Australia). The polycarbonate material blocks most

UV radiation (200–400 nm) and has a very high (90%) and uniform transmittance between 400 and 1600 nm. Consecutive units were fastened together during vineyard installation using plastic "zip" cable ties and each unit was independently anchored to the ground using 30 cm pegs. Ambient temperature and relative humidity at bunch height were recorded at 15 min intervals using TinyTag Ultra2 loggers (Hastings Dataloggers, Port Macquatie, Australia) which were shielded in Stevenson-type screens. Vapour pressure deficit was calculated as a function of temperature and relative humidity (Monteith and Unsworth 1990). The design of these chambers aimed at, and achieved (i) increasing daytime temperature, (ii) tracking diurnal and seasonal temperature cycles, (iii) affecting vapour pressure deficit, rather than relative humidity, and (iv) having no secondary effects on vine and fruit growth and development (Sadras and Soar, 2009). The open-top structure ensured canopies including fruit were exposed to natural radiation and wind. To directly test for experimental artefacts from chambers, we used the F-statistical test of Potvin et al. (1990) to compare curves of berry traits vs time on chronological and thermal-time scales. On chronological scales curves of berries from heated treatments where ahead of curves from unheated controls, but on thermal-time scale, the curves of both treatments were statistically undisinguishible; this was verified for berry total soluble sugars (Sadras and Moran 2013b), cell-death in berry mesocarp (Bonada et al. 2013), and organic acids (Sweetman et al. 2014). Hence, we interpret the differences between heated and control treatment as a true effect of temperature with no confounding factors (Bonada and Sadras 2015).

Temperature treatments were initiated on the 15th of January 2013, when vines were at stage E-L 35. Pruning treatments were initiated in the following winter (July) and spring (September). To corroborate block uniformity in the assignation of treatments, we measured yield and bunch number in February 2013, shortly after establishment of temperature treatments. Yield (P=0.83) and bunch number (0.94) were similar in the vines allocated to heated and control treatments. Likewise, yield (P = 0.43) and bunch number (P = 0.81) were similar in the vines allocated to future pruning treatments.

Relative to the controls, daily mean ambient temperature in the heated treatments increased by 0.40 °C (SD = 0.119) in 2013-14, and 0.68 °C (SD = 0.252) in 2014-15; these effects were associated with increased maximum temperature, with no significant change in minimum temperature or relative humidity. Fig. 1 in Moran et al. (2019) shows the monthly average temperature of control treatments in comparison to long-term records, and the daily maximum temperature of heated and control treatments. In most cases, heating advanced phenology including the harvest. It only increased yield in 2013-14. Berry anthocyanin to TSS ratio decreased in heated vines by about 10% in 2013-14 and 2014-15 (Moran et al. 2019).

Harvest and winemaking

The harvest was targeted at TSS of 14°Be, and varied between 13.6 and 14.6°Be among treatments (Table 1). The aim of harvesting all treatments at a similar TSS was therefore partially achieved due to both sampling logistics and the difficulty to predict the trajectory of

TSS. The small differences in fruit maturity among treatments at harvest may have influenced wine attributes.

A small-scale wine was made from fruit harvested from each replicate sub-plot. Each sub-plot yielded ~30 kg, and within 4 h a random subsample of 25 kg of fruit was crushed using a hand operated crusher-destemmer (https://www.grifomarchetti.com/). Active dry wine yeast (EC 1118 Lalvin, La Champagne, France) was rehydrated as per manufacturer's directions and inoculated into must at 200 mg/L. Fermentation was carried out in 75-l open-top containers, placed in a room with minimum air temperatures of 20 ^{o}C and maximum temperature of 28 ^{o}C. Under these conditions, ferment temperature was between 25 and 29 ^{o}C, and the time to complete fermentation was 6-7 d, which is comparable to commercial wineries. During active fermentation, the cap was gently plunged twice a day for 30 seconds and daily measurements of sugar consumption were taken with a hydrometer (Alla France; 49120 Chemillé en Anjou, France). After a week, ferments were pressed in a 50-l basket press and wine was transferred into 10-l glass demijohns with an immediate addition of 1 g/l of tartaric acid. The pH was further adjusted with tartaric acid to approximately 3.5 prior to malolactic fermentation. Malolactic bacteria (Lalvin VP41, *Oenococus oeni*, France) were inoculated as per packet instructions once the wines completed primary fermentation and the residual sugar was below 3 g/l. Residual sugar was measured with a colorimeter (Bayer Clinitest copper sulfate tablets, Vintessential, Orange, NSW, Australia) in 2014 and an infrared method (OenoFoss, FOSS, Hillerød, Denmark) in 2015. Malolactic fermentation was assumed to be complete when wines were below 0.1 g/l of malic acid as measured with Accuvin colour strip test (Napa, CA, USA). After completion of malolactic fermentation, the wine was racked off from gross lees, free sulphur was adjusted to 40 ppm (potassium metabisulphite ~0.1g/l), and wine cold stabilised at ~2 °C for approximately two weeks before bottling. The wines were bottled manually by gravity-feed in 375-ml Burgundy bottles that were prefilled with nitrogen to minimise oxidation, then closed with screw cap closure (http://www.novatwist.com), packed in cartons, and aged for 4 to 5 months at 15 °C in a controlled temperature room prior chemistry and sensory analysis.

Wine chemical analysis

Duplicate measures were made of: (i) pH, (ii) titratable acidity at pH end-point 8.2, (iii) alcohol, (iv) volatile acidity, (v) free and total sulphur by aspiration (Iland et al. (2004), and (vi) residual sugars using colorimetry (Bayer® Clinitest copper sulphate tablets, Copyright 1995 by Bayer Corp., Elkhart, IN USA) in 2014, and with infrared method (OenoFoss, FOSS, Hillerød, Denmark) in 2015. Wines were considered dry when residual sugars were <3g/l. Wine colour spectra were assessed by Cie-Lab returning four parameters: L* (lightness), a* (from green to red), b* (from blue to yellow), and C* (chroma or saturation) (Ohno 2000). Modified Somers (Mercurio et al. 2007) was used to measure: (i) chemical age 1 ($A_{520sulfite}/A_{520acetal;}$ A=absorbance) and chemical age 2 ($A_{520sulfite}$ / (5*A_{520HCl}) which are spectral ratios defined as the extent of displaced monomers (anthocyanin) by polymeric pigments during the ageing process; (ii) colour density; (iii) hue (SO_2 corrected); (iv) pigmented tannin (meaning SO_2-resistant pigments); (v) anthocyanin; and (vi) phenolic substances. Tannin was measured by the methylcellulose precipitable technique (Mercurio et al. (2007). To account for tannin-anthocyanin associations, copigmentation was measured as described in Gutiérrez (2003).

Wine sensory analysis

Descriptive analysis was used to assess wine sensory attributes following the protocol of Bastian et al. (2010). Prior to formal assessment, a panel of 12 individuals was trained in ten 2-h sessions using reference standards (Supplementary Table 1). Wines were assessed in triplicate for each treatment replicate, in a total of three sessions. In each session a set of 18 wines were assessed in 215 ml clear wine glasses XL5 (ISO standard) with 5-min breaks between every 6 wines. Each attribute was scored on a linear scale between 5% (low) and 95% (high), with the aid of a computer (Fizz software, Version 1.3, Biosystemes, Couternon, France) in individual booths under fluorescent light. The attributes were scored in the following order: 1-colour, 2-aroma, 3-taste, 4-palate, 5-mouthfeel and 6-aftertaste. There were 35 attributes evaluated in 2014 and 30 in 2015 (Supplementary Table 2 and 3). The scale for mouthfeel was anchored as medium body to full bodied, for tannin quantity from non-drying to very drying and tannin quality from smooth (silky) to harsh (sandpaper), with three intervening categories, velvet, suede and chalky. The aftertaste attributes non-fruit, fruit and alcohol were described after expectoration; non-fruit was defined as any taste, non-fruit flavour or mouthfeel sensation other than fruit or alcohol, fruit as any fruit perceived, and alcohol as heat perception from low to high. Aftertaste was measured as attributes that lingered for short (10 s), medium (30 s) or long periods after expectoration (60 s).

Statistical Analysis

Chemical attributes were assessed with two-way ANOVA with pruning time and temperature as fixed effects (Statview, SAS Institute, Cary, NC, USA). The sensory descriptors were analysed with a mixed model two-way ANOVA with pruning time and temperature as fixed factors, and assessor and assessor-by-temperature-pruning interaction as random effects (XLSTAT Version 2015.5.01.23654, Addinsoft, Paris, France). Fisher LSD test was used to account for differences between treatments when ANOVA returned significant factor effects.

Principal component analysis (PCA) was used to explore associations among chemical and sensory traits. Chemical traits were used as active, and sensory and yield components as supplementary variables; the analysis was constrained to those variables that responded to pruning treatment according to ANOVA. The responsive variables changed with vintage, hence separate PCAs were performed for 2014 and 2015. Linear regressions were fitted to explore associations between wine attributes and temperature (mean, maximum and minimum) in different phenological windows, as explained in Moran et al. (2018).

Results

The effects of late pruning and heating on vine phenology, yield components, and berry composition have been reported in Moran et al. (2019). Here we present a summary of measured vine traits relevant to the interpretation of wine chemical and sensory analysis. In 2013-14, late pruning delayed veraison of unheated vines in relation to winter-pruned vines by 2 days in BB, and for treatment 2-3L, by 2 days in node 2 and 7 days in node 1. In heated vines, late pruning only delayed veraison by 1 day in BB and by 3 days in 2-3L at both nodes 1 and 2. In 2014-15, late pruning delayed veraison by 7 days in both BB and 2-3L node 2, and by 15 days in 2-3L node 1 in unheated controls. Late pruning delayed veraison by 9 days in both BB and 2-3L node 2, and by 16 days in 2-3L node 1 in heated vines (Moran et al. 2019).

Seasonal mean temperature (September to March) at mid-canopy level was raised by the heated treatment, relative to the control, by 0.40°C (SD = 0.119) in 2013-14 and 0.68°C (SD = 0.252) in 2014-15. In 2013-14, mean temperature 2 weeks post-veraison averaged 25.2°C in WP, 25.9°C in BB and 26.2 in 2-3L in the unheated environment, and 26.1°C in WP, 26.7°C in BB, 27.8C in 2-3L in heated environment. In 2014-15, mean temperature at 2 weeks post-veraison was 20.4°C in WP, 20.6°C in BB, and 19.8°C in 2-3L in the unheated environment, and 24.29°C in WP, 21.9° in BB, and 20.4°C in 2-3L in the heated environment (Moran et al. 2019).

In 2013-14, heating the vine environment increased yield by approximately 60% due to higher bunch number in relation to unheated controls, with no change in yield-to-cane weight ratio. In 2014-15, late pruning at BB increased bunch number and yield by 50%, and the yield-to-cane weight ratio by about 45% in comparison to WP. Elevated temperature increased berry weight up to 15%. Late pruning reduced berry weight by 10% in unheated vines pruned at 2-3L and by 25% in heated vines pruned at BB or 2-3L (Moran et al. 2019).

Wine composition

Table 1 shows wine attributes after 6 months of bottling. There were small differences in pH and alcohol among heating and pruning treatments in 2013-14, and no differences in 2014-15.

In 2013-14, heating increased L* by 10%, and decreased a* and C* by about 15% in comparison to unheated controls (Table 2). Late pruning did not affect Cie-Lab parameters. In 2014-15, heating increased the yellow tones (b*>1) 2.3-fold, and decreased the hue angle h* 2.7-fold in comparison to unheated controls.

In 2014-15, late pruning increased a* and C* by about 12% in wines made from both heated and unheated vines. It increased the hue angle h* by 1.33-fold in wines made from heated treatment and 2.42-fold in their unheated counterparts (Table 2).

Heating decreased concentration of anthocyanin by 15% in 2013-14 and by 10% in 2014-15 in relation to unheated controls (Table 3). It also decreased colour density by 15% in 2013-14 and by 10% 2014-15. Heating also decreased the berry anthocyanin-to-TSS ratio by about 10% in 2013-14 and 2014-15 (Moran et al. 2019).

In 2013-14, late pruning decreased anthocyanin under heating conditions by 20%. In 2014-15, however, late pruning increased anthocyanin in unheated vines pruned at BB by 16% and in heated vines pruned at 2-3L by 24%. Yet, late pruning did not affect the berry anthocyanin-to-TSS ratio (Moran et al. 2019).

In 2013-14, late pruning increased tannin concentration in wines made from unheated vines by 2.8-fold (pruned at BB) and 1.6-fold (pruned at 2-3L) against 1.4 fold (pruned at BB) in wines made from heated fruit. Heating and late pruning had negligible effect on tannin in 2014-15. Heating decreased total phenolics in 2013-14 by about 40%. In 2014-15, it decreased percent copigmentation by 14%. In 2014-15, heating increased chemical age 1 by 20%.

Wine sensory attributes

In 2013-14, heating, late pruning and their interaction impacted on 17 out of 35 wine attributes (Table 4). Heating decreased colour hue by 19%; opacity by 29%; dried fruit, black olives and savoury aromas and flavours by about 11%; body, tannin quantity and quality by about 10%, and the non-fruit aftertaste by 6%. Heating increased floral aromas by 22%. Pruning at BB increased the colour hue by about 5% and savoury flavour by 10%; and reduced berry aroma and bitter taste by about 9%. Colour opacity, vegetal green flavour and body responded to interaction effects. Late pruning increased colour opacity by 9% in wines made from heated vines. Late pruning decreased vegetal green palate flavour by 4% in wines made from heated vines and it increased by 13% in wines made from unheated vines pruned at BB. Late pruning increased body by 5% in wines made from heated vines.

In 2014-15, heating treatment, timing of pruning and their interaction impacted on 10 out of 30 wine attributes (Table 5). Heating decreased colour opacity by 8%, pepper flavour by 13%, savoury flavour by 26%, and aftertaste by 6%; and increased dried fruit aroma by 5%. Pruning at 2-3L increased floral aroma and red fruit flavour by about 10% and 7%, respectively. Three sensory traits: colour opacity, red fruit aroma and savoury flavour, responded to interactions. Late pruning increased colour opacity by 7% in unheated, and 9% in the heated (2-3L) treatment. Late pruning increased red fruit flavour by 10% in unheated, and 4% in the heated (2-3L) environment. Late pruning increased savoury flavour in unheated by 40%, and it remained unchanged under heated conditions.

Associations between wine composition, yield components and sensory attributes

Principal component analysis (Figure 1) and correlations (Supplementary Tables 4 and 5) show associations between wine chemical and sensory traits, and yield components. Chemical traits, including Somers measures and tannin by MCP, were used as active variables, and yield components, Cie-Lab, pH, alcohol, TA, and sensory traits were used as supplementary variables.

In 2013-14, the first and second components accounted for 80% of the total variation of wine chemical traits (Figure 1A). Temperature treatments accounted for 56% of the variation (PC1), and timing of pruning by 24% (PC2). Unheated treatments resulted in higher concentrations of anthocyanin. Wine tannin was positively correlated to sensory traits such as colour hue ($r = 0.76$, $p = 0.0002$), and opacity intensity ($r = 0.71$, $p = 0.0006$), tannin quantity ($r = 0.54$, $p= 0.002$) and tannin quality ($r = 0.49$, $p=0.039$). Colour density was positively correlated to tannin quantity ($r = 0.57$, $p = 0.017$) and negatively correlated to yield ($r = -0.76$, $p<0.0001$), bunch number (-0.66, $p = 0.002$), berry number ($r = -0.62$, $p = 0.005$) and cane weight ($r = -0.720$, $p =0.0004$). Total phenolic ($r = -0.48$, 0.04) and total pigments ($r = -0.62$, $p = 0.005$), were also negatively correlated to yield.

In 2014-15, the first and second components accounted for 83% of the total variation of wine chemical traits. Traits were similarly separated by thermal treatments, which accounted for 42 of the variation (PC1) and by timing of pruning accounting for 41% of the variation (PC2). There was an interaction between temperature and time of pruning treatments whereby wine anthocyanin and colour density increased in wines made from 2-3L in heated treatment, reflected in a displacement from negative to positive in the x-axis of Figure 1B. Total anthocyanin correlated positively with colour intensity ($r =0.76$, $p = 0.0002$) and pepper flavour ($r = 0.57$, $p = 0.015$), and negatively with acid taste ($r = -0.50$, $p = 0.040$). Colour density was positively correlated to colour intensity ($r = 0.749$ $p = 0.0003$) and negatively with berry weight ($r = -0.61$, $p = 0.0085$). Chemical (colour spectrum parameters by Somers) and CieLab parameters showed strong correlations. Chemical age 1 and 2 were positively correlated with yellow tones (*b) (r=0.67, p=0.002). Total anthocyanin was positively correlated with red tones (a*) (r=0.73, p=0.0006), chroma (C*) (r=0.71, p=0.001), and negatively correlated to lightness (L*) (r=-0.54p=0.02).

Anthocyanins, colour density and total phenolics in relation to temperature during the 14-d window after veraison

Late pruning delayed veraison from 1 to 2 weeks in relation to vines pruned in winter, and heating advanced veraison up to 1 week in comparison to unheated controls. As a consequence, developing fruit was exposed to different temperatures during this critical stage. For the pooled data capturing both the phenology-driven shift in temperature with pruning time, and the direct effects of heating, we found that anthocyanins, colour density and total phenolics declined with higher mean temperature in the 14-d window after veraison (Figure 2).

Discussion

Late pruning of Shiraz in Barossa Valley shifted vine phenology, with neutral or positive effects for vine yield and berry properties, and no noticeable carry-over effects over 3-4 seasons (Moran et al. 2018a, Petrie et al. 2017). Here we tested the condition of neutral or positive effects on wine required for this practice to be useful in commercial vineyards, with a particular focus on the interactions between pruning time and elevated ambient temperature.

Effects of temperature and timing of pruning on wines were season-dependent

The effects of temperature and timing of pruning on wine attributes varied with season, as expected because vine yield, berry and wine attributes are strongly influenced by seasonal conditions, often interacting with multiple viticultural and environmental factors. For example, in a comparison of Merlot, Cabernet franc and Cabernet Sauvignon in Bordeaux, sources of variation for berry traits ranged: season > soil > cultivar (Van Leeuwen et al. 2004). A meta-analysis of the impact of viticultural practices (fruit thinning, defoliation, deficit irrigation, pruning severity, crop cover) on vine yield and fruit traits (TSS, pH, titratable acidity, tannin concentration, antocyanin concentration) showed the dominant effect of seasonal conditions compared to the smaller and more variable effects of practices (Kendall 2019). In a comprehensive comparison of heated and unheated vines in the Barossa Valley interacting with seasons, varieties, fruit loads, pruning times, and water regimes, elevated temperature had no significant effect on yield in 32 out of 37 comparisons, reduced yield in 2 and increased yield in 3 (Sadras et al. 2017). Among other factors, the effect of experimentally increasing temperature depends on the background temperature of the control treatment, which is strongly dependent on season (Sadras and Moran 2013a). Owing to the season-dependent response to treatments, wines were analysed separately for 2013-14 and 2014-15 (Tables 2-5, Fig. 1).

In 2013-14, heating increased yield by 60%, slowed ripening and produced wines with lower alcohol. The higher yield possibly contributed to slower ripening (Moran et al. 2018b) and reduced wine alcohol than unheated vines; this kind of association is common (Kliewer and Dokoozlian 2005, Uriarte et al. 2016). Wine total phenolics decreased with increasing yield, despite the unchanged yield-to-pruning weight ratio in Shiraz (Moran et al. 2018b). Therefore, elevated ambient temperature led to wines with less colour, more intense floral and red fruit, lighter in body, less savoury flavours, and lower in both tannin intensity and tannin sub-quality (rougher on the top of the palate) in comparison to control wines. Whereas elevated temperature usually advances the onset of ripening, source:sink relations modulate the actual response of berry development to temperature (Sadras and Moran 2013b); in this particular case, a substantial increase in yield may have offset the advancement of ripening with temperature. The resulting wine contrasted with the typical full-bodied Barossa Valley Shiraz (Iland et al. 2002).

In 2014-15, wines made from heated vines pruned at 2–3 leaves preserved wine colour, likely due to a shift of developing berries into a cooler temperature window shortly after veraison. However, colour density was positively correlated to a lighter berry weight but it has previously been shown that a higher skin-to-pulp ratio typical of small berries might enhance wine colour (Downey et al. 2006). Late pruning and heating did not change the mouthfeel of the wines with the exception of length of aftertaste. There was no change in total polyphenols or tannins; however, there was a decrease of total polyphenols that correlated to higher temperatures shortly after veraison (next section). Heating, nevertheless, decreased the length of aftertaste, pepper and savoury flavour and increased dried fruit aromas. Consistent with this finding, pepper aroma and flavour are enhanced in cooler climates (Zhang et al. 2015).

Anthocyanins, colour density and total phenolics correlated negatively with mean temperature in a 2-week window after veraision

In the study in rainfed vineyards by Van Leeuwen et al. (2004)), where vines were subjected to large variation in water supply associated with seasonal variation in rainfall and variation in soil water holding capacity, vine water status explained a large part of the variation in berry traits. In our study with vines grown under supplementary irrigation, temperature was the dominant source of variation in anthocyanins, colour density and total phenolics. These traits correlated negatively with mean temperature in a short, 2-week developmental window after veraison (Fig. 2), with no apparent influence of yield on anthcyanin extraction (Fig. 1). The correlation in Fig. 2 highlights the importance of environmental conditions shortly after veraison, as opposed to the whole ripening period. Our finding is consistent with both an independent study with Shiraz wine in Barossa Valley (Moran et al. 2018a), and with early work reporting a short window of constant heating (30 vs 20°C) after the onset of colour reducing colour in skin berry of Aki Queen, with no effect of heating after 3 weeks (Yamane et al. 2006). Sadras and Moran (2012) were the first to show that elevated temperature decouples berry anthocyanin from TSS in Shiraz and Cabernet franc, and that this decoupling stems from a shift in the onset of pigmentation; this observation was later verified in other varieties and environments (Balda and Martínez de Toda 2015,Movahed et al. 2016). The parameters of linear regressions in Fig. 2 could be useful as a coarse approach to model the impact of warming on wine anthocyanin. As with any empirical relationship, caution needs to be used in extrapolations, e.g. there might be a low temperature threshold influencing the colouring of Shiraz berries in cooler climates, and genotypic differences could be important (Herderich et al. 2012).

In conclusion, this study and our previous work suggest that late pruning, up to 2–3 unfolded leaves, could be a useful practice to deal with the undesirable effects of warming in Shiraz vineyards under current and projected thermal regimes of the Barossa Valley.

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Table 1. Wine composition as affected by heating and pruning time, and harvest dates in 2013-14 and 2014-15. Values are the mean scores ± standard error; P values are from ANOVA.

Temperature	Pruning Time	Season	Harvest date	Total Acidity (g/l)	pH	Alcohol (% v/v)	Volatile acidity (g/L)	Free SO2 (mg/L)	Total SO2 (mg/L)	Residual Sugars (g/L)
Control	WP	2013-14	11-Mar	6.5 ± 0.25	3.63 ± 0.012	15.0 ± 0.87	0.52 ± 0.075	12.4 ± 1.04	19.2 ± 2.40	<3g/L‡
	BB		12-Mar	6.5 ± 0.23	3.56 ± 0.023	14.7 ± 0.34	0.59 ± 0.047	11.1 ± 0.46	11.5 ± 1.87	<3g/L
	2-3L		12-Mar	6.2 ± 0.15	3.65 ± 0.024	14.7 ± 0.16	0.54 ± 0.042	8.0 ± 2.8	20.8 ± 0.92	<3g/L
Heated	WP		13-Mar	6.3 ± 0.02	3.57 ± 0.002	13.9 ± 0.13	0.57 ± 0.017	9.9 ± 0.23	19.7 ± 0.93	<3g/L
	BB		13-Mar	6.5 ± 0.07	3.52 ± 0.026	13.7 ± 0.14	0.63 ± 0.034	10.8 ± 0.83	17.9 ± 0.58	<3g/L
	2-3L		13-Mar	6.5 ± 0.12	3.58 ± 0.002	14.1 ± 0.10	0.58 ± 0.019	9.2 ± 0.87	19.2 ± 0.92	<3g/L
P-Value	Temperature		0.5787	0.5787	0.0026†	0.0028	0.2139	0.5322	0.117	
	Pruning Time		0.6479	0.6479	0.003	0.6285	0.2611	0.0495	0.023	
	Interaction		0.2524	0.2524	0.7632	0.6008	0.9652	0.2378	0.0208	
Control	WP	2014-15	10-Feb	6.6 ± 0.03	3.77 ± 0.013	14.7 ± 0.19	0.59 ± 0.002	23.2 ± 0.44	46.3 ± 0.60	0.37 ± 0.073
	BB		17-Feb	6.2 ± 0.06	3.76 ± 0.007	14.7 ± 0.17	0.54 ± 0.018	23.8 ± 0.17	43.3 ± 0.88	0.60 ± 0.104
	2-3L		25-Feb	6.5 ± 0.15	3.72 ± 0.048	14.4 ± 0.29	0.53 ± 0.013	19.3 ± 3.94	45.5 ± 3.12	0.33 ± 0.044
Heated	WP		06-Feb	6.7 ± 0.14	3.77 ± 0.009	14.2 ± 0.24	0.56 ± 0.025	18.8 ± 2.35	41.2 ± 3.18	0.27 ± 0.008
	BB		12-Feb	6.5 ± 0.20	3.73 ± 0.018	14.9 ± 0.29	0.51 ± 0.09	21.8 ± 0.60	45.8 ± 0.60	0.40 ± 0.050
	2-3L		19-Feb	6.6 ± 0.03	3.77 ± 0.014	15.0 ± 0.22	0.62 ± 0.02	20.3 ± 3.98	42.2 ± 6.93	0.53 ± 0.101
P-Value	Temperature		0.1026	0.1026	0.7926	0.5558	0.7787	0.4009	0.4851	0.5799
	Pruning Time		0.1728	0.1728	0.4813	0.254	0.3559	0.5013	0.9643	0.0701
	Interaction		0.6204	0.6204	0.2902	0.0942	0.2365	0.5791	0.519	0.0413

†The pH was corrected during winemaking therefore difference between treatments do not necessarily reflect true treatment effect.

‡Colorimeter method described in methods.

Table 2. Cie-Lab parameters of Shiraz wine as affected by heating and pruning time in 2013-14 and 2014-15. L* indicates lightness, a* red hue, b* yellow, C* chroma and h* hue angle. Values are mean ± standard error. P values are from ANOVA.

Season	Temperature	Pruning Time	L*	a*	b*	C*	h*
2013-14	Control	WP†	54.6 ± 4.39	41.2 ± 3.69	5.9 ± 0.98	41.6 ± 3.79	7.0 ± 0.5
		BB	52.2 ± 1.27	44.5 ± 1.23	4.3 ± 0.12	44.7 ± 1.23	10 ± 0.4
		2-3L	56.4 ± 1.98	39.8 ± 0.10	2.6 ± 1.00	39.9 ± 0.16	28 ± 16.9
	Heated	WP	63.5 ± 0.74	35.9 ± 0.73	2.4 ± 0.48	36.0 ± 0.76	16 ± 3.7
		BB	63.3 ± 1.01	36.5 ± 0.66	4.7 ± 2.75	37.0 ± 1.08	16 ± 8.3
		2-3L	63.7 ± 1.50	35.9 ± 1.42	2.2 ± 0.45	36.0 ± 1.40	18 ± 4.4
	P-Value	Temperature	<0.0001	0.0003	0.3134	0.0004	0.8274
		Pruning Time	0.4248	0.1562	0.2836	0.1406	0.3979
		Interaction	0.5458	0.3073	0.3741	0.4048	0.5118
2014-15	Control	WP	57.9 ± 1.14	34.2 ± 1.32 b‡	3.0 ± 0.59	34.3 ± 1.36 b	12 ± 2.5 b
		BB	57.8 ± 2.56	39.6 ± 1.27 a	2.0 ± 0.45	39.7 ± 1.29 a	22 ± 4.5 b
		2-3L	59.4 ± 2.99	39.3 ± 1.37 a	1.4 ± 0.63	39.4 ± 1.38 a	38 ± 11.5 a
	Heated	WP	64.6 ± 1.88	32.6 ± 1.85 y	5.0 ± 1.20	33.0 ± 1.98 y	7 ± 1.9 y
		BB	63.9 ± 3.37	34.3 ± 2.66 y	4.1 ± 0.56	34.6 ± 2.70 x	9 ± 0.7 y
		2-3L	58.4 ± 2.03	39.4 ± 1.07 x	3.6 ± 0.36	39.5 ± 1.07 x	11 ± 1.2 x
	P-Value	Temperature	0.073	0.122	0.003	0.164	0.042
		Pruning Time	0.599	0.013	0.134	0.018	0.048
		Interaction	0.25	0.302	0.994	0.323	0.159

†WP, winter control; BB, late pruning at budbreak; 2-3 L, late pruning at 2 to 3 L leaves.

‡When the heating × pruning time interaction was not significant, different letters for control (a, b, c) and heated (x, y) pruning treatment means indicate that differences were significant ($p < 0.05$, Tukey's honest significant difference [HSD] post-hoc test).

Table 3. Color spectrum and % copigmentaion as affected by heating, pruning time and its interaction, in 2014 and 2015.

Tempera-ture/season	Pruning Time	Chemical age 1	Chemical age 2	Total anthocyanin (mg/L)	Color density (au)	Total phenolic (au)	Pigmented tannin (au)	Hue (unitless)	Tannin (g ECAT/L)	Copigmen-tation (%)
2013-14										
Control	WP[†]	0.38 ± 0.010	0.19 ± 0.007	271 ± 14.6 a‡	16.2 ± 1.35	37.3 ± 3.32 a	3.7 ± 0.41	0.70 ± 0.002	0.5 ± 0.05 c	14.7 ± 0.83
	BB	0.36 ± 0.014	0.19 ± 0.021	270 ± 26.5 a	17.1 ± 0.68	36.9 ± 1.94 a	3.7 ± 0.26	0.67 ± 0.007	1.3 ± 0.02 a	17.0 ± 1.75
	2-3L	0.32 ± 0.011	0.18 ± 0.021	231 ± 12.9 ab	15.9 ± 1.49	28.4 ± 1.43 b	3.1 ± 0.35	0.67 ± 0.012	0.7 ± 0.07 b	19.7 ± 2.44
Heated	WP	0.31 ± 0.009	0.13 ± 0.006	261 ± 5.7 a	11.9 ± 0.59	31.2 ± 0.06 x[ʃ]	2.2 ± 0.10	0.68 ± 0.004	0.6 ± 0.04 b	21.9 ± 0.42
	BB	0.41 ± 0.05	0.28 ± 0.09	190 ± 2.4 c	13.0 ± 0.76	25.9 ± 1.43 x	3.2 ± 0.55	0.70 ± 0.014	0.8 ± 0.08 a	19.9 ± 4.32
	2-3L	0.34 ± 0.021	0.17 ± 0.007	208 ± 4.9 b	11.8 ± 0.92	25.3 ± 0.57 y	2.4 ± 0.12	0.68 ± 0.011	0.7 ± 0.07 ab	18.2 ± 0.34
P-Value	Temperature	0.9721	0.8596	0.0037	0.0003	0.0002	0.0084	0.154	0.061	0.1693
	Pruning Time	0.1253	0.2067	0.0087	0.4155	0.0019	0.1201	0.8429	<0.0001	0.9548
	Interaction	0.1154	0.244	0.0414	0.9926	0.0636	0.3883	0.3339	0.0014	0.2363
2014-15										
Control	WP	0.21 ± 0.003	0.12 ± 0.004	446 ± 10.2 b	24.3 ± 0.24 a	55.8 ± 1.69	3.4 ± 0.07	0.50 ± 0.006	1.3 ± 0.09	37.0 ± 0.17
	BB	0.19 ± 0.013	0.11 ± 0.009	518 ± 7.8 a	26.3 ± 1.18 a	56.5 ± 3.33	3.4 ± 0.35	0.46 ± 0.009	1.3 ± 0.16	39.9 ± 0.04
	2-3L	0.21 ± 0.029	0.12 ± 0.017	470 ± 32.1 ab	24.2 ± 1.93 a	53.5 ± 4.54	3.5 ± 0.53	0.47 ± 0.019	1.1 ± 0.19	34.9 ± 2.38
Heated	WP	0.26 ± 0.025	0.14 ± 0.014	395 ± 18.8 b	20.9 ± 0.22 b	52.3 ± 1.30	3.7 ± 0.29	0.49 ± 0.016	1.0 ± 0.07	30.4 ± 1.50
	BB	0.24 ± 0.022	0.13 ± 0.014	413 ± 16.5 b	20.7 ± 1.39 b	53.0 ± 5.01	3.5 ± 0.60	0.45 ± 0.022	1.1 ± 0.22	34.2 ± 1.70
	2-3L	0.23 ± 0.014	0.13 ± 0.007	489 ± 13.3 a	25.4 ± 0.50 a	57.7 ± 3.28	3.9 ± 0.19	0.47 ± 0.013	1.4 ± 0.16	33.1 ± 1.42
P-Value	Temperature	0.0198	0.0982	0.0098	0.0132	0.7444	0.401	0.8942	0.5912	0.0020
	Pruning Time	0.4825	0.5721	0.0181	0.1789	0.9109	0.8	0.0508	0.8976	0.0750
	Interaction	0.5684	0.7291	0.0168	0.027	0.4655	0.923	0.9070	0.3097	0.2570

[†]WP, winter control; BB, late pruning at budbreak; 2-3 L, late pruning at 2 to 3 L leaves.

[‡]When the heating × pruning time interaction was significant, different letters (a, b, c) indicate significant differences among means ($p < 0.05$, Tukey's HSD multiple range test).

[ʃ]When the heating × pruning time interaction was not significant, different letters for control (a, b, c) and heated (x, y) indicate a significant difference between treatment pruning means ($p < 0.05$, Tukey's honest significant difference [HSD] post-hoc test).

Table 4. Wine sensory traits affected by heating (H), pruning time (P) and its interaction in 2013-14. Values are the mean scores ± standard error on a 0-100 scale; difference between treatment is indicated by * (P<0.05), ** (P<0.01) and *** (P<0.001).

		Control			Heated			Significance		
Sense	Trait	WP†	BB	2-3L	WP	BB	2-3L	H	P	TxP
Color	Hue	89.0 ± 1.79 b	93.3 ± 0.96 a	87.0 ± 1.51 b	72.0 ± 1.72 y∫	75.0 ± 1.83 x	71.2 ± 1.95 y	***	**	ns
	Opacity	93.0 ± 1.34 a‡	93.0 ± 1.06 a	84.9 ± 1.64 b	60.3 ± 1.93 d	68.2 ± 1.97 c	63.5 ± 1.95 d	***	***	***
Aroma	Berry Fruit	57.5 ± 2.52 a	52.3 ± 2.25 b	53.5 ± 2.15 b	58.0 ± 2.00x	54.1 ± 2.10 y	54.1 ± 2.11 y	ns	*	ns
	Dried Fruit	44.8± 2.64	43.9 ± 3.83	45.9 ± 2.21	38.1 ± 2.01	41.8 ± 2.13	40.4 ± 2.19	**	ns	ns
	Black Olive	33.4 ± 2.20	31.9 ± 2.12	32.1 ± 2.07	27.7 ± 1.85	31.0 ± 1.90	28.4 ± 1.89	**	ns	ns
	Savoury	19.2 ± 2.57 b	20.9 ± 2.20 a	18.1 ± 2.03 b	16.4 ± 1.77 y	18.5 ± 1.92 x	15.7 ± 1.76 y	***	**	ns
	Floral	11.2 ± 1.47	11.8 ± 1.20	12.5 ± 1.30	15.7 ± 1.44	14.8 ± 1.47	13.1 ± 1.29	**	ns	ns
Taste	Bitter	28.2 ± 3.14 a	25.3 ± 2.27 b	25.9 ± 2.19 b	25.4 ± 2.19 x	23.4 ± 2.14 y	25.8 ± 2.41 x	*	*	ns
Palate	Dried Fruit	43.9 ± 2.78	41.0 ± 2.09	40.3 ± 2.04	38.0 ± 2.11	38.0 ± 2.14	36.5 ± 2.14	***	ns	ns
	Black Olives	28.5 ± 3.32	29.2 ± 3.53	25.5 ± 3.07	25.0 ± 3.05	26.7 ± 3.00	25.8 ± 3.25	*	ns	ns
	Savoury	19.0 ± 2.53	18.5 ± 1.97	16.5 ± 1.77	16.3 ± 1.75	17.5 ± 1.80	16.8 ± 1.79	*	ns	ns
	Veg Green	11.3 ± 1.73	12.8 ± 1.43	10.7 ± 1.27	12.1 ± 1.40	11.4 ± 1.40	11.8 ± 1.37	ns	ns	*
	Chocolate	11.5 ± 1.55	10.7 ± 1.26	11.0 ± 1.29	10.4 ± 1.21	10.4 ± 1.24	9.4 ± 1.14	*	ns	ns
Mouthfeel	Body	64.7 ± 2.53	66.4 ± 2.13	61.8 ± 2.20	57.6 ± 2.18	60.9 ± 2.24	60.5 ± 2.14	***	ns	*
	Tannin Quantity	56.3 ± 2.29	54.8 ± 1.90	51.5 ± 1.92	48.2 ± 1.93	48.9 ± 2.02	47.3 ± 1.86	***	ns	ns
	Tannin Quality	52.0 ± 2.34	51.7 ± 2.06	47.4 ± 1.97	43.9 ± 1.92	44.8 ± 2.01	45.1 ± 2.29	***	ns	ns
After taste	Non Fruit	76.6 ± 2.16	75.7 ± 1.76	74.7 ± 1.82	72.3 ± 1.92	70.3 ± 1.95	72.3 ± 1.87	**	ns	ns

†WP, winter control; BB, late pruning at budbreak; 2-3 L, late pruning at 2 to 3 L leaves.

‡When the heating × pruning time interaction was significant, different letters (a, b, c) indicate significant differences among means ($p < 0.05$, Tukey's HSD multiple range test).

∫When the heating × pruning time interaction was not significant, different letters for control (a, b, c) and heated (x, y) indicate a significant difference between treatment pruning means ($p < 0.05$, Tukey's honest significant difference [HSD] post-hoc test).

Table 5. Wine sensory traits affected by heating (H), pruning time (P) and its interaction (TxP) in 2014-15. Values are the mean scores ± standard error on a 0-100 scale; difference between treatment is indicated by *(P<0.05), **(P<0.01) and ***P<0.001).

Sense	Trait	Control			Heated			Significance		
		WP†	BB	2-3L	WP	BB	2-3L	H	P	TxP
Color	Opacity	64.9 ± 1.47 b‡	70.4 ± 1.21 a	69.1 ± 1.37 a	62.2 ± 1.85 b	58.8 ± 1.87 b	67.9 ± 1.39 a	***	***	***
Aroma	Red fruit	47.3 ± 1.91 b	47.8 ± 1.88 ab	51.1 ± 2.19 a	46.5 ± 2.26 y∫	46.9 ± 2.07 y	50.4 ± 1.90 x	ns	**	ns
	Savoury	25.9 ± 1.93 a	24.5 ± 1.67 a	21.9 ± 1.46 b	26.4 ± 1.90 x	25.4 ± 2.04 x	23.2 ± 1.71 y	ns	**	ns
	Floral	27.7 ± 2.11 b	29.6 ± 2.06 b	32.0 ± 2.42 a	28.6 ± 2.12 y	28.1 ± 2.15 y	30.3 ± 2.13 x	ns	*	ns
	Dried fruit	46.7 ± 2.07	45.1 ± 1.91	45.9 ± 2.20	48.0 ± 2.26	50.8 ± 1.87	46.2 ± 2.08	*	ns	ns
Taste	Acidity	55.6 ± 1.31 a	50.3 ± 1.12 b	48.5 ± 1.05 b	53.9 ± 1.60 x	55.1 ± 1.21 x	52.3 ± 1.87 y	ns	*	ns
Palate	Red fruit	46.5 ± 2.13 b	51.1 ± 1.79 a	50.8 ± 1.86 a	48.9 ± 2.08 ab	46.8 ± 1.77 b	50.9 ± 1.86 a	ns	**	**
	Pepper	31.4 ± 0.93	33.4 ± 1.29	32.8 ± 1.27	27.3 ± 1.40	29.6 ± 1.12	29.5 ± 1.10	***	ns	ns
	Savoury	20.9 ± 0.94 c	27.2 ± 1.41 b	31.4 ± 1.47 a	20.5 ± 0.80 c	21.2 ± 0.88 c	21.6 ± 1.03 c	***	***	***
Aftertaste	Aftertaste	55.5 ± 1.77	55.5 ± 1.67	52.2 ± 1.50	50.8 ± 1.06	51.0 ± 1.52	51.9 ± 1.48	*	ns	ns

†WP, winter control; BB, late pruning at budbreak; 2-3 L, late pruning at 2 to 3 L leaves.

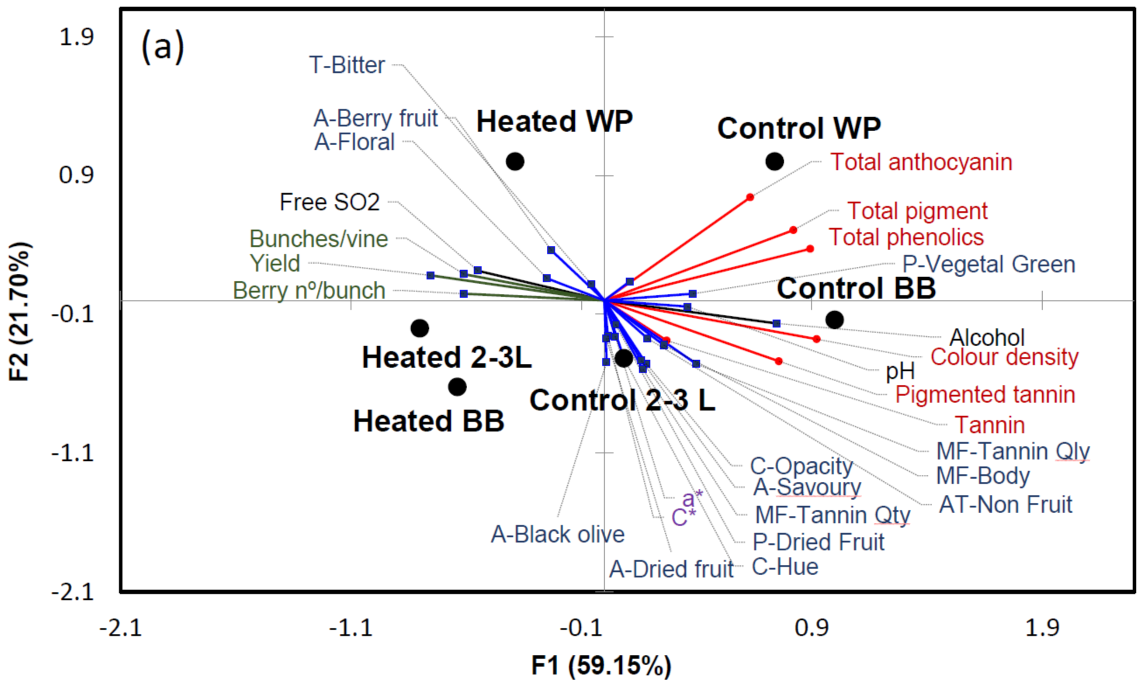
‡When the heating × pruning time interaction was significant, different letters (a, b, c) indicate significant differences among means (p < 0.05, Tukey's HSD multiple range test).

∫When the heating × pruning time interaction was not significant, different letters for control (a, b, c) and heated (x, y, z) indicate a significant difference between treatment pruning means (p < 0.05, Tukey's honest significant difference [HSD] post-hoc test).

Captions to Figures

Figure 1. Principal component analysis of the wine composition (red font, active variable), yield components (green font, supplementary variable), Cie-Lab parameters (purple font, supplementary variable) and sensory attributes (blue font, supplementary variable) that responded to pruning treatments and heating during the (a) 2013-14 and (b) 2014-15 season. A, aroma; AT, aftertaste; BB, budbreak; C, colour; MF, mouthfeel; P, palate; T, taste; WP, winter; and 2–3 L, 2 to 3 leaves.

Figure 2. Correlations between daily mean temperature during a 2-week period after veraison and wine (a) anthocyanin, (b) colour density, and (c) total polyphenols. Sources of variation are season, pruning time, and temperature treatments. Fitted lines are least square regressions ($r^2>0.89$; $P<0.001$). Circles (2–3 L) and down triangles (budburst) represent late pruning treatments, and squares winter pruning in 2013-2014 (open symbols) and 2014-15 (close symbols). Blue symbols are ambient temperature and red symbols are heated.



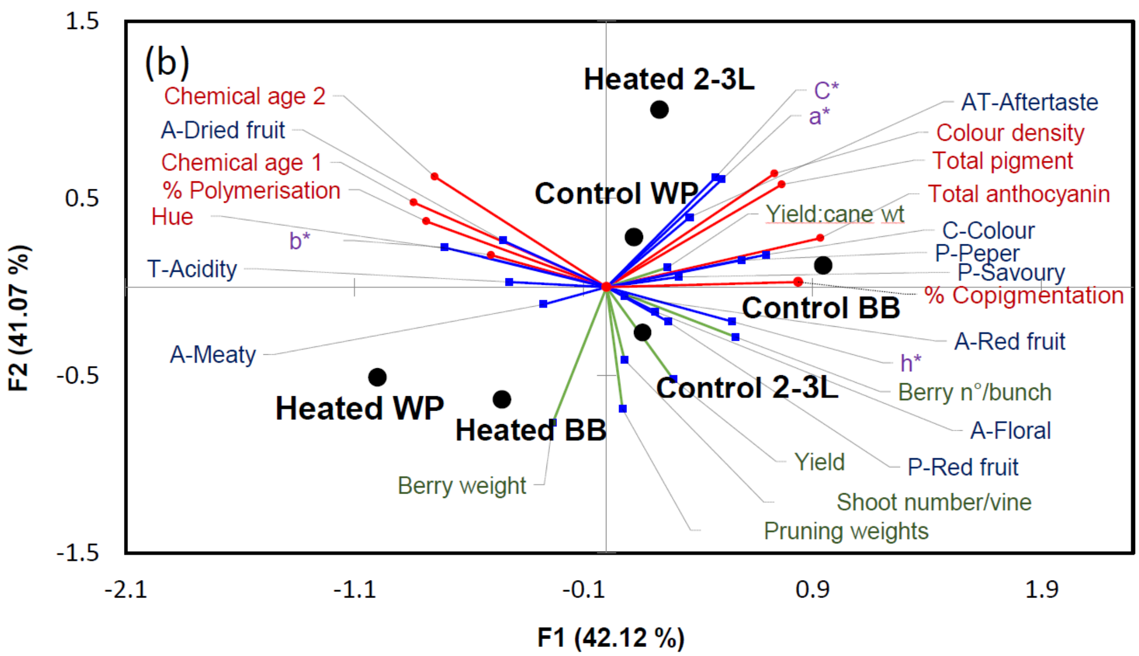


Figure 1a and 1b

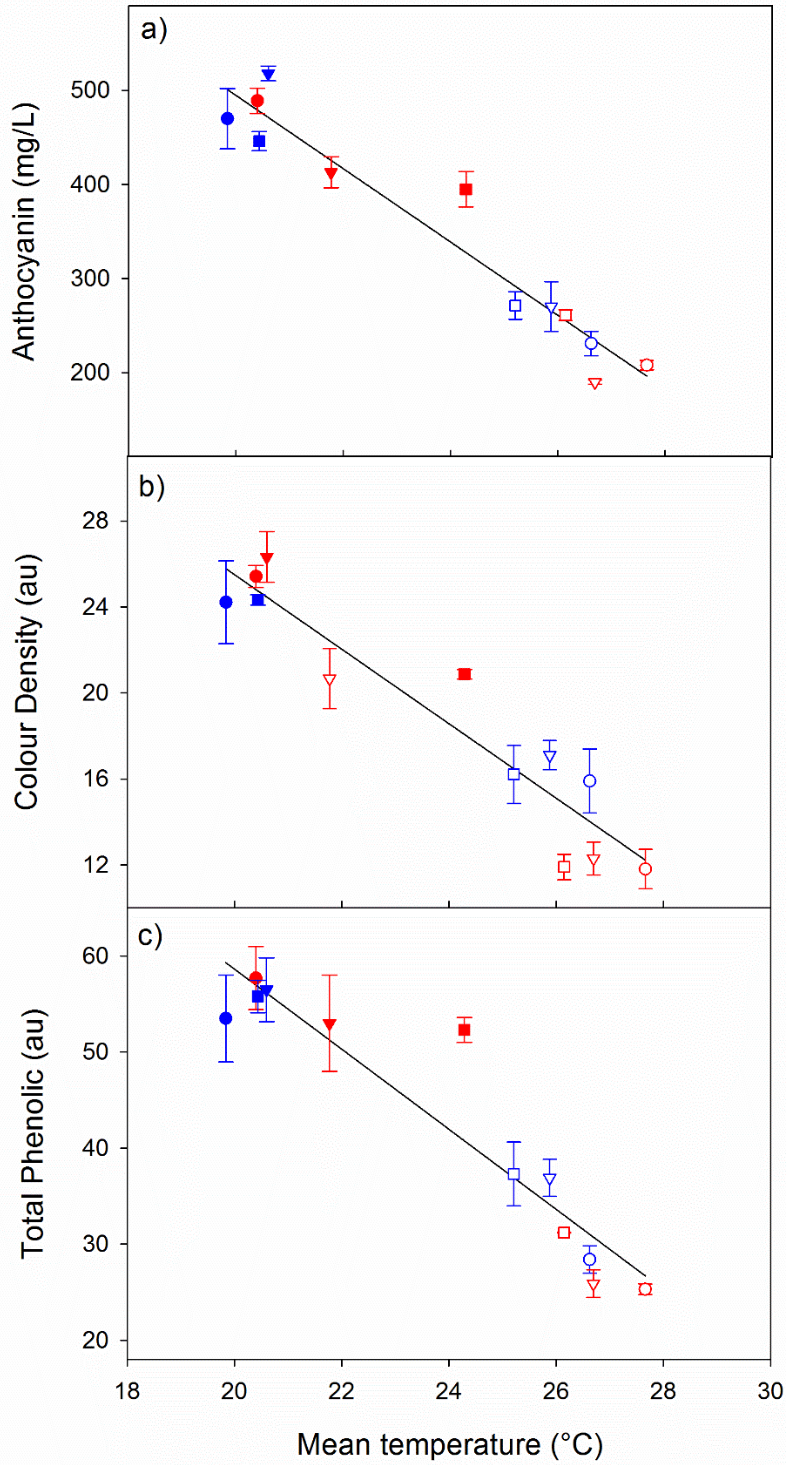

a)
b)
c)
Anthocyanin (mg/L)
Colour Density (au)
Total Phenolic (au)
500
400
300
200
28
24
20
16
12
60
50
40
30
20
18
20
22
24
26
28
30
Mean temperature (°C)

Figure 2

Supplementary material

Table S1. Reference Standards Recipes

Attribute	**Definition**
Red fruits	One each of frozen: 1 raspberry, 2 red currant, ½ strawberry, mashed
Dark fruit	1 blackberry, 2 blueberries, ¼ plum, 4 black currants, all frozen and mashed
Dried fruits	6 raisins + 1 prune
Ripe fruits	One teaspoon each of: plum jam, blackberry jam, raspberry jam.
Savoury	2 pieces each of: salami, pepperoni, bacon
Floral	One drop of rosewater
Vegetal Green	1cm piece of tomato stem + 1cm green capsicum
Mint/menthol	A pinch dried mint + 3 drop from a “fresh wine solution” consisted of 1 drop peppermint dissolved in 30ml of wine.
Spice	Pinch of mixed spice
Aniseed/liquorice	5 Fennel seeds + 1cm piece liquorice
Pepper	Pinch of both black and white pepper (or mixed pepper)
Olives	One black olive and drop of olive brine
Chocolate	Chocolate
Earthy	Teaspoon of earth
Vanilla	Drop of vanilla
Mocha	Tsp mocha (use 1/10 of teaspoon of ground coffee and half a piece of dark chocolate).
Leather	Leather (no wine)
Confectionery	Strawberry and cream lolly

Table S2. Wine sensory traits affected by heating (H), pruning time (Pt) and its interaction. Values are the mean scores ± standard error in a 0-100 scale; P values are from ANOVA, and different letters indicate means difference according to Tukey HSD at $p < 0.05$.

Sense	Trait	Control			Heated			Significance		
		WP[a]	**BB**	**2-3L**	**WP**	**BB**	**2-3L**	**Heating**	**Pruning**	**H X P**
Colour	Hue	87.6 ± 2.39	93.3 ± 1.37	86.4 ± 2.53	69.2 ± 3.20	74.5 ± 2.94	69.7 ± 3.25	**<.0001**	**0.0023**	**0.8271**
	Opacity	94.0 ± 1.62	93.0 ± 1.80	83.9 ± 3.00	54.5 ± 4.08	65.1 ± 3.71	58.0 ± 6.25	<.0001	**<.0001**	0.0003
Aroma	Berry Fruit	53.1 ± 4.02	52.3 ± 3.82	53.5 ± 3.37	58.0 ± 3.62	54.1 ± 3.38	54.1 ± 0.25	0.1005	0.407	0.4829
	Dried Fruit	43.6 ± 3.98	43.9 ± 3.83	45.9 ± 4.02	38.1 ± 3.41	41.8 ± 3.53	40.4 ± 9.25	**0.0013**	0.2966	0.4756
	Ripe Fruit	45.2 ± 4.68	43.4 ± 4.19	42.9 ± 3.83	44.4 ± 3.74	44.4 ± 3.60	44.4 ± 6.25	0.7054	0.7972	0.7801
	Black Olive	33.4 ± 3.50	31.9 ± 3.57	32.1 ± 3.39	27.7 ± 3.33	31.0 ± 2.99	28.4 ± 5.25	0.0017	0.6383	0.1871
	Cola	12.6 ± 2.72	13.8 ± 2.73	14.4 ± 2.72	16.1 ± 3.11	13.5 ± 2.86	15.9 ± 7.25	0.0807	**0.3682**	0.2066
	Pepper	32.5 ± 3.35	34.5 ± 3.38	31.8 ± 3.28	31.0 ± 3.18	31.9 ± 3.01	31.8 ± 3.25	0.18	0.4216	0.5958
	Licorice	15.8 ± 2.14	16.6 ± 1.86	16.8 ± 1.97	16.3 ± 1.88	16.6 ± 1.86	15.6 ± 1.25	0.7899	0.9112	0.7922
	Savoury	20.8 ± 4.10	20.9 ± 4.14	18.1 ± 3.74	16.4 ± 3.38	18.5 ± 3.58	15.7 ± 1.25	<.0001	0.0064	0.4206
	Floral	11.2 ± 1.90	11.8 ± 1.88	12.5 ± 2.04	15.7 ± 2.42	14.8 ± 2.49	13.1 ± 9.25	0.004	0.8393	0.2456
	Veg Green	9.9 ± 2.39	10.5 ± 2.41	10.9 ± 2.53	10.9 ± 2.36	10.0 ± 2.38	10.5 ± 3.25	0.8515	0.7914	0.3958
	Minty	11.7 ± 2.54	11.9 ± 2.46	12.2 ± 2.52	11.4 ± 2.38	12.3 ± 2.47	11.0 ± 5.25	0.451	**0.6225**	0.386
	Chocolate	13.0 ± 2.42	12.4 ± 2.22	13.0 ± 2.34	12.8 ± 2.18	13.1 ± 2.40	11.5 ± 4.25	0.6874	0.7679	**0.4401**
	Earthy	13.1 ± 2.40	11.1 ± 2.05	10.6 ± 2.18	12.0 ± 2.16	12.1 ± 2.24	12.1 ± 4.25	0.4617	**0.2997**	**0.2312**
	Leather	12.9 ± 3.08	12.7 ± 2.80	13.0 ± 2.89	12.6 ± 2.71	12.9 ± 2.75	13.7 ± 0.25	0.8008	0.7659	0.8773
	Faulty	6.1 ± 2.89	3.2 ± 1.18	2.6 ± 0.70	1.5 ± 0.48	3.2 ± 1.03	2.3 ± 8.25	0.1464	0.605	0.1729
Taste	Acid	57.8 ± 3.28	55.8 ± 3.31	55.4 ± 3.06	54.2 ± 2.95	57.2 ± 2.85	54.0 ± 2.25	0.1742	0.2307	0.0861
	Sweet	20.6 ± 3.98	20.3 ± 4.10	21.1 ± 4.39	21.3 ± 4.58	19.3 ± 3.93	20.2 ± 9.25	0.588	0.4932	0.6547
	Bitter	28.2 ± 4.85	25.3 ± 4.36	25.9 ± 4.16	25.4 ± 4.24	23.4 ± 4.10	25.8 ± 8.25	**0.0292**	0.0265	0.3417
Palate	Berry Fruit	48.1 ± 4.11	49.9 ± 3.88	50.1 ± 3.88	51.9 ± 3.42	51.5 ± 3.48	50.9 ± 1.25	0.0965	0.8887	0.5879
	Dried Fruit	43.9 ± 4.25	41.0 ± 3.51	40.3 ± 3.51	38.0 ± 3.57	38.0 ± 3.45	36.5 ± 2.25	**0.0006**	**0.2322**	**0.6134**
	Ripe Fruit	40.7 ± 4.29	39.4 ± 4.25	39.4 ± 3.98	41.7 ± 3.91	40.4 ± 3.72	38.8 ± 2.25	0.6826	**0.3312**	0.7943
	Blk Olives	28.5 ± 3.32	29.2 ± 3.53	25.5 ± 3.07	25.0 ± 3.05	26.7 ± 3.00	25.8 ± 3.25	0.036	0.131	0.2211
	Spice	36.0 ± 4.11	36.8 ± 4.28	35.6 ± 4.15	35.9 ± 4.07	34.6 ± 3.68	35.4 ± 1.25	0.3631	0.9367	0.5899
	Savoury	19.0 ± 4.10	18.5 ± 3.80	16.5 ± 3.44	16.3 ± 3.41	17.5 ± 3.50	16.8 ± 1.25	0.0502	0.1342	0.1026

	Veg Green	11.0 ± 2.59	12.8 ± 2.47	10.7 ± 2.42	12.1 ± 2.43	11.4 ± 2.42	11.8 ± 5.25	0.4202	0.2001	0.0117
	Minty	9.8 ± 2.49	10.2 ± 2.51	9.3 ± 2.49	9.9 ± 2.52	9.2 ± 2.52	9.8 ± 4.25	0.5793	0.7502	0.1422
	Chocolate	11.5 ± 2.55	10.7 ± 2.29	11.0 ± 2.33	10.4 ± 2.23	10.4 ± 2.29	9.4 ± 5.25	0.0302	0.4184	0.561
Mouthfeel	Body	61.9 ± 3.97	61.4 ± 4.12	57.8 ± 4.14	51.7 ± 4.17	54.3 ± 4.31	56.6 ± 9.25	**<.0001**	0.8161	0.0283
	Tan Qty	57.5 ± 3.31	54.8 ± 3.32	51.5 ± 3.32	48.2 ± 3.07	48.9 ± 3.46	47.3 ± 3.25	<.0001	0.0776	0.2483
	Tan Qly	52.6 ± 3.37	51.7 ± 3.64	47.4 ± 3.54	43.9 ± 3.37	44.8 ± 3.56	45.1 ± 3.25	<.0001	0.2587	0.0568
After taste	Fruit	45.9 ± 4.96	45.6 ± 4.55	44.6 ± 4.55	44.3 ± 4.29	46.0 ± 3.97	43.8 ± 9.25	**0.6098**	**0.5926**	**0.8151**
	Alcohol	70.0 ± 4.18	70.9 ± 3.68	69.9 ± 4.29	67.9 ± 4.30	68.6 ± 4.31	69.0 ± 1.25	0.0962	**0.8393**	0.8549
	Non Fruit	77.0 ± 2.52	75.7 ± 2.39	74.7 ± 2.58	72.5 ± 3.05	70.3 ± 3.12	72.3 ± 5.25	0.0013	0.5091	0.5948

[a]WP, winter pruning, BB, budburst, 2-3L, 2 to 3 leaves.

Table S3. Wine sensory traits affected by heating (H), pruning time (Pt) and its interaction. Values are the mean scores ± standard error in a 0-100 scale; P values are from ANOVA, and different letters indicate means difference according to Tukey HSD at $p < 0.05$.

Sense	Trait	……………..	Control	………………	………………	Heated	………..…	Significance		
		WP†	BB	2-3L	WP	BB	2-3L	Heating	Pruning	H X P
Colour	Opacity	64.9 ± 1.47	70.4 ± 1.21	69.1 ± 1.37	62.2 ± 1.85	58.8 ± 1.87	67.9 ± 1.39	**<.0001**	**0.0001**	**<.0001**
Aroma	Red fruit	47.3 ± 1.91	47.8 ± 1.88	51.1 ± 2.19	46.5 ± 2.26	46.9 ± 2.07	50.4 ± 1.90	0.4613	**0.0074**	0.9983
	Dark fruit	54.7 ± 1.91	55.8 ± 1.90	56.3 ± 2.13	53.6 ± 1.57	55.0 ± 1.79	55.8 ± 1.59	0.4087	0.27	0.9662
	Dried fruit	46.7 ± 2.07	45.1 ± 1.91	45.9 ± 2.20	48.0 ± 2.26	50.8 ± 1.87	46.2 ± 2.08	0.0436	0.4471	0.1635
	Cooked fruit	42.9 ± 2.48	44.0 ± 2.47	44.8 ± 2.47	43.9 ± 2.62	43.7 ± 2.56	43.1 ± 2.67	0.7609	0.9052	0.5989
	Black olive	36.7 ± 1.80	37.6 ± 1.92	35.5 ± 1.73	38.6 ± 1.98	36.8 ± 2.19	35.5 ± 1.96	0.7427	0.2253	0.5775
	Floral	27.7 ± 2.11	29.6 ± 2.06	32.0 ± 2.42	28.6 ± 2.12	28.1 ± 2.15	30.3 ± 2.13	0.437	**0.0337**	0.4855
	Vegetal Green	23.2 ± 1.89	21.7 ± 1.73	22.3 ± 1.85	23.0 ± 1.90	22.4 ± 2.03	21.9 ± 1.72	0.9252	0.4123	0.8006
	Pepper	29.1 ± 1.65	30.5 ± 1.71	28.9 ± 1.43	27.3 ± 1.35	27.8 ± 1.39	29.9 ± 1.69	0.1429	0.4755	0.1392
	Minty	31.7 ± 2.39	30.4 ± 2.71	34.0 ± 3.32	31.5 ± 2.33	29.8 ± 2.70	33.6 ± 2.96	0.7661	0.0645	0.9939
	Spice	36.1 ± 1.93	35.7 ± 1.69	35.8 ± 1.55	33.2 ± 1.97	36.6 ± 1.71	34.4 ± 1.85	0.2035	0.3796	0.2096
	Confectionery	22.4 ± 1.68	23.0 ± 1.45	24.9 ± 1.74	24.5 ± 1.73	23.2 ± 1.54	24.9 ± 1.62	0.3661	0.1518	0.4961
	Meaty	25.9 ± 1.93	24.5 ± 1.67	21.9 ± 1.46	26.4 ± 1.90	25.4 ± 2.04	23.2 ± 1.71	0.2564	**0.002**	0.9161
	Liquorice	29.1 ± 1.66	26.9 ± 1.47	25.7 ± 1.27	26.6 ± 1.49	28.5 ± 1.84	27.7 ± 1.63	0.6999	0.5501	0.0943
Palate	Red fruit	46.5 ± 2.13	51.1 ± 1.79	50.8 ± 1.86	48.9 ± 2.08	46.8 ± 1.77	50.9 ± 1.86	0.5042	0.0093	**0.0051**
	Dark fruit	53.4 ± 2.15	53.7 ± 1.58	54.1 ± 2.00	52.1 ± 1.73	53.9 ± 1.50	53.1 ± 1.90	0.3896	0.5326	0.6915
	Cooked fruit	47.1 ± 2.42	47.6 ± 2.54	49.1 ± 2.69	45.6 ± 2.68	45.9 ± 2.25	47.6 ± 2.88	0.1301	0.2516	0.9952
	Black olive	39.8 ± 1.85	38.8 ± 1.39	41.6 ± 1.27	38.9 ± 1.63	42.6 ± 1.52	39.5 ± 1.55	0.8573	0.6643	0.1716
	Vegetal	24.8 ± 1.33	23.6 ± 1.24	23.6 ± 1.47	25.2 ± 0.92	25.4 ± 1.17	25.6 ± 1.10	0.2075	0.9185	0.79
	Pepper	31.4 ± 0.93	33.4 ± 1.29	32.8 ± 1.27	27.3 ± 1.40	29.6 ± 1.12	29.5 ± 1.10	**0.0005**	0.2143	0.9472
	Spice	38.4 ± 1.23	38.7 ± 1.58	37.8 ± 2.03	33.7 ± 1.55	35.9 ± 1.29	37.6 ± 1.75	0.0608	0.5874	0.3948
	Meaty	20.9 ± 0.94	27.2 ± 1.41	31.4 ± 1.47	20.5 ± 0.80	21.2 ± 0.88	21.6 ± 1.03	**<.0001**	**<.0001**	**0.0004**
Taste	Acidity	55.6 ± 1.31	50.3 ± 1.12	48.5 ± 1.05	53.9 ± 1.60	55.1 ± 1.21	52.3 ± 1.87	0.0572	**0.0142**	0.0608
	Bitterness	38.8 ± 1.56	35.5 ± 1.76	35.3 ± 1.55	40.3 ± 1.89	38.6 ± 1.58	38.9 ± 2.26	0.0854	0.3204	0.8455
	Salty	29.3 ± 1.64	30.5 ± 1.59	31.5 ± 1.62	31.0 ± 1.80	30.4 ± 1.49	31.0 ± 1.65	0.7802	0.8178	0.7908
	Sweetness	47.7 ± 1.82	45.5 ± 1.73	42.4 ± 1.93	45.5 ± 2.36	44.5 ± 1.48	45.6 ± 1.79	0.9798	0.4035	0.3452
Mouthfeel	Body	43.8 ± 1.76	44.9 ± 1.61	45.2 ± 1.26	44.7 ± 1.77	46.1 ± 1.70	40.4 ± 1.55	0.5171	0.2761	0.1442
	Alcohol heat	49.8 ± 1.57	50.9 ± 1.16	49.1 ± 1.39	50.3 ± 1.57	49.4 ± 1.58	50.3 ± 1.62	0.9469	0.9575	0.6327
	Astringency	51.5 ± 2.12	54.4 ± 2.00	47.3 ± 1.74	49.2 ± 2.19	47.8 ± 2.29	50.2 ± 2.67	0.2999	0.5889	0.1294
After taste	Aftertaste	55.5 ± 1.77	55.5 ± 1.67	52.2 ± 1.50	50.8 ± 1.06	51.0 ± 1.52	51.9 ± 1.48	**0.0186**	0.7283	0.3198

†WP, winter pruning, BB, budburst, 2-3L, 2 to 3 leaves.

Table S4. Correlation matrix (Pearson (n)) between chemical (black) and sensory attributes (blue), and vine performance (green) in 2014-15.

Variables	Total anthocyanins (mg/L)	Colour density (au)	Total pheno-lics (au)	Total Pig-ment (au)	Pigmented tannin (au)	Tannin (g ECAT/L)	Alcohol v/v %	pH	YIELD /R	BUNC H/R	berry nº	Shoot wt. (Kg/plant)	C-Hue	C-trans	AT-Non Fruit
Total anthocyanins (mg/L)	**1**	0.388	**0.811**	**0.894**	0.127	0.035	0.379	0.183	-0.328	-0.277	-0.416	-0.415	-0.191	-0.147	-0.029
Colour density (au)	0.388	**1**	**0.677**	**0.621**	**0.764**	0.248	**0.782**	**0.513**	**-0.762**	**-0.662**	**-0.615**	**-0.720**	0.213	0.328	0.288
Total phenolics (au)	**0.811**	**0.677**	**1**	**0.888**	**0.565**	0.116	**0.563**	0.219	**-0.639**	-0.456	-0.427	**-0.615**	-0.149	-0.098	-0.005
Total Pigment (au)	**0.894**	**0.621**	**0.888**	**1**	0.378	0.154	**0.518**	0.269	**-0.479**	-0.429	**-0.559**	**-0.646**	-0.076	-0.010	0.019
Pigmented tannin (au)	0.127	**0.764**	**0.565**	0.378	**1**	0.301	**0.570**	0.085	**-0.796**	**-0.618**	-0.432	-0.440	0.165	0.305	0.185
Tannin (g ECAT/L)	0.035	0.248	0.116	0.154	0.301	**1**	-0.036	-0.460	-0.229	-0.384	-0.436	-0.253	**0.757**	**0.711**	0.409
Alcohol v/v %	0.379	**0.782**	**0.563**	**0.518**	**0.570**	-0.036	**1**	**0.593**	-0.359	-0.261	-0.420	**-0.508**	-0.041	0.075	0.037
pH	0.183	**0.513**	0.219	0.269	0.085	-0.460	**0.593**	**1**	-0.280	-0.201	-0.373	-0.435	-0.282	-0.206	-0.015
Yield/vine	-0.328	**-0.762**	**-0.639**	**-0.479**	**-0.796**	-0.229	-0.359	-0.280	**1**	**0.800**	**0.583**	**0.528**	-0.068	-0.169	-0.194
Bunch	-0.277	**-0.662**	-0.456	-0.429	**-0.618**	-0.384	-0.261	-0.201	**0.800**	**1**	**0.558**	0.220	-0.283	-0.381	-0.293
berry nº	-0.416	**-0.615**	-0.427	**-0.559**	-0.432	-0.436	-0.420	-0.373	**0.583**	**0.558**	**1**	**0.611**	-0.267	-0.317	-0.221
Shoot wt. (Kg/plant)	-0.415	**-0.720**	**-0.615**	**-0.646**	-0.440	-0.253	**-0.508**	-0.435	**0.528**	0.220	**0.611**	**1**	-0.102	-0.150	-0.154
C-Hue	-0.191	0.213	-0.149	-0.076	0.165	**0.757**	-0.041	-0.282	-0.068	-0.283	-0.267	-0.102	**1**	**0.954**	**0.762**
C-trans	-0.147	0.328	-0.098	-0.010	0.305	**0.711**	0.075	-0.206	-0.169	-0.381	-0.317	-0.150	**0.954**	**1**	**0.757**
A-berry fruit	0.190	-0.334	-0.118	-0.060	-0.427	-0.245	-0.123	-0.032	0.369	0.281	0.320	0.276	-0.378	-0.370	-0.184
A-Floral	-0.051	-0.315	-0.138	-0.195	-0.397	-0.369	-0.090	-0.038	0.404	**0.525**	0.437	0.142	**-0.578**	**-0.638**	**-0.526**
A-savoury	-0.143	0.294	-0.093	0.038	0.154	**0.803**	-0.011	-0.074	-0.159	-0.409	**-0.471**	-0.312	**0.769**	**0.761**	**0.486**
A-Black olive	-0.273	0.126	-0.215	-0.148	0.280	**0.625**	-0.005	-0.252	-0.112	-0.351	-0.231	0.033	**0.654**	**0.749**	0.253
A-dried fruit	-0.173	0.092	-0.104	-0.110	0.294	**0.550**	-0.196	-0.361	-0.279	**-0.484**	-0.096	0.188	**0.636**	**0.638**	0.349
T-bitter	0.141	-0.046	-0.095	0.002	-0.044	0.047	0.025	-0.044	0.074	0.029	0.080	0.103	0.331	0.415	**0.615**
MF-body	-0.042	0.418	0.046	0.075	0.279	0.382	0.104	0.119	-0.404	**-0.608**	-0.363	-0.087	**0.733**	**0.726**	**0.711**
MF-tannin Qty	-0.199	0.344	-0.105	-0.082	0.224	**0.540**	0.183	0.060	-0.171	-0.396	-0.261	-0.104	**0.771**	**0.780**	**0.751**
MF-tannin Qly	-0.032	**0.547**	0.130	0.121	0.413	**0.486**	0.381	0.189	-0.350	**-0.595**	-0.345	-0.182	**0.687**	**0.744**	**0.716**
P-Dried Fruit	-0.011	0.133	-0.138	-0.004	0.139	**0.480**	-0.100	-0.195	-0.181	-0.390	-0.288	0.026	**0.687**	**0.760**	**0.525**
P-Veg Grn	0.364	0.255	0.355	0.365	0.240	0.315	0.246	0.037	-0.282	-0.279	-0.411	-0.280	-0.140	-0.083	0.044
AT-Non Fruit	-0.029	0.288	-0.005	0.019	0.185	0.409	0.037	-0.015	-0.194	-0.293	-0.221	-0.154	**0.762**	**0.757**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table S5. Correlation matrix (Pearson (n)) between chemical (black & purple) and sensory attributes (blue), and vine performance (green) in 2014-15.

Variables	Chemical Age 1	Chemical Age 2	Free Anthocyanin	Hue	Colour Density	Total Pigment	% Pigmented Tannin	Tannin	% Polymerisation	Total phenolics FC	Berry N°	Berry wt.	Fruit: Shoot	Pruning wt.	Shoot n°	Yield	a*	b*	C*	hab	C-Colour	A-Floral	A-Red fruit	A-Dried fruit	A-Meaty	P-Red fruit	P-Pepper	P-Meaty	T-Acidity	AT-aftertaste	L*	%Copigmentation
Chemical Age 1	**1.0**	**1.0**	**-0.6**	0.4	-0.3	-0.3	**1.0**	0.3	**0.8**	-0.1	**-0.5**	-0.1	-0.1	-0.3	-0.2	-0.4	-0.1	**0.8**	0.0	**-0.6**	**-0.5**	-0.2	0.0	**0.5**	0.2	-0.3	-0.5	-0.3	0.3	-0.2	-0.1	**-0.7**
Chemical Age 2	**1.0**	**1.0**	**-0.5**	0.4	-0.2	-0.2	**1.0**	0.4	**0.8**	0.0	**-0.5**	-0.3	-0.2	-0.4	-0.2	**-0.5**	0.0	**0.7**	0.1	**-0.6**	-0.4	-0.2	0.0	**0.5**	0.1	-0.3	-0.4	-0.2	0.3	-0.1	-0.3	**-0.6**
Free Anthocyanin	**-0.6**	**-0.5**	**1.0**	-0.4	**0.9**	**0.9**	**-0.5**	0.4	**-0.6**	**0.6**	0.5	-0.4	0.3	-0.1	-0.1	0.2	**0.7**	**-0.6**	**0.7**	**0.5**	**0.8**	0.2	0.1	-0.4	-0.3	0.3	**0.6**	0.4	**-0.5**	0.4	**-0.5**	**0.7**
Hue	0.4	0.4	-0.4	**1.0**	-0.1	-0.4	0.4	0.1	0.5	0.0	**-0.8**	0.0	-0.2	-0.4	-0.3	**-0.5**	-0.3	0.2	-0.3	-0.4	-0.1	-0.3	-0.1	0.0	0.2	-0.1	-0.2	-0.1	0.3	0.0	-0.1	-0.4
Colour Density	-0.3	-0.2	**0.9**	-0.1	**1.0**	**1.0**	-0.2	**0.7**	-0.3	**0.8**	0.1	**-0.6**	0.2	-0.4	-0.2	-0.2	**0.8**	-0.4	**0.8**	0.3	**0.7**	0.1	0.1	-0.3	-0.3	0.2	**0.5**	0.3	-0.4	0.5	**-0.8**	**0.5**
Total Pigment	-0.3	-0.2	**0.9**	-0.4	**1.0**	**1.0**	-0.2	**0.7**	-0.4	**0.7**	0.3	**-0.6**	0.3	-0.3	-0.2	0.0	**0.9**	-0.4	**0.8**	0.3	**0.7**	0.2	0.1	-0.2	-0.3	0.2	**0.5**	0.3	-0.5	0.4	**-0.7**	**0.5**
% Pigmented Tannin	**1.0**	**1.0**	**-0.5**	0.4	-0.2	-0.2	**1.0**	0.4	**0.8**	0.0	**-0.5**	-0.2	-0.2	-0.4	-0.2	-0.5	0.0	**0.8**	0.1	**-0.6**	-0.4	-0.2	0.0	**0.5**	0.1	-0.3	-0.4	-0.2	0.3	-0.1	-0.3	**-0.6**
Tannin	0.3	0.4	0.4	0.1	**0.7**	**0.7**	0.4	**1.0**	0.2	**0.8**	-0.2	**-0.8**	0.2	**-0.7**	-0.4	**-0.5**	**0.6**	0.0	**0.6**	-0.1	0.3	-0.2	-0.2	0.2	-0.1	-0.3	0.2	0.1	0.1	**0.6**	**-0.9**	0.3
% Polymerisation	**0.8**	**0.8**	**-0.6**	0.5	-0.3	-0.4	**0.8**	0.2	**1.0**	-0.1	**-0.5**	-0.2	0.0	-0.4	**-0.5**	**-0.5**	-0.1	**0.5**	-0.1	-0.3	**-0.5**	-0.2	-0.1	0.4	0.1	-0.2	-0.4	-0.1	0.3	-0.1	-0.1	**-0.6**
Total phenolics FC	-0.1	0.0	**0.6**	0.0	**0.8**	**0.7**	0.0	**0.8**	-0.1	**1.0**	0.1	**-0.8**	0.2	**-0.6**	-0.3	-0.3	**0.5**	-0.2	**0.5**	0.1	0.3	-0.2	-0.1	0.0	-0.1	-0.2	**0.5**	0.1	0.0	**0.6**	**-0.9**	**0.6**
% Copigmentation	**-0.7**	**-0.6**	**0.7**	-0.4	**0.5**	**0.5**	**-0.6**	0.3	**-0.6**	**0.6**	**0.5**	-0.4	0.3	-0.1	-0.1	0.1	0.3	**-0.6**	0.3	**0.5**	0.4	0.0	-0.2	-0.2	0.1	0.0	**0.6**	0.3	-0.1	**0.6**	-0.3	**1.0**
Berry N°	**-0.5**	**-0.5**	0.5	**-0.8**	0.1	0.3	**-0.5**	-0.2	**-0.5**	0.1	**1.0**	0.1	0.3	**0.5**	0.2	**0.7**	0.4	-0.3	0.3	0.4	0.2	**0.5**	0.4	-0.1	-0.3	0.4	0.3	0.3	-0.3	0.2	0.1	**0.5**
berry wt	-0.1	-0.3	-0.4	0.0	**-0.6**	**-0.6**	-0.2	**-0.8**	-0.2	**-0.8**	0.1	**1.0**	-0.3	**0.8**	**0.7**	**0.6**	**-0.7**	0.3	**-0.6**	-0.2	-0.3	0.2	0.1	-0.2	0.2	0.3	**-0.6**	-0.5	0.1	-0.5	**0.8**	-0.4
Fruit:Shoot	-0.1	-0.2	0.3	-0.2	0.2	0.3	-0.2	0.2	0.0	0.2	0.3	-0.3	**1.0**	-0.2	-0.4	0.4	0.3	-0.2	0.3	0.2	0.0	-0.1	-0.2	0.1	0.2	0.1	0.2	0.2	0.0	0.0	-0.1	0.3
Pruning wts	-0.3	-0.4	-0.1	-0.4	-0.4	-0.3	-0.4	**-0.7**	-0.4	**-0.6**	**0.5**	**0.8**	-0.2	**1.0**	**0.8**	**0.7**	-0.3	0.1	-0.3	-0.1	-0.1	0.4	0.3	-0.2	-0.1	0.4	-0.3	-0.3	-0.1	-0.3	**0.7**	-0.1
Shoot n°	-0.2	-0.2	-0.1	-0.3	-0.2	-0.2	-0.2	-0.4	**-0.5**	-0.3	0.2	**0.7**	-0.4	**0.8**	**1.0**	0.4	-0.4	0.2	-0.4	-0.3	0.0	0.2	0.0	-0.2	0.1	0.0	-0.2	**-0.5**	0.0	-0.1	0.4	-0.1
Yield	-0.4	**-0.5**	0.2	**-0.5**	-0.2	0.0	-0.5	**-0.5**	**-0.5**	-0.3	**0.7**	**0.6**	0.4	**0.7**	0.4	**1.0**	0.0	0.0	0.0	0.0	-0.1	0.3	0.2	-0.1	0.1	0.4	-0.1	-0.1	-0.2	-0.3	0.5	0.1
a*	-0.1	0.0	**0.7**	-0.3	**0.8**	**0.9**	0.0	**0.6**	-0.1	**0.5**	0.4	**-0.7**	0.3	-0.3	-0.4	0.0	**1.0**	-0.2	**1.0**	0.3	**0.5**	0.4	0.4	-0.1	-0.4	0.4	**0.5**	**0.6**	**-0.6**	0.3	**-0.7**	0.3
b*	**0.8**	**0.7**	**-0.6**	0.2	-0.4	-0.4	**0.8**	0.0	**0.5**	-0.2	-0.3	0.3	-0.2	0.1	0.2	0.0	-0.2	**1.0**	-0.1	**-0.8**	**-0.7**	-0.2	0.1	**0.6**	0.4	-0.1	**-0.6**	**-0.6**	0.4	-0.3	0.1	**-0.6**
C*	0.0	0.1	**0.7**	-0.3	**0.8**	**0.8**	0.1	**0.6**	-0.1	**0.5**	0.3	**-0.6**	0.3	-0.3	-0.4	0.0	**1.0**	-0.1	**1.0**	0.3	**0.5**	0.4	0.4	0.0	-0.4	0.4	**0.5**	**0.5**	**-0.5**	0.3	**-0.7**	0.3
hab	**-0.6**	**-0.6**	**0.5**	-0.4	0.3	0.3	**-0.6**	-0.1	-0.3	0.1	0.4	-0.2	0.2	-0.1	-0.3	0.0	0.3	**-0.8**	0.3	**1.0**	**0.5**	0.3	0.1	-0.4	-0.4	0.3	0.4	**0.6**	**-0.5**	0.2	-0.1	**0.5**
L*	-0.1	-0.3	**-0.5**	-0.1	**-0.8**	**-0.7**	-0.3	**-0.9**	-0.1	**-0.9**	0.1	**0.8**	-0.1	**0.7**	0.4	0.5	**-0.7**	0.1	**-0.7**	-0.1	-0.4	0.0	-0.1	0.0	0.2	0.1	**-0.5**	-0.3	0.1	**-0.6**	**1.0**	-0.3
C-Colour	**-0.5**	-0.4	**0.8**	-0.1	**0.7**	**0.7**	-0.4	0.3	**-0.5**	0.3	0.2	-0.3	0.0	-0.1	0.0	-0.1	**0.5**	**-0.7**	**0.5**	**0.5**	**1.0**	0.4	0.2	**-0.7**	**-0.5**	0.3	**0.5**	**0.5**	-0.4	0.4	-0.4	0.4
A-Floral	-0.2	-0.2	0.2	-0.3	0.1	0.2	-0.2	-0.2	-0.2	-0.2	**0.5**	0.2	-0.1	0.4	0.2	0.3	0.4	-0.2	0.4	0.3	0.4	**1.0**	**0.7**	-0.4	**-0.6**	**0.7**	0.1	0.4	-0.4	0.0	0.0	0.0
A-Red fruit	0.0	0.0	0.1	-0.1	0.1	0.1	0.0	-0.2	-0.1	-0.1	0.4	0.1	-0.2	0.3	0.0	0.2	0.4	0.1	0.4	0.1	0.2	**0.7**	**1.0**	-0.3	**-0.6**	**0.7**	0.1	0.3	-0.4	-0.2	-0.1	-0.2
A-Dried fruit	**0.5**	**0.5**	-0.4	0.0	-0.3	-0.2	**0.5**	0.2	0.4	0.0	-0.1	-0.2	0.1	-0.2	-0.2	-0.1	-0.1	**0.6**	0.0	-0.4	**-0.7**	-0.4	-0.3	**1.0**	0.4	-0.4	-0.4	-0.4	0.3	0.0	0.0	-0.2
A-Meaty	0.2	0.1	-0.3	0.2	-0.3	-0.3	0.1	-0.1	0.1	-0.1	-0.3	0.2	0.2	-0.1	0.1	0.1	-0.4	0.4	-0.4	-0.4	**-0.5**	**-0.6**	**-0.6**	0.4	**1.0**	-0.4	-0.1	-0.4	0.4	-0.1	0.2	0.1
P-Red fruit	-0.3	-0.3	0.3	-0.1	0.2	0.2	-0.3	-0.3	-0.2	-0.2	0.4	0.3	0.1	0.4	0.0	0.4	0.4	-0.1	0.4	0.3	0.3	**0.7**	**0.7**	-0.4	-0.4	**1.0**	0.0	0.3	-0.4	-0.2	0.1	0.0
P-Peper	-0.5	-0.4	**0.6**	-0.2	**0.5**	**0.5**	-0.4	0.2	-0.4	**0.5**	0.3	**-0.6**	0.2	-0.3	-0.2	-0.1	**0.5**	**-0.6**	**0.5**	0.4	**0.5**	0.1	0.1	-0.4	-0.1	0.0	**1.0**	**0.7**	-0.4	0.4	**-0.5**	**0.6**
P-Meaty	-0.3	-0.2	0.4	-0.1	0.3	0.3	-0.2	0.1	-0.1	0.1	0.3	-0.5	0.2	-0.3	**-0.5**	-0.1	**0.6**	**-0.6**	**0.5**	**0.6**	**0.5**	0.4	0.3	-0.4	-0.4	0.3	**0.7**	**1.0**	**-0.5**	0.2	-0.3	0.3
T-Acidity	0.3	0.3	**-0.5**	0.3	-0.4	-0.5	0.3	0.1	0.3	0.0	-0.3	0.1	0.0	-0.1	0.0	-0.2	**-0.6**	0.4	**-0.5**	**-0.5**	-0.4	-0.4	-0.4	0.3	0.4	-0.4	-0.4	**-0.5**	**1.0**	0.2	0.1	-0.1
AT-aftertaste	-0.2	-0.1	0.4	0.0	0.5	0.4	-0.1	**0.6**	-0.1	**0.6**	0.2	-0.5	0.0	-0.3	-0.1	-0.3	0.3	-0.3	0.3	0.2	0.4	0.0	-0.2	0.0	-0.1	-0.2	0.4	0.2	0.2	**1.0**	**-0.6**	**0.6**

Values in bold are different from 0 with a significance level alpha=0.05

Chapter 6

Conclusions and Future Research

Conclusions and Future Research

Advancement of maturity due to warming have been thoroughly studied in the wine industry (Jones 2005, Petrie and Sadras 2008, Webb et al. 2012, Schultz 2016). Earlier harvest can compress vintages within and between varieties, affecting logistics to process fruit thus creating bottlenecks (Sadras et al. 2014). In this thesis, late pruning was proposed as a practical management tool to counteract effects of warming on advancing vine phenology and harvest time, disrupting fruit and wine composition and sensory attributes in Shiraz produced in the Barossa Valley. This thesis achieved five goals from objectives defined in Chapter 1. First, late pruning was a beneficial tool to delay maturity (TSS ~14°Be) with neutral effects on yield in 7 out of 10 cases in two sites (Chapter 2 and 4). The maturity delay in late pruning vines retarded the harvest up to 16 days. Fermentation process is likely to take five to seven days therefore delaying maturity about one week in the vineyard will allow to free up ferments space in the wineries. This way the wineries can schedule the harvest without compromising ferments availability in order to achieve a desired wine style. Yield response to late pruning was unchanged on average in the long term analysis from the pooled data across seasons from both sites; on a site by season basis three outcomes were observed: yield increased, yield was maintained, and yield decreased. Second, negligible carry-over effects were observed on vine phenology, yield components and fruit composition, after four consecutive years of delaying the pruning in the same vines (Chapter 2). This reinforces that delaying the winter pruning up to 2-3 leaves have developed is unlikely to reduce yields in the long term. Third, wine chemical composition and sensory attributes were improved in two vintages in a commercial vineyard (Chapter 3). Improving these wine attributes provides to growers the opportunity to upscale the fruit grade and to wineries to objectively increase their wine quality. Fourth, wine chemical and sensory attributes were negatively affected by heating. Interactive effects between late pruning and heating demonstrated that under heating conditions late pruning had the potential to restore loss of wine colour, and enhanced chemical and sensory traits that are beneficial in Shiraz wine styles produced in the Barossa Valley (Chapter 5). In addition this research has identified a phenological window, immediately after veraison (1 to 2 weeks) that was sensitive to an increase in mean temperature. In this window, higher mean temperature was negatively correlated to wine colour, colour density and total phenolics (Chapter 3 and 5).

Research aimed to delay maturity is paramount to counteract warming impact on advancing vine phenology, disrupting fruit and wine composition, and compressing the harvest. The potential impacts of late pruning should be tested across wine regions, management systems, cultivars and clones. Finally, reducing heat load in vineyards during and short period after veraison could be a beneficial outcome to preserve wine typicity in regions that often face extreme heat events.

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