A window into hotter and drier futures:

phenological shifts and adaptive practices

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Abstract

Temperature is one of the main environmental factors influencing vine physiology, berry composition and wine attributes. However, much of our understanding on the effects of temperature on vine physiology and berry composition has been gained from experiments under controlled conditions. Almost all of our knowledge on temperature effects on wine is indirect, this means, it is based on comparisons between thermally contrasting locations or vintages. Controlled conditions often generate experimental artefacts and indirect methods are bound to be inconclusive.

This project measured the effects of elevated temperature on vine physiology, berry composition and wine attributes using open-top chambers to manipulate temperature under realistic vineyard conditions. These experiments confirmed some but challenged many of the text-book expectations of temperature effects on grape composition and wine attributes. Direct assessment of temperature effects on vine performance, berry and wine attributes provides the industry with a window into hotter futures and suggests technological adaptations in the vineyard and winery.

Executive Summary

Background and Objectives

This project belongs to Program 3 Grapes for Purpose, with a dominant element of Vine Performance and some elements of Berry Composition. The key strategy is “Collaborative investments in vine physiology” and the measure of success is “A better industry understanding of temperature, water and vine relationships”. Against this background, three major R&D needs had been identified:

(i) Scaling-up to the 3-way interaction between heat, water and variety.
(ii) Variety-dependent tools for in-season prediction of ripening for logistic purposes, i.e. harvest, transport and winery scheduling, and irrigation planning.
(iii) Viticultural practices to reduce heat damage.

This project had four objectives[1]:

Enabling objectives:

1. To quantify the responses of Shiraz vines and wines to the combined effects of high temperature and water deficit under realistic vineyard conditions.
2. To quantify variety-dependent responses to high temperature and the interaction between temperature and fruit load per unit canopy size.

Practical objectives:

3. To develop user-friendly, robust models for variety-specific prediction of maturity. This will assist in harvest and winery scheduling, reduce costs, and help irrigation trusts in planning regional demand accounting for shifts in phenology and peak demand of irrigation water.
4. To develop management guidelines with emphasis on the interaction between heat stress, water supply and fruit load per unit canopy size.

Achievements

This report is organised in chapters, all of which have been exposed to the process of peer-review in recognised scientific journals, hence allowing for independent quality control of results and interpretations. Chapters 1 to 8 deal with the experimental work related to objectives 1 and 2. Chapters 9 to 11 deal with objective 3. The viticultural and oenological implications of the main findings of this project and management guidelines (objective 4) are discussed in detail in the individual chapters.

[1] The original project spanned from to 1 July 2009 to 30 June 2012. An extension was granted until 31 December 2013, which included additional objectives and activities; these are reported separately.

1. In viticulture, indirect methods that compare seasons or regions to infer temperature effects are often inconclusive because temperature is confounded with other climatic factors, management practices and soils. Unequivocal results require direct comparisons where temperature is manipulated in realistic vineyard conditions. In this project, we developed a new large-scale, automated open-top system to elevate temperature by ~ 2 ^{o}C above ambient, hence allowing for warming projections in Australia. A system that combines passive heating (clear days) and active heating by circulating hot water (nights and overcast days) was designed that largely achieves five attributes: (1) reproduces the daily and seasonal cycles of temperature and vapour pressure deficit, (2) has small effect on relative humidity, hence allowing for increased vapour pressure deficit, (3) minimises biologically important secondary effects, as the canopies are exposed to the natural light environment (4) has structural strength to withstand the weather, particularly wind, and (5) allows for number and size of replicates required for statistical resolution and viticultural needs, including sufficient fruit for meaningful wine production (Chapter 1).

2. Phenological shifts are one of the most conspicuous biological effects of global warming; observational data need benchmarking against experimental data. Grapevine phenology was monitored weekly between budswell and maturity in three factorial field experiments during two seasons. We present, for the first time, evidence of nonlinearity of thermal effects on grapevine phenology; this is explained by two complementary factors. Firstly, temperature-driven shifts of sensitive events (e.g. onset of sugar accumulation in berries) moved the timing of subsequent events into cooler conditions, hence dampening warming effects. Secondly, thermal effects on phenology seemed to be modulated by the interplay between resource-driven growth and temperature-driven development; an enhanced thermal effect on berry ripening associated with high source:sink ratio supports this proposition. Measured shifts in maturity were smaller than expected from reported time series analysis. During berry ripening towards maturity, the phenological trajectories of heated and control vines largely overlapped on thermal time scales; differences in phenology between treatments were therefore attributed to actual thermal regimes. We thus conclude that thermal sensitivities from time series (6 to 9 d $^{o}C^{-1}$) over-estimate thermal effects on grapevine maturity (Chapter 2).

3. Yield of untreated control vines ranged from 1.2 to 10.5 kg vine^{-1} across seasons and experiments. Warming had asymmetric effects on fruit yield and fruit/pruning weight ratios: yield response to elevated temperature varied from 46% reduction to 177% increase in relation to controls, and the response of fruit/pruning weight ratio varied from 56% reduction to 145% increase. Yield response to temperature was primarily explained by the response of bunch number (r^2=0.92, $P < 0.0001$). The effect of elevated temperature on fruit/pruning weight ratio was closely related to the response of fruit yield (r^2=0.79, $P < 0.0001$) and unrelated to the response of pruning weight ($P = 0.18$). Elevated temperature reduced concentration of starch in trunks but not in roots. We conclude that the asymmetric responses of yield and fruit/pruning weight ratios resulted from seasonal influences on warming effects, whereby the largest responses of these traits were mediated by the promotion of bunch formation. This highlights the need to assess warming effects in the light of

both the background temperature and the distribution of thermally contrasting season in particular locations. Lower source : sink ratio and reduced starch concentration in trunks are two important elements of the carbon balance of the vine with long-term physiological and viticultural implications (Chapter 3).

4. We tested the hypothesis that elevated temperature decouples anthocyanins and total soluble solids (TSS) in developing berries and explored the effects of fruit load and water supply on the putative decoupling. For Shiraz and Cabernet Franc, the relationship between anthocyanins and TSS had a lag phase where TSS increased with no change in anthocyanins and an approximately linear phase where both traits increased in parallel for TSS > 11.4 ^{o}Brix. Mean residuals in the linear phase were 0.065 mg g^{-1} in controls compared to -0.064 mg g^{-1} in the heated treatment hence demonstrating the thermal decoupling of anthocyanins and TSS across varieties fruit loads and water regimes ($P<0.0001$). Delayed onset of anthocyanin accumulation at elevated temperature explained the decoupling. Water deficit increased the anthocyanin : TSS ratio. We demonstrated for the first time that elevated temperature decouples anthocyanins and sugars in berries by a relative shift in onset rather than rate of accumulation of these berry components. Warming trends are likely to disrupt the anthocyanin : sugar ratio in berries with consequences for colour : alcohol balance in red wines. Water deficit established shortly before veraison could partially restore these ratios (Chapter 4).

5. Thermal decoupling results from the differential effect of temperature on different components of biological systems from molecular to ecosystem scales. We tested the hypotheses that elevated temperature decouples berry sensory traits and that this effect is variety dependent. We assessed 20 sensory traits in berries from a factorial field experiment combining two temperature regimes (elevated temperature, control) and four varieties (Cabernet Franc, Chardonnay, Semillon, Shiraz) over two thermally contrasting seasons in the Barossa Valley. The thermal response had two elements: offset, which accounts for the average advancement (or delay) across traits, and decoupling, which measures the scatter in the response of the different traits. Large decoupling was generally associated with large offset (e.g. Cabernet Franc) but large decoupling was also observed with minor offset (e.g. Semillon). Two types of decoupling were identified: decoupling mostly driven by differential response between seed and other berry components and decoupling with a dominant variation in traits within berry parts. In conclusion, elevated temperature decoupled berry sensory traits and this effect was variety and season dependent. Awareness of the magnitude and type of decoupling would likely contribute to viticultural and winemaking adaptations in warmer regimes (Chapter 5).

6. The effects of vineyard temperature on wine attributes are largely inferred from indirect comparisons between thermally contrasting regions or vintages. Here we directly assessed wine attributes in trials where manipulation of temperature in the field minimised confounded effects typical of indirect comparisons. Cabernet Franc, Chardonnay, Semillon and Shiraz vines were exposed to two thermal regimes (elevated temperature, control) over two growing seasons. Juice titratable acidity (TA) and pH, and wine sensory attributes were assessed. Three types of responses

to elevated temperature were found for juice TA and pH: (i) pH increased and TA decreased (Cabernet Franc, Chardonnay), (ii) both traits were unresponsive (Shiraz) and (iii) pH increased but acidity was unresponsive (Semillon). Elevated temperature consistently reduced green and citrus aromas and enhanced rich mouth feel and tropical flavours in Semillon. Thermal effects on sensory traits of Shiraz and Cabernet Franc were strongly season dependent. Responses of juice TA and pH to elevated vineyard temperature in Shiraz and Semillon did not conform to expectations from indirect comparisons. Owing to confounded environmental factors, the effect of temperature on wine attributes inferred from regional or seasonal comparisons needs to be interpreted with caution. Direct evaluation of the effect of elevated vineyard temperature on wine attributes can help developing winemaking adaptations for warmer futures (Chapter 6).

7. Berry water loss at late stages of ripening is a variety dependent trait correlated with mesocarp cell death. We tested the hypotheses that elevated temperature anticipates the onset and increases the rate of mesocarp cell death. The implications of these putative effects on the time-course of berry shrivel were also investigated. We assessed the progression of mesocarp cell vitality and the degree of shrivelling in berries from a factorial field experiment combining two thermal regimes (elevated temperature and control) and two varieties (Shiraz and Chardonnay). A bilinear model was used to objectively discriminate the onset of cell death and to quantify the rates of cell death before and after the inflection point in chronological and thermal scales. Elevated temperature advanced the onset of mesocarp cell death of berries and increased the rate of cell death in the period onset-harvest for both varieties. There was a close correlation between the percentage of living tissue and shrivel for Shiraz but not shrivel was observed in Chardonnay despite significant mesocarp cell death. In conclusion, elevated temperature accelerated both mesocarp cell death and berry shrivelling in Shiraz and accelerated mesocarp cell death but had no impact on shrivel in Chardonnay. Mesocarp cell death seems necessary but not sufficient to explain berry shrivelling. Awareness of functional links between berry shrivel and mesocarp cell death, and the environmental modulation of these traits would likely contribute to management practices that could reduce the severity of shrivel in a context of warmer conditions (Chapter 7).

8. We measured the effect of elevated temperature, and its interaction with fruit load and water supply, on the stomatal conductance (g_s) of *Vitis vinifera*, cv. Shiraz. Thermal regimes (control vs. elevated temperature using open-top chambers) were initiated in spring, thus affecting leaf development, and were maintained during the whole growing season. We used a top-down approach where reaction norms were derived that relate g_s for each treatment and the mean g_s across treatments; the slopes of reaction norms quantify phenotypic plasticity. Stomatal conductance responded to neither the interaction between temperature and fruit load ($P = 0.37$) nor the interaction between temperature and water regime ($P = 0.33$). We therefore dealt with each factor separately. Reaction norms of g_s under elevated temperature and control treatments diverged, i.e. elevated temperature had no effect on g_s under conditions conducive to low conductance (below ~ 100 mmol m^{-2} s^{-1}) but increased g_s in relation to controls under conditions favouring high conductance.

Stomata density was 159 ± 6.7 mm^{-2}, and was unaffected by temperature. Stomata length increased from 20.6 µm in controls to 23.2 µm in the heated treatment ($P < 0.0001$) and width increased from 14.4 µm in controls to 16.8 µm in the heated treatment ($P < 0.0001$). Thus, longer and wider stomata contributed to the enhanced plasticity of stomatal conductance under elevated temperature. Consistently, reaction norms of light-saturated photosynthesis were divergent, i.e. elevated temperature did not affect photosynthesis under conditions conducive to low g_s and photosynthesis, but increased photosynthesis in relation to controls under more favourable conditions. A high source:sink ratio reduced the plasticity of stomatal conductance. Water regime had a minor effect on the plasticity of stomatal conductance, but the reaction norms for irrigated and water deficit treatments were offset by ~ 60 mmol m^{-2} s^{-1} thus indicating a consistent effect of water deficit across environmental conditions. We conclude that the responses of stomata derived from transient temperature treatments in fully expanded leaves are unsuitable to model the dynamics of gas exchange in response to projected warming. Our experiments support a conceptual model where current stomatal conductance is a function of (i) the prevailing environmental conditions during early stages of leaf development that set the upper limit of conductance through the modulation of stomatal density and size, (ii) the current environmental conditions and (iii) the source:sink ratio (Chapter 8).

9. To predict the trajectory of sugar accumulation in berries for planning of harvest and post-harvest operations, we derived a simple model that is consistent with the known biological and viticultural drivers of berry ripening and requires inputs that are readily available to industry. We used data from three vintages in climatically contrasting regions of Australia to derive a relationship between total soluble solids (TSS) and thermal time. A linear-plateau function was fitted to the 90^{th} percentile data, hence providing a boundary function representing the potential for each variety-environment combination. Biologically relevant parameters were derived including the rate of change in TSS, maximum TSS (TSS_{max}), the threshold thermal time when berries reached $TSS_{max,}$ the onset and the duration of the period of linear increase in TSS. Gaps, calculated as the difference between actual TSS and the boundary function, correlated positively with vapour pressure. A model with the observed onset of ripening as an input and a variety and site specific rate as parameter accounted for 81-92% of the variation in TSS for independent data sets. In conclusion, the trajectory of TSS in berries can be modelled “as if” the main environmental and management sources of variation affect the onset rather than the rate of sugar accumulation. Our onset-rate model can be coupled with (i) short-term temperature forecasts to predict the trajectory of TSS for management purposes, and (ii) long-term records of temperature to produce probabilistic profiles of maturity date (Chapter 9).

10. Early maturity of grapevine associated with recent warming is well documented but poorly understood. Here we ask the question: is early maturity associated with early onset of ripening or with increased rate of sugar accumulation? We used records of total soluble solids (TSS) for Chardonnay, Shiraz and Cabernet Sauvignon collected in commercial vineyards between 1995 and 2009. The combination of 15 vintages and three climatic regions yielded a range of average seasonal

temperature between 15.5 and 20.5 ^{o}C. Using boundary functions of TTS vs time, we derived the onset and rate of increase in TSS, and the time of maturity set at 12 ^{o}Bé for comparative purposes. Pooled across varieties, the rate of change in maturity was -9.8 ± 0.94 ^{o}Cd^{-1}. Shifts in onset accounted for 86% of the variation in time of maturity, and rate of ripening for the remaining variation. In conclusion, early maturity associated with higher temperature (and related factors including higher radiation and higher vapour pressure deficit) is primarily driven by early onset of ripening under a wide range of production systems in south-eastern Australia. Viticultural practices aiming at delaying maturity to counteract the effect of high temperature, high radiation and high vapour pressure deficit are more likely to be successful if they target the onset of ripening (Chapter 10).

11. To predict harvest time for logistic applications in vineyards and wineries, measurements of total soluble solids (TSS) are combined with projections assuming TSS increases at 1 ^{o}Bé wk^{-1}. Here we ask three questions. (1) What is the magnitude and direction of the bias in estimates of ripening assuming 1 ^{o}Bé/wk across varieties, regions and seasons? (2) What are the climatic drivers of this bias? (3) Could thermal rates improve predictions? Actual TSS of Chardonnay, Shiraz and Cabernet Sauvignon was measured in commercial vineyards in Coonawarra, Barossa Valley and Riverland. Predictions used (a) the default 1 ^{o}Bé wk^{-1}, (b) variety and location specific chronological rates between 0.8 and 1.2 ^{o}Bé wk^{-1}, and (c) variety and location specific thermal rates between 0.009 to 0.017 ^{o}Bé ^{o}Cd^{-1}. In 8 out of 9 cases, 1 ^{o}Bé wk^{-1} biased ripening predictions. Seasonal bias correlated with evaporative demand in the Riverland and Coonawarra, and with minimum temperature in the Barossa. Thermal rates were not superior to calibrated chronological rates to predict ripening. In conclusion, locally calibrated rates significantly improved ripening predictions for major grapevine varieties in regions with contrasting climates and viticultural profiles. Improved prediction of maturity time would allow for better allocation of key resources in vineyards and wineries – labour, time, machinery, transport, analytical services, chemicals, storage (Chapter 11).

12. The project established an active programme of communication; it delivered two industry wine tasting workshops and 39 presentations and publications targeting industry and scientific audiences (Chapter 12). The Australian Society of Viticulture and Oenology awarded the paper titled “Elevated temperature decouples anthocyanins and sugar in berries of Shiraz and Cabernet Franc” the title of AJGWR Viticulture paper of the year for the most significant impact on the Australian wine industry.

Chapter 1. A large-scale, open-top system to increase temperature in realistic vineyard conditions

VO Sadras, R Bubner and MA Moran

Abstract

In viticulture, indirect methods that compare seasons or regions to infer temperature effects are often inconclusive because temperature is frequently confounded with other climatic factors, management practices and soils. Unequivocal results require direct comparisons where temperature is manipulated in realistic vineyard conditions. Here we describe a large-scale, automated open-top system to elevate temperature by ~ 2 ^{o}C above ambient, hence allowing for warming projections in Australia. A system that combines passive heating (clear days) and active heating by circulating hot water (nights and overcast days) was designed that largely achieves five attributes: (1) reproduces the daily and seasonal cycles of temperature and vapour pressure deficit, (2) has small effect on relative humidity, hence allowing for increased vapour pressure deficit, (3) minimises biologically important secondary effects, as the canopies are exposed to the natural light environment (4) has structural strength to withstand the weather, particularly wind, and (5) allows for number and size of replicates required for statistical resolution and viticultural needs, including sufficient fruit for meaningful wine production.

1. Introduction

The effects of temperature on vines and wines have been investigated with a range of methods. Indirect methods compare seasons or regions using statistical tools to infer putative effects of temperature (Cozzolino et al., 2011; Duchêne and Schneider, 2005; Elmendorf et al., 2011; Grifoni et al., 2006; Jones and Davis, 2000; Jones et al., 2005; Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Sadras and Petrie, 2011b; Soar et al., 2008; Urhausen et al., 2011). However valuable and irrespective of the sophistication of statistics used to unscramble main effects and interactions, this indirect approach is inconclusive because temperature is confounded with other climatic factors, and in many cases with management practices and soils (Calviño and Sadras, 1999; Rodriguez and Sadras, 2007; Soar et al., 2008). Unequivocal results require direct comparisons. Manipulation of temperature in controlled environments has been favoured (e.g.Kadir et al., 2007; Liu et al., 2008; Mori et al., 2005b; Wang et al., 2004; Wen et al., 2008; Zhang et al., 2008). Soar et al. (2009) and Tarara et al. (2000) outlined the limitations of controlled environments. Artefacts from potted plants in controlled conditions mean this approach is unsuitable for traits such as vine water relations (Passioura, 2002; Passioura, 2006). Other traits such as phenological development up to flowering can be sensibly measured in controlled environments (Moncur et al., 1989).

Several systems have been developed to manipulate temperature of vines or vine parts in the field. Tarara and co-workers (2008a; 2000) developed a chamber-free system whereby heated or cooled air was blown across bunches to achieve up to 10 ^{o}C temperature differential. Petrie and Clingeleffer (2005) used mini-chambers combined with shade cloth and reflective foil to manipulate the temperature and irradiance of individual buds. Bowen et al. (2004a; 2004b) enclosed cordons or canes in polyethylene sleeves which increased maximum temperature by 5-8°C and decreased minimum temperature by 1-2°C. All these systems targeted specific plant parts, rather than the whole canopy. Ezzahouani (2003) and Soar et al. (2009) used plastic enclosures to increase temperature of whole vines; these closed systems reduce total radiation, increase diffuse radiation and alter the boundary layer of the canopy. Collectively, these secondary effects constrain the usefulness of closed systems as research tools, particularly for long-term studies (Soar et al., 2009; Tarara et al., 2000).

Expanding the criteria of Marion et al. (1997) and Hall and Sadras (2009), desirable attributes of a system for manipulation of vine temperature in the field include:

1. Reproduces the daily and seasonal cycles of temperature and vapour pressure deficit.
2. Does not increase relative humidity, hence allowing for increased vapour pressure deficit.
3. Minimises biologically important secondary effects.
4. Has structural strength to withstand the weather (particularly wind) to ensure a reasonable longevity (2-3 years or more) and allows for access to vines for sampling and maintenance operations.
5. Allows for number and size of replicates required for statistical resolution and viticultural needs, including sufficient fruit for meaningful wine production. The number of treated vines also needs to account for buffer vines at the edges of the treated area and between heated and control sections of the vineyard.

A further criterion that is not essential for temperature studies but is desirable for realistic simulation of anticipated environments over the next five decades is the compatibility of elevated temperature and enhancement of ambient CO_2 concentration in FACE-type systems (Bindi et al., 2001a). This desirable attribute and the critical criterion 3 require open-top systems. The open-top, passive heating system of Sadras and Soar (2009) largely meets all five criteria, but has two main deficiencies: it overheats in clear, mild days and, being dependent on sunlight for heating, does not work at night and during overcast days.

This paper describes the construction, performance, advantages and limitations of an automated open-top system that combines passive heating (clear days) and active heating (nights and overcast days) and regulates the size of the opening to conserve or release heat as required. The system has been designed to increase temperature by ~ 2 ^{o}C above ambient to represent warming projections for wine growing regions of Australia (Webb et al., 2007b).

2. Heating system

Figure 1 shows an overview of the system. It involves three conceptual components: passive heating in clear days, overheating control in clear days and active heating at night and during overcast days. The system is automated to target ~ 2 ^{o}C difference between heated and control treatments at canopy level.

2.1. Passive heating in clear days and opening-closing system to regulate heat exchange

Under the prevailing conditions of the Barossa Valley, an early passive-heating system with fixed panels had the following features (Sadras and Soar, 2009): (i) reproduced the daily cycle of temperature, (ii) increased maximum temperature by 2-4 ^{o}C, except early in spring when temperature increase relative to controls was up to 8 ^{o}C, (iii) had small effects on relative humidity at canopy level, hence allowed for the daily tracking of vapour pressure deficit, and (iv) had insignificant effects on night temperature.

The early, fixed panel system was upgraded into an actuated panel arrangement (Fig. 1bc, Fig. 2). Modular frames were made from 25 mm square tube steel (Stratco, Australia) and fastened solid polycarbonate sheeting (Standard-Clear-Greca, Suntuf, Australia). This material blocks most UV radiation (200 to 400 nm) and has a very high (90%) and uniform transmittance between 400 and 1600 nm. The panels are connected to pushrods, which are in turn connected to a central 25-mm water pipe arrangement that spans across four panel sections. Star dropper supports were located midway along the length of each pipe section to provide additional support. A single rotary actuator (SM24P-SR Belimo, Switzerland) mounted to the wooden post drives a group of four panels and is connected to the water pipe via a 25-mm solid rod, inserted into the pipe. Bearings secured to the wooden posts at each end of the set of four panels are used to locate the water pipe and actuator. Terminal boxes, located adjacent to each actuator, were terminated at the building management system (BMS); details of automated control are explained below.

2.1. Active heating at night and during overcast days

Fig. 3 outlines the active heating component of the system, scaled to heat three large replicates (criterion 5). It included a twin boiler arrangement (27 kW Lamborghini Calorelima, Italy and 80 kW Hurlcon, Victoria, Australia) and 1000 L insulated hot-water storage vessel. The boilers, water storage vessel and control box were mounted on a concrete plinth and contained beneath a weatherproof roof structure (Fig. 1).

Hot water distribution was controlled with a set of primary pumps to circulate water between the boilers and storage vessel, and one secondary pump per replicate to circulate water in the field (Fig. 1b). The secondary pumps aimed at the maintenance of a high flow rate (4.6 L s^{-1}) hence reducing the thermal gradient between the extremes of the plot. The pipes (high-density polyethylene, 50 mm diameter) used to transfer heat under the vines were mounted on wooden planks to avoid soil heating by conduction (Fig. 1bc).

2.2. Control strategy

The experiment was laid out in a randomised block design with two treatments (heated, control) and three replicates. Heating and panel closing/opening decisions for the heated plots were managed on the basis of the pair-wise difference in temperature between heated and control treatments for each block individually. Screens with temperature and humidity sensors were placed at canopy level in the centre of each replicate (Fig. 1c). A BMS control system (BACview6, AutomatedLogic, Georgia, USA) was programmed to manage the operation of all equipment; it was located within a control box adjacent to the boilers and water storage vessel. The major rules to manage the system are as follows. If the temperature in a heated replicate is greater than 2.5°C above its paired control, the actuators gradually drive the panels towards the vertical, open position. If the temperature in the heated replicate is less than 2.5°C above the paired control, the panels gradually close. If the panels are already closed and the set point is still not met, the solenoid valves open and the pumps start and circulate hot water through the pipes.

3. System performance

Here we assess the performance of the system from two perspectives. From a physical perspective, we evaluate the capacity of the system to reproduce daily and seasonal cycles of temperature, its effects on relative humidity and other secondary effects. From a viticultural perspective, we illustrate the responses of key grapevine traits.

Criteria 1 and 2: temporal cycles of temperature and vapour pressure deficit
The heating system captured the daily and seasonal cycles of temperature (Fig. 4). Comparison of 15-minute temperature records shows the heating system was able to elevate temperature consistently up to the target 2 ^{o}C above controls in a range of ambient temperature between 0 and 47 ^{o}C (Fig. 5a). In mild, clear spring days the system tended to overheat (green points in Fig. 5). Inset of Fig. 5a highlights the difference in temperature between treatments aggregated over a nine-month period.

The horizontal gradient of temperature between the extreme positions of the heating system was small, i.e. 0.003 ± 0.0005 ^{o}C m^{-1}. The vertical gradient (0.2 to 1 m above soil level) increased from 0.10 ± 0.022 ^{o}C m^{-1} in controls to 2.2 ± 0.029 ^{o}C m^{-1} in the heated treatment.

The impact of increased ambient temperature on the thermal regime of the topsoil, which concentrates most biological activity, is highly dynamic because the local coupling between atmosphere and land is influenced by the nature of the landscape, the vegetation, soil moisture and topography (Evans et al., 2011). Top soil (0.10 m) in the heated treatment was on average 2.2 ± 0.02 ^{o}C hotter than in controls during the day and 1.8 ± 0.01 ^{o}C hotter during the night. Thus, top-soil warming was in the same order of magnitude than air warming for our particular combination of soil, climate, vegetation and management.

The heating system did not increase humidity in comparison with controls. If anything, relative humidity at canopy level was slightly lower in heated treatments; it averaged 66 ± 0.4 % in heated *vs.* 72 ± 0.4% in control treatments in Spring, 54 ± 0.4 % *vs.* 61 ± 0.5% in Summer, 73 ± 0.4% *vs.* 80 ± 0.4% in Autumn and 82 ± 0.3 % *vs.* 88 ± 0.3% in Winter. The heating system therefore tracked the daily and seasonal cycles of vapour pressure deficit, which was consistently increased in relation to controls as a result of significant increases in temperature (Figures 4 and 5b).

Criterion 3: biologically important secondary effects
The open-top chambers were designed for the canopy to be exposed to the natural light environment. Vines with different shape and size, as driven by pruning method and conduction system, would require adjustment in the shape and dimension of the heating system to allow for fully exposed canopies. In our experimental system, the trunk is under the panels (Fig. 1bd) whereas the bulk of shoots (> 90%) and all fruit are concentrated on the central part of the chambers. Few shoots may be trapped below the line of the panels and some may jut out of the panels (Fig. 1d). We observed occasional damage of leaves in contact with the hot surface of the metal frames, but the number of leaves affected was negligible in comparison with, for example, the number of leaves removed for the measurement of water potential.

The exposition of the canopy to natural light intensity and quality is critical to avoid undesirable reduction in photosynthesis from reduced light intensity or enhanced photosynthesis from increased proportion of diffuse radiation (Roderick and Farquhar, 2003; Rodriguez and Sadras, 2007; Spitters, 1986; Wohlfahrt et al., 2008). Likewise, maintenance of the natural spectral composition of light helps to avoid biologically important secondary effects, e.g. effects of red/far-red ratio on development and

functionality of stomata (Casson et al., 2009; Talbott et al., 2003; Wang et al., 2010) or effect of UV on anthocyanin synthesis (Ubi et al., 2006).

Heating systems that use fans to deliver and distribute heat in chambers generate turbulence and promote dust deposition on leaves and bunches (Soar et al., 2009). Even the blowers used to mildly spread CO_2 in FACE systems alter the microclimate of the crop, e.g. reducing dew duration, particularly in still nights (PinterJr et al., 2000). In contrast, a positive feature of our system is that it gently delivers heat by radiation, thus avoiding turbulence and dust that potentially affects canopy function and fruit traits.

Potentially important secondary effects of our open-top chambers include changes in both wind speed and rainfall distribution. The effect on wind and hence the boundary layer of the canopy is unavoidable. Measurements of gas exchange at the leaf and whole-plant may therefore be reliable, but the system would be less reliable for scaling up beyond the plant (Jarvis and McNaughton, 1986). To test for the effect of the panels on rain distribution and its putative consequences for vine water relations, we measured pre-dawn leaf water potential periodically in the growing season 2010-11. We found no statistical difference between pre-dawn leaf water potential in heated and control treatments, even after a series of significant rainfall events (Fig. 6). Differences in distribution of rainfall from the disposition of panels cannot be excluded but if these differences existed, they were biologically negligible under the conditions tested in our trial. The system is indeed leaky, and the panels can be open to vertical position in rainy periods as required. A long-term, cumulative change in rainfall distribution might affect the root pattern of vines but irrigation during the growing season when roots are actively growing would reduce the likelihood of this artefact (Keller, 2010b; Soar and Loveys, 2007).

Criterion 4: structural strength and access to vines for sampling

The robustness of the system was tested on 23 May 2011, when wind speed reached 90 km h^{-1}. This event damaged the simpler chambers of Sadras and Soar (2009) in a nearby experiment, but did not affect the system described here; better anchorage and heavier components contributed to its robustness.

Practical constraints of the system include access to the space between chambers and vines for sampling, harvesting, pruning and weed control. Thermal images can be captured with no difficulty from outside the chambers; other traits such as stomatal conductance can be measured by leaning on the metal frames, and closer access to vines required for berry sampling is facilitated by temporarily shifting the panels to vertical position. An *ad-hoc* extension of a standard sprayer boom allows tractors to be driven through neighbouring rows, hence allowing for the normal spraying regime of the whole vineyard.

Excessive canopy vigour interferes with two aspects of system performance. First, foliage reduces light transmission through the panels, hence reducing the passive heating component. Second, shoots may entangle with the mobile mechanisms; periodic inspection and occasional trimming are required. Trimming that removes significant amount of shoot obviously needs to be reproduced in controls. To maintain the heating capacity of the system, occasional washing of the panels might be required to remove dust deposits; in

our environment, we did not need to clean our panels in trials where they have been installed for over two years.

Criterion 5: number and size of replicates for statistical resolution
The projected increase in temperature during the next decades involves two relevant elements for viticulture (Sadras, 2010). One is the putative shift in the incidence of heat waves. The second, the target of our heating system, is the gradual increase in mean temperature. The long-term cumulative effect of a moderate increase in temperature could be substantial, as illustrated in the inset of Fig. 5a. To capture these effects, our heating system was designed to allow for number and size of replicates required for meaningful sampling and statistical resolution, including buffer vines at the edges of the treatments. In its current design, each replicate includes 21 vines spaced at 2.25 m (Fig. 1a). Here we present a few examples of viticulturally important responses which are fully developed in subsequent papers.

Accumulated over time, the increase in background temperature alters phenological development and vine physiology with direct consequences for production of quality grapes. The cumulative effect of temperature (inset in Fig. 5a) accelerated phenological development (Fig. 7a), increased the phenotypic plasticity of stomatal conductance (Fig. 7b), increased pH and reduced total acid concentration in grape juice (Fig. 7c). In contrast to studies where these types of effects are inferred by comparison of seasons or sites or where controlled conditions involve confounding factors, the effects illustrated in Fig. 7 are, with a great degree of confidence, attributable to temperature in realistic vineyard conditions.

In conclusion, we designed and deployed a large-scale, open-top heating system that combines passive and active components that do not create unrealistic turbulence. The system largely achieves five important criteria: it reproduces the daily and seasonal cycles of temperature and vapour pressure deficit, does not increase relative humidity, hence allowing for increased vapour pressure deficit, minimises biologically important secondary effects, has structural strength to withstand the weather, particularly wind, and allows for number and size of replicates required for statistical resolution and viticultural needs, including sufficient fruit for meaningful wine production.

Acknowledgements

This work was funded by the Grape and Wine Research and Development Corporation, Department of Agriculture, Fisheries and Forestry and Complementary State NRM Program. The system was designed and constructed with the assistance of Scott Perry, Tony Edmond, Peter McPhee, Sam James, Grant Mc Evoy, Rusty Smith, and Dale Reeve. Treva Hebberman adapted standard machinery to maintain the regular crop protection program in the vineyard.

Captions to figures

1. (a) Overview of large-scale heating system. A large number of vines per replicate allow for buffer vines at the edges, robust sampling and statistics, and amount of fruit required for meaningful wine production. In the background: gas tank (G) and shelter (S) including boiler, water tank and automated control system. (b) Detail of system showing hot-water pipes, pump (P), actuator (A) and shielded temperature and humidity sensors in control treatment (T). (c) Detail of articulated panels; inset shows shielded temperature and humidity sensor. (d) The open-top system allows for the canopy to be exposed to the natural light environment.

2. (a) Section and (b) top views of panel arrangements.

3. Hot water component of heating system.

4. Daily and seasonal patterns of temperature and vapour pressure deficit in control and heated treatments. Each point is the average of three replicates with independent temperature regulation, measured at 15-minute intervals.

5. Comparison of (a) temperature and (b) vapour pressure deficit in heated and control treatments. Each point is the average of three independent replicates representing spring (green), summer (red), autumn (grey) and winter (blue) conditions. Both temperature and humidity were recorded at 15 min intervals. Reference lines are $y = x$. Inset shows cumulative temperature between the establishment of the treatment (early October 2010) and end of June 2011.

6. Heating systems did not affect the dynamics of predawn leaf water potential in relation to controls. The closed arrow head is the beginning of irrigation and open arrow heads indicate rainfall events of 22, 35 and 29 mm, respectively. Error bars are two standard errors of the mean. *Method*: pre-dawn leaf water potential was measured on 12 leaves per treatment (four leaves per replicate x three replicates) using a Scholander pressure bomb following the procedures in Hsiao (1990).

7. Elevated temperature (a) accelerated development, (b) increased phenotypic plasticity of stomatal conductance, and (c) increased pH and reduced titratable acidity in mature Shiraz berries. *Methods.* (a) Phenological development was monitored weekly in 9 tagged vines per treatment (3 vines per replicate x 3 replicates) using the E-L scale described in Coombe (1995); asterisks indicate temperature effects at $P < 0.05$. (b) Stomatal conductance was measured five times during the growing season on 9 tagged leaves per treatment (3 leaves per replicate x 3 replicates) using a steady-state porometer (Decagon SC1, Pullman, Washington, USA). Measurements were taken on clear days between 11 am and 3 pm (local time). Plasticity is the slope of the lines relating stomatal conductance for each thermal regime and the environmental mean across thermal regime; for details of method see Sadras and Trentacoste (2011). (c) Titritable acidity and pH were measured on the juice of ripe berries (100 berries per replicate x 3 replicates) using the procedures outlined by Iland et al.(2000). In (a,b,c) error bars are two standard errors of the mean and were not plotted when smaller than symbol.

Fig. 1

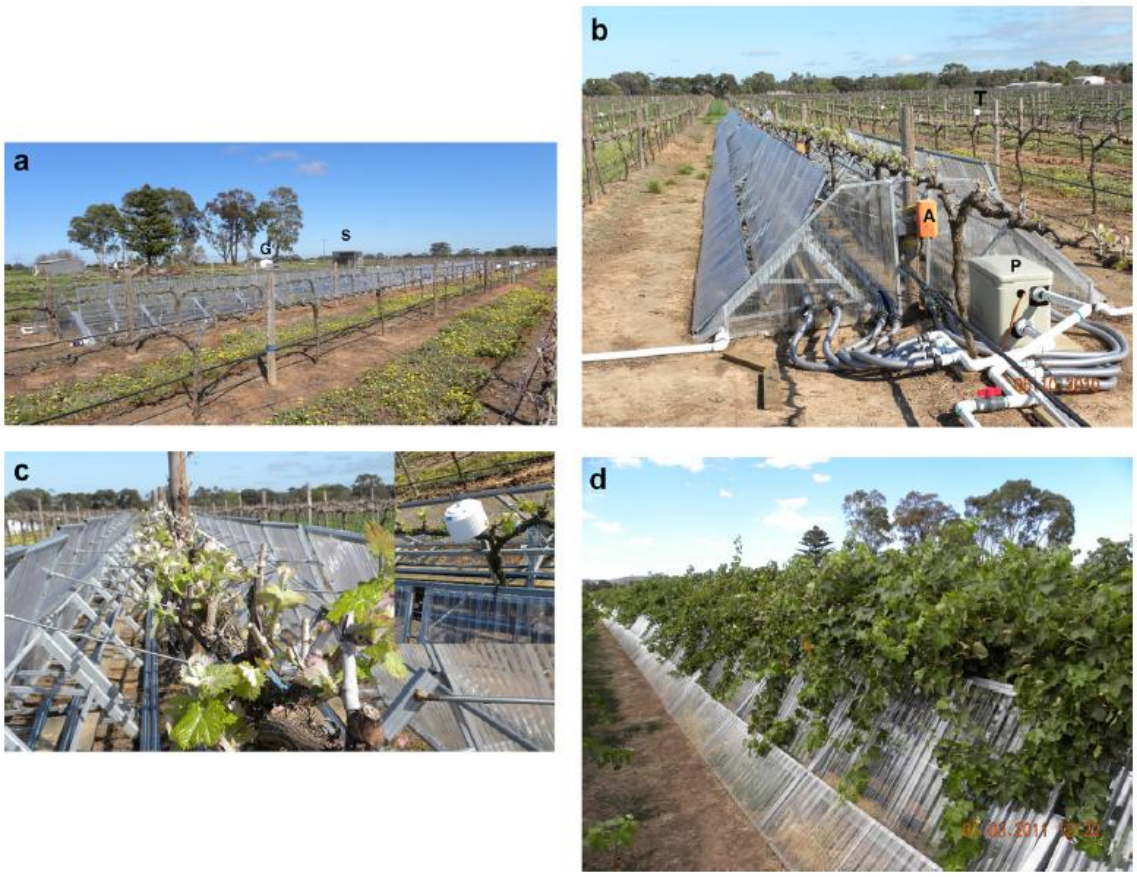
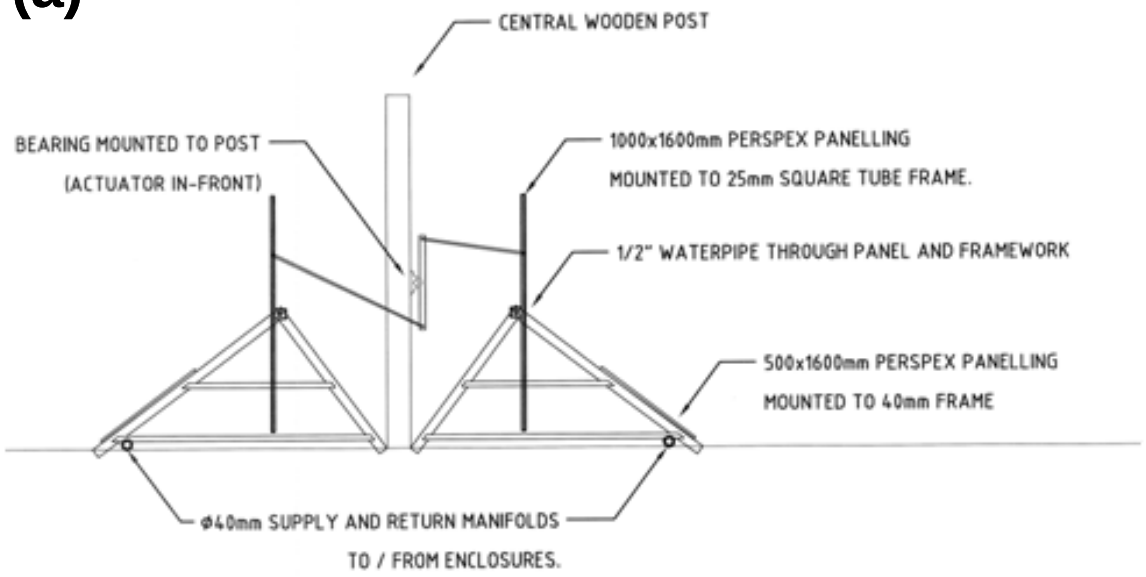


Fig. 2

(a)


CENTRAL WOODEN POST
BEARING MOUNTED TO POST
(ACTUATOR IN-FRONT)
1000x1600mm PERSPEX PANELLING
MOUNTED TO 25mm SQUARE TUBE FRAME.
1/2" WATERPIPE THROUGH PANEL AND FRAMEWORK
500x1600mm PERSPEX PANELLING
MOUNTED TO 40mm FRAME
ø40mm SUPPLY AND RETURN MANIFOLDS
TO / FROM ENCLOSURES.

(b)

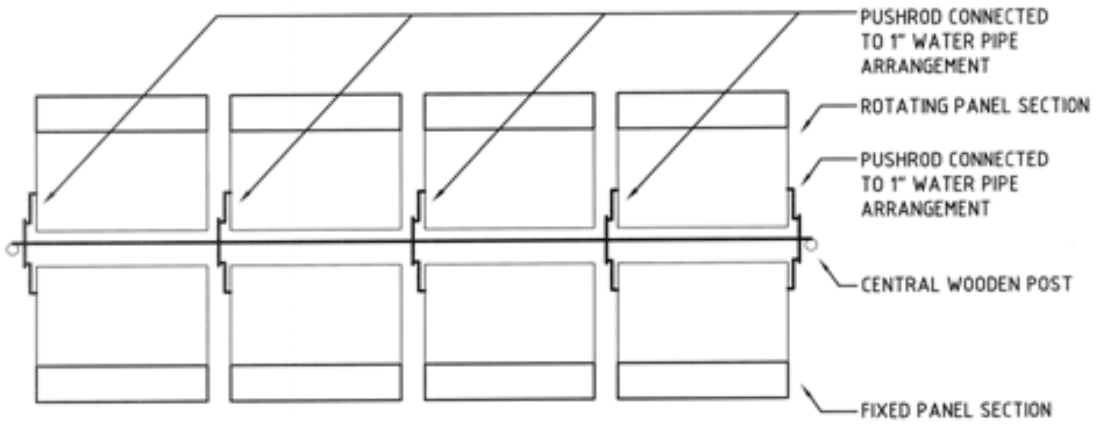

PUSHROD CONNECTED
TO 1" WATER PIPE
ARRANGEMENT
ROTATING PANEL SECTION
PUSHROD CONNECTED
TO 1" WATER PIPE
ARRANGEMENT
CENTRAL WOODEN POST
FIXED PANEL SECTION

Fig. 3

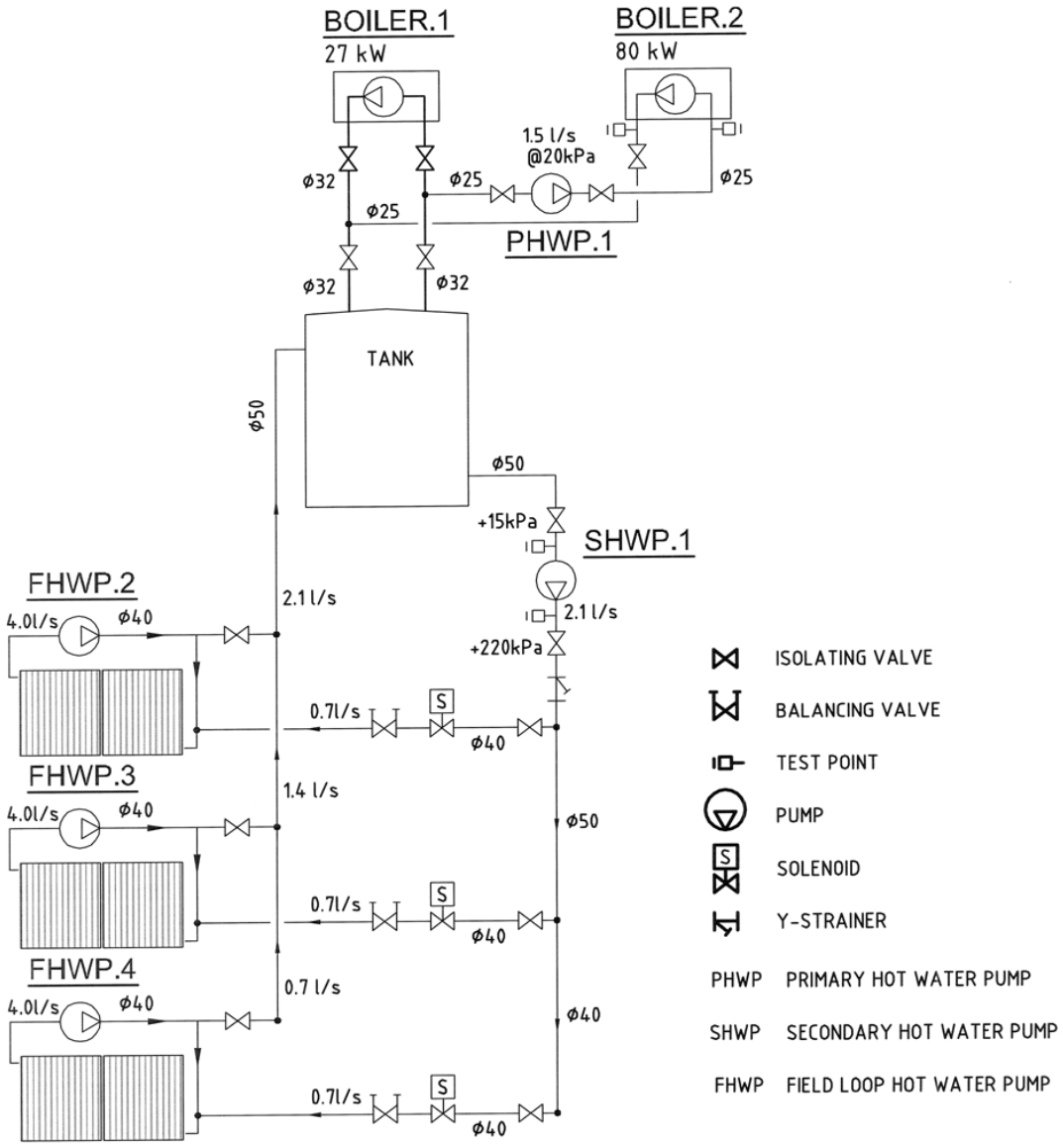
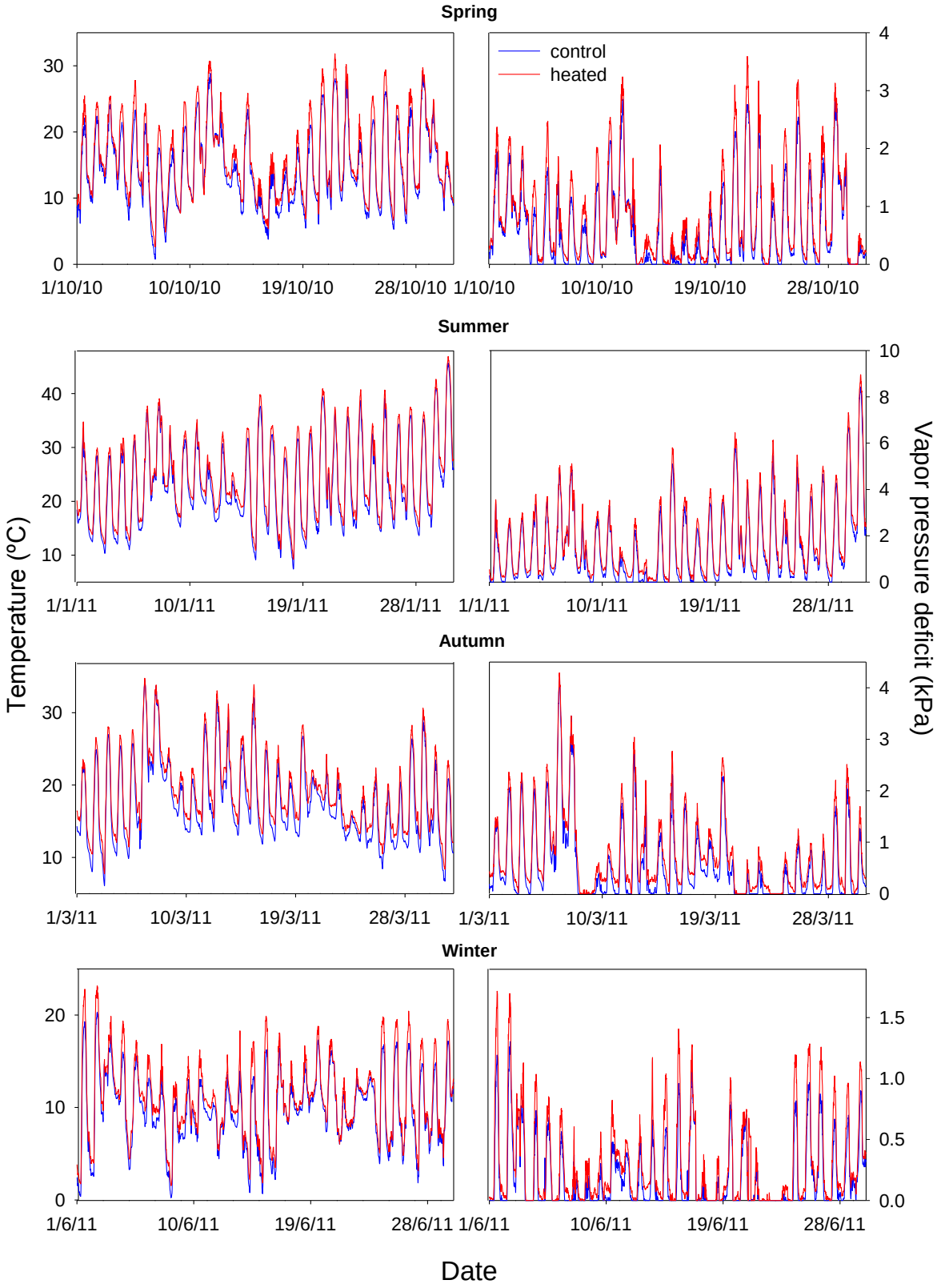

BOILER.1
27 kW
BOILER.2
80 kW
1.5 l/s
@20kPa
ø32
ø25
ø25
ø25
PHWP.1
ø32
ø32
TANK
ø50
ø50
+15kPa
SHWP.1
FHWP.2
4.0l/s
ø40
2.1 l/s
2.1 l/s
+220kPa
0.7l/s
ø40
FHWP.3
4.0l/s
ø40
1.4 l/s
ø50
0.7l/s
ø40
FHWP.4
4.0l/s
ø40
0.7 l/s
ø40
0.7l/s
ø40
ISOLATING VALVE
BALANCING VALVE
TEST POINT
PUMP
SOLENOID
Y-STRAINER
PHWP PRIMARY HOT WATER PUMP
SHWP SECONDARY HOT WATER PUMP
FHWP FIELD LOOP HOT WATER PUMP

Fig. 4


Spring
Summer
Autumn
Winter
control
heated
Temperature (°C)
Vapor pressure deficit (kPa)
Date
1/10/10
10/10/10
19/10/10
28/10/10
1/1/11
10/1/11
19/1/11
28/1/11
1/3/11
10/3/11
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19/6/11
28/6/11

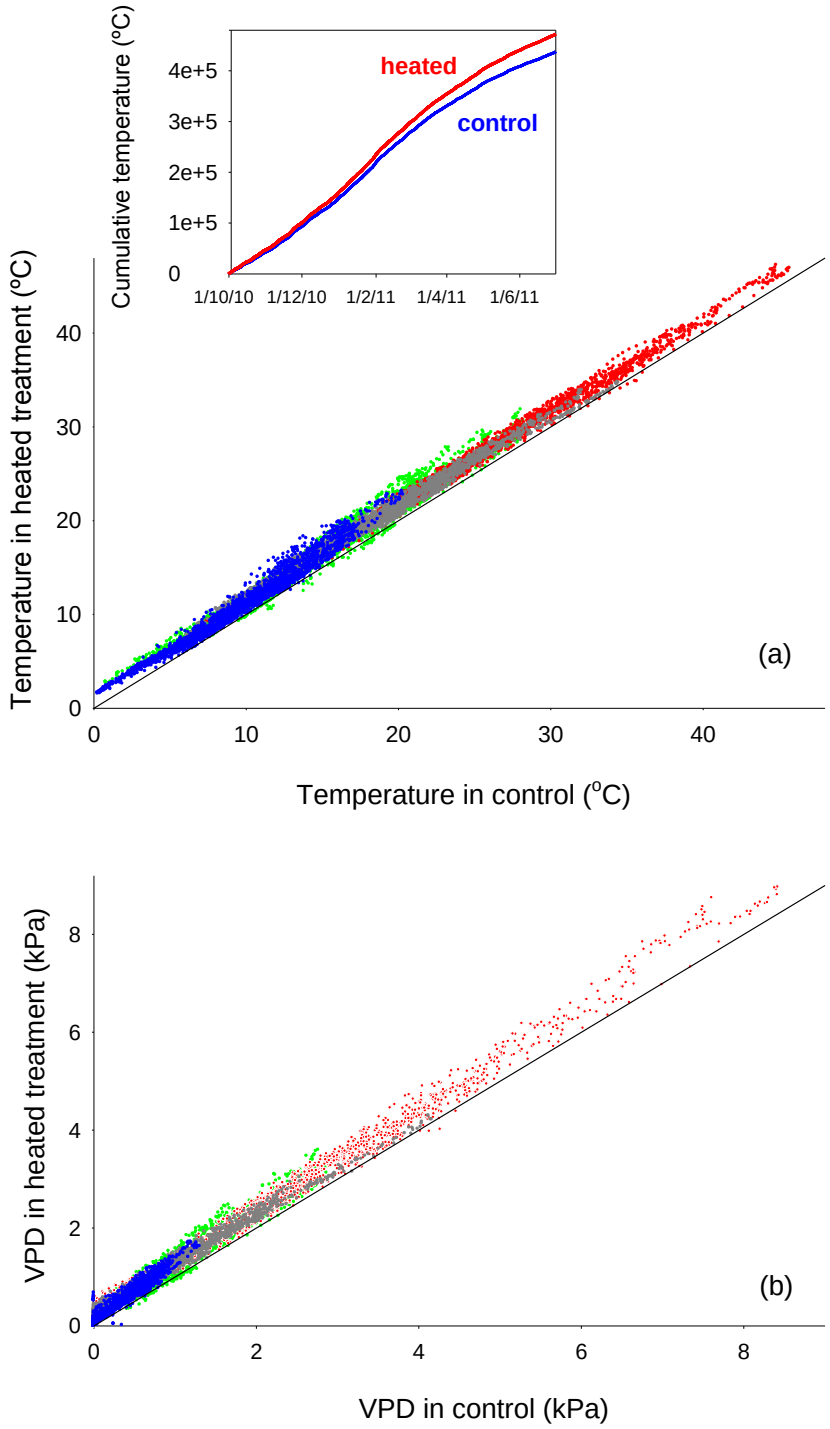
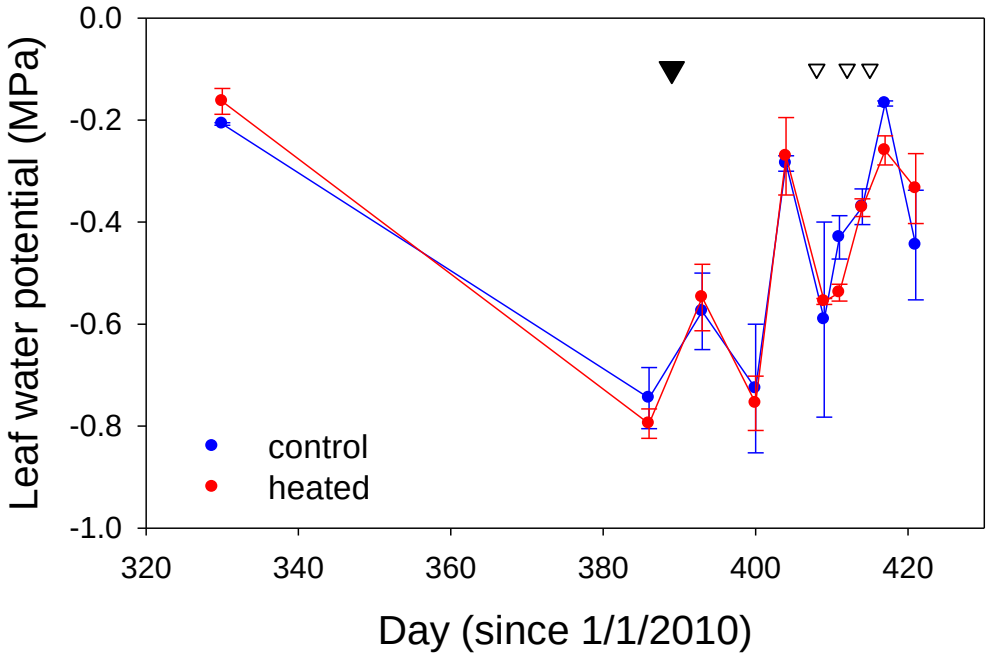

Cumulative temperature (°C)
4e+5
3e+5
2e+5
1e+5
0
heated
control
1/10/10
1/12/10
1/2/11
1/4/11
1/6/11
Temperature in heated treatment (°C)
40
30
20
10
0
(a)
0
10
20
30
40
Temperature in control (°C)
VPD in heated treatment (kPa)
8
6
4
2
0
(b)
0
2
4
6
8
VPD in control (kPa)

Fig. 5

Fig. 6


0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Leaf water potential (MPa)
320
340
360
380
400
420
Day (since 1/1/2010)
control
heated

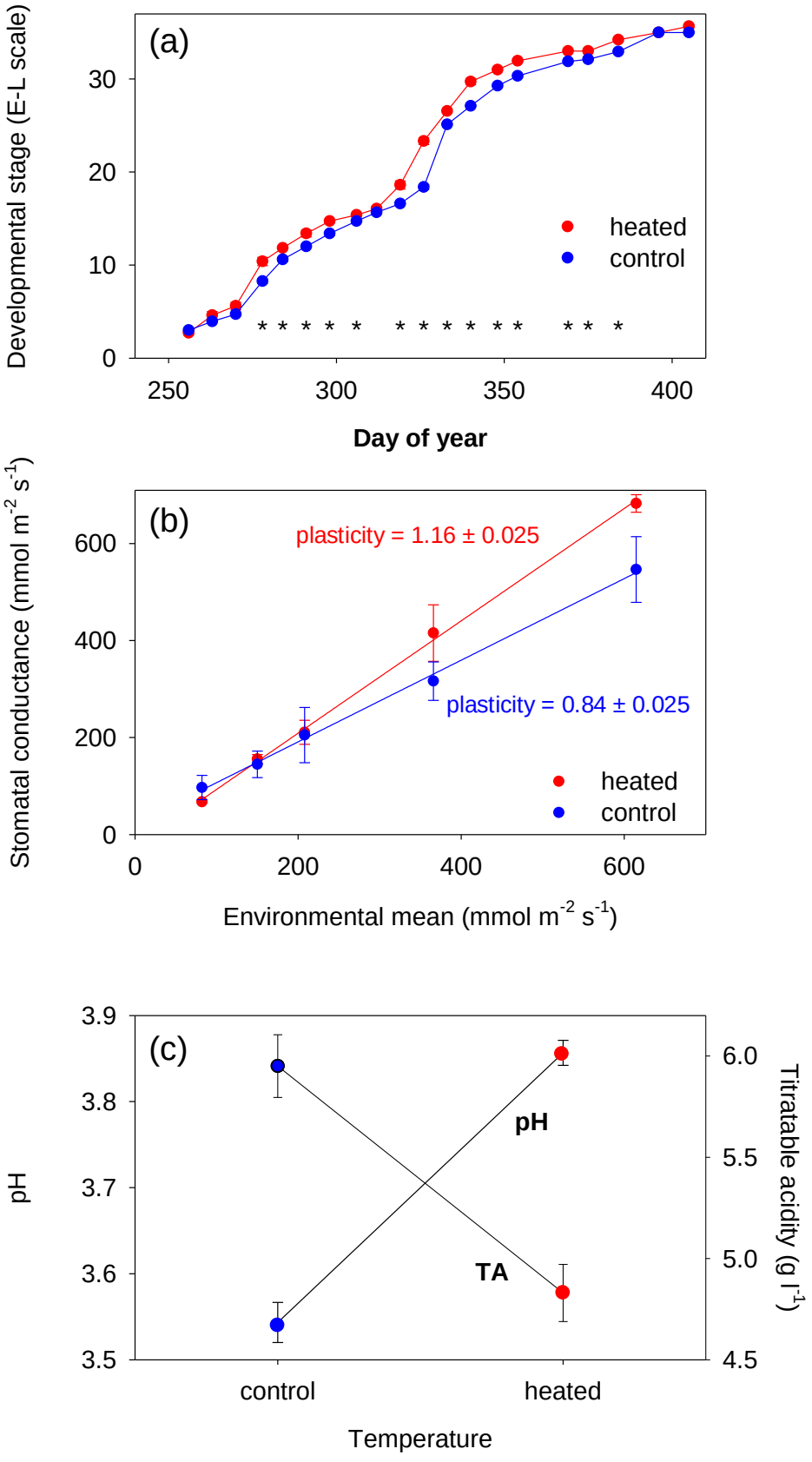

(a)
Developmental stage (E-L scale)
0
10
20
30
250
300
350
400
Day of year
heated
control
(b)
plasticity = 1.16 ± 0.025
plasticity = 0.84 ± 0.025
Stomatal conductance (mmol m-2 s-1)
0
200
400
600
Environmental mean (mmol m-2 s-1)
(c)
pH
TA
3.5
3.6
3.7
3.8
3.9
4.5
5.0
5.5
6.0
Titratable acidity (g l-1)
control
heated
Temperature

Fig. 7

Chapter 2. Nonlinear effects of elevated temperature on grapevine phenology

VO Sadras, MA Moran

Abstract

Phenological shifts are one of the most conspicuous biological effects of global warming; observational data need benchmarking against experimental data.

Grapevine phenology was monitored weekly between budswell and maturity in three factorial field experiments during two seasons. Two thermal regimes (heated *vs* control) were combined with two irrigation regimes (exp. 1), two source:sink ratios (exp. 2) and four varieties (exp. 3). Open-top chambers were used to elevate temperature.

We present evidence of nonlinearity of thermal effects on phenology that is explained by two complementary factors. Firstly, temperature-driven shifts of sensitive events (e.g. onset of sugar accumulation in berries) moved the timing of subsequent events into cooler conditions, hence dampening warming effects. Secondly, thermal effects on phenology seemed to be modulated by the interplay between resource-driven growth and temperature-driven development; an enhanced thermal effect on berry ripening associated with high source:sink ratio supports this proposition.

Measured shifts in maturity were smaller than expected from reported time series analysis. During berry ripening towards maturity, the phenological trajectories of heated and control vines largely overlapped on thermal time scales; differences in phenology between treatments were therefore attributed to actual thermal regimes. We thus conclude that thermal sensitivities from time series (6 to 9 d $^{o}C^{-1}$) over-estimate thermal effects on grapevine maturity.

1. Introduction

Grapevine is an economically important crop and a biological model of long-lived perennials widely used in climate change studies. Analysis of time series combined with robust theory supports a link between phenological shifts of grapevines and increasing ambient temperature in both the northern and southern hemispheres (Duchêne et al., 2010; Duchêne and Schneider, 2005; Etien et al., 2009; Jones and Davis, 2000; Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Urhausen et al., 2011; Webb et al., 2012; Wolfe et al., 2005). Comparisons across species and varieties, phenological stages and environments require a common measure of temperature sensitivity, defined as the change in days per ^{o}C (Wolkovich et al., 2012). Unrelated time series in Australia and Italy returned temperature sensitivities for grapevine maturity between 7 and 9 d $^{o}C^{-1}$ (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Tomasi et al., 2011). Whereas the direction of the phenological shifts is clear (i.e. early maturity), their magnitude needs experimental benchmarking; significant mismatches between warming experiments and observational data have been reported (Wolkovich et al., 2012).

Three elements contribute to the uncertainty in the reported temperature sensitivity of phenology derived from time series: (i) confounded factors in the observational data (Büntgen and Schweingruber, 2010; Ellwood et al., 2012; Freed and Cann, 2010; Keenan, 2007; Sheehy et al., 2006; Wolkovich et al., 2012), (ii) a focus on discrete events such as maturity which overlooks the thermal responses of the intervening phenostages (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Tomasi et al., 2011) and (iii) the lack of distinction between resource-driven growth and non-resource driven developmental processes. Growth of plants or plant parts is the irreversible increase in size (Loomis and Connor, 1996) whereas development is the continuous change in plant form and function with characteristic transition phases (Amzallag, 1999). Growth is closely associated with the ability of plants to capture resources including radiation, water and nutrients whereas development is primarily driven by non-resource environmental factors including temperature, photoperiod and light quality (Aphalo and Ballaré, 1995; Loomis and Connor, 1996; Monteith et al., 1994). Overlooking the role of resources, including radiation and water, has led to the overestimation of thermal effects in analysis of time series (Sheehy et al., 2006). Despite the diffuse limit between growth and development in certain phenostages, the physiological distinction between these processes is conceptually important for the attribution of warming effects to observed changes in plant phenology.

Understanding the effect of elevated temperature on the intervening phenostages up to maturity is both important for the implementation of viticultural practices and relevant for the understanding and attribution of trends in the context of climate change. For example, late pruning can be used to delay development and reduce frost risk in cool regions (Friend and Trought, 2007). Between 1952 and 1997, Bordeaux vineyards showed a significant trend for earlier harvest, but statistically undetectable trends for budburst, flowering and veraison (Jones and Davis, 2000). This means that either the approach used in that study lacked resolution to detect shifts in intermediate phenostages, or more likely, that development after veraison fully accounted for early maturity (Sadras and Petrie, 2011b). Snap-shot characterisation of plant and animal phenology is common (Loomis and Connor, 1996; Parmesan, 2006), but overlooks the temporal continuity of the phenotype (West-Eberhard, 2003). For example, the photoperiodic modulation of flowering time only makes sense when the intervening stages towards flowering are considered (Rawson and Hindmarsh, 1982).

Here we report thermal effects on grapevine phenology in three factorial experiments established in the Barossa Valley of Australia. Elevated temperature and control treatments were factorially combined with: two water regimes (exp. 1), two source:sink ratios (exp. 2) and four varieties (exp. 3). The rationale of these factorial combinations is two-fold. First, phenology is variety dependent and can be modulated by water supply and source:sink ratio (Bravdo et al., 1985; Iland et al., 2011; Pearce and Coombe, 2005). Second, these factors are embedded sources of variation in phenological time series used in climate change studies (Duchêne and Schneider, 2005; Jones and Davis, 2000; Petrie and Sadras, 2008b; Webb et al., 2012). Our aims were to (i) measure the effect of elevated temperature on grapevine phenology, (ii) evaluate variety-dependent phenological responses and interactions of thermal regime with water supply and source:sink ratio, and (iii) compare temperature sensitivity derived from time series and warming experiments.

2. Method

2.1. Experiments

The site, plant material, experimental design, growing conditions and heating methods have been described elsewhere (Sadras et al., 2012a; Sadras et al., 2012b; Sadras and Moran, 2012; Sadras and Soar, 2009). Briefly, three factorial experiments were established in vineyards of the Barossa Valley (34^{o}S, 139^{o} E), Australia. Experiment 1 combined two thermal regimes [elevated temperature *vs.* control] and two water regimes [fully irrigated *vs.* water deficit after veraison]. Experiment 2 combined two thermal regimes [elevated temperature *vs.* control] and two source:sink ratios [untreated control *vs.* increased source:sink ratio by removing 66-50% of bunches at phenological stage E-L 32 (Coombe, 1995)]. Experiment 1 used own-rooted Shiraz (clone 1654), and experiment 2 used Shiraz (clone 1654) on Paulsen 1103 rootstock, both planted in 2004. Experiment 3 combined two thermal regimes [elevated temperature *vs.* control] and four own-rooted varieties: Cabernet Franc clone Penfold 58 planted in 1988, Chardonnay clone 277 planted in 1995, Semillon clone LRC147 planted in 1989 and Shiraz clone NSW 15 planted in 1997.

In all three experiments, treatments were laid out in a split-plot design with three replicates. Thermal regime was assigned to main plot and irrigation (exp. 1) and fruit load (exp. 2) to sub-plots. In exp. 3, main plot was assigned to variety and subplot to thermal regime. Each replicate comprised nine vines, two buffer vines within each replicate and at least three further buffer vines between replicates, thus minimising border effects.

Experiment 1 used a system that combines passive and active heating (Sadras et al., 2012a) and experiments 2 and 3 used passive heating systems (Sadras and Soar, 2009). The construction, capacity and limitations of these large scale, open-top heating systems have been described in detail (Sadras et al., 2012a; Sadras and Soar, 2009). The differential thermal regimes were maintained continuously during two growing seasons and the intervening dormant period in winter (experiments 1 and 2) or during three growing seasons and the two intervening winter periods (experiment 3). Here we focus on the two seasons (2010-11 and 2011-12) common to all three experiments where phenology was intensively monitored.

2.2. Measurements and data analysis

In all three experiments, temperature was measured continuously and recorded at 15 minute intervals using shielded sensors that were located in the centre of each replicate at mid-canopy level (TinyTag Ultra2 loggers, Hastings Dataloggers, Port Macquarie, New South Wales, Australia). Figure 1 shows the thermal regimes in all three experiments. Pre-dawn leaf water potential was used to characterise the water regimes in experiment 1 (Supplementary Figure 1).

2.2.1. Phenological scales

Phenology was monitored weekly during 2010-11 and 2011-12. From budswell to veraison, phenological stage was quantified in three tagged vines per replicate (i.e. nine vines per treatment) using the E-L scale (Coombe, 1995). The dynamics of berry growth comprises two successive sigmoidal curves designated “berry formation” and “berry ripening” (Coombe and Iland, 2005). Between the beginning of berry ripening and harvest, phenology was quantified with berry sugar concentration using the method in Sadras *et al*. (2008). There was a period of ~50 d when phenology was assessed with both scales.

2.2.2. Calculation of temperature effects

Owing to the qualitative nature of the E-L scale in contrast to the quantitative characterisation of berry sugar concentration during berry ripening, we used different approaches to characterise the effect of temperature on phenology. For the period using the E-L scale, we calculated the date (date of year, DOY) when each treatment reached a certain stage. The difference in DOY between control and heated treatments was taken as a measure of temperature effect on phenology. Analysis of variance was used to assess the variation of temperature effect with developmental stage. Analysis of variance was also used to assess differences among varieties in exp. 3. Differential water regimes (exp. 1) and source:sink ratios (exp. 2) were established late during the season, hence no attempt was made to analyse these effects on early developmental stages using the E-L scale.

During the period of berry ripening, temperature effect was calculated as the difference in sugar concentration between heated and control treatments. We used piecewise regression (De los Riosa and Garcia, 2011; Freed and Cann, 2010) to characterise the relationship between temperature effect and sugar concentration in controls. Regressions were fitted to the data pooled across experiments to probe for general patterns whereas analysis of residuals (Berg and Grace, 2011; Ericsson et al., 2009; Freed and Cann, 2010; Mayo et al., 2009; Monzon et al., 2006; Sadras et al., 2003; Tang et al., 2011) was used to statistically assess the effect of water regime (exp. 1), source:sink ratio (exp. 2) and variety (exp. 3) on phenological responses to temperature.

To verify that observed differences in phenology were attributable to thermal regimes, we plotted the trajectories of berry sugar concentration on chronological and thermal-time scales. The expectation is that differences between phenological trajectories of heated and control treatments on chronological scales should disappear on a thermal time scale, unless experimental artefacts caused systematic phenological shifts (Bowen et al., 2004b). The thermal scale used a base temperature of 10°C (Bowen et al., 2004b; Williams et al., 1985)

as a convenient default, despite evidence of its variation with both variety and stage of development (Duchêne et al., 2010; Moncur et al., 1989). Phenological trajectories were statistically compared with the *F-test* of Potvin et al. (1990).

3. Results

3.1. Nonlinear effects of temperature on vine phenology between early vegetative growth and veraison

Between early vegetative growth and veraison, there was a non-linear effect of temperature on phenology; this was captured in the significant ($P < 0.0001$) variation of thermal effect with vine phenostage (Fig. 2). Four phases were apparent. First, heated vines reached stages E-L 4 (first leaf tissue visible) to E-L 9 (2-3 leaves separated) 2.4 ± 0.33 d earlier than controls. Second, between E-L 11 (4 leaves separated) and E-L 26 (100% cap fall), the difference increased to 4.4 ± 0.19 d. Third, the difference between heated and control vines increased further between E-L 27 (berry setting) and E-L32 (beginning of bunch closure). In the fourth phase after E-L 32, the difference between heated and controls declined to 2.5 ± 0.58 d at E-L 35 (veraison).

Experiment 3 allowed for the assessment of variety-dependent responses to temperature. Temperature effect was significantly different among varieties ($P = 0.016$) and varied with phenological stage ($P < 0.0001$) but was unaffected by the interaction of variety and phenological stage ($P = 0.99$); averaged across stages, the thermal effect on phenology was largest for Semillon, smallest for Shiraz and intermediate for Cabernet Franc and Chardonnay (Fig. 3a).

3.2. Nonlinear effects of temperature on vine phenology during berry ripening

Figure 4 illustrates the trajectories of berry sugar concentration on chronological and thermal time scales. On a chronological scale, sugar concentration in control berries lagged behind that of their heated counterparts during the phase of rapid sugar accumulation (left panels Fig 4). On a thermal time scale, the trajectories largely overlapped (right panels in Fig. 4). Applying Potvin et al. (1990) *F-test* to all experiments, the trajectories of berry sugar concentration on a thermal time scale for heated and control treatments overlapped ($P > 0.05$) in 14 out of 16 cases (= 2 water regimes x 2 seasons in exp. 1; 2 source:sink ratios x 2 seasons in exp. 2, 4 varieties x 2 seasons in exp. 3).

Piecewise regression between temperature effect and phenological stage in controls returned a two-phase model with $R^2 = 0.43$ ($P < 0.0001$) (Fig. 5). Temperature effect increased during the lag-phase and peaked when controls reached 7.6 ± 0.37 ^{o}Brix (vertical dashed line in Fig. 5). Temperature effect declined gradually between the onset of rapid sugar accumulation and maturity (Fig. 5).

Experiment 1 allowed for the interaction between thermal and water regimes. Despite significant effects of water regime on plant water status (Supplemental Figure 1),

temperature effects on development during berry ripening under full irrigation and water deficit were statistically undistinguishable (P > 0.14).

Experiment 2 allowed for the interaction between temperature and source:sink ratio. The dotted horizontal line in Fig. 5 accounts for the lack of response (slope not different from zero, P = 0.47) in Shiraz vines where severe fruit thinning increased source:sink ratio; excluding these data the R^2 of the fitted model increased slightly (from 0.42 to 0.47; both P < 0.0001) but the threshold did not change (7.61 ± 0.372 for the complete data set vs 7.59 ± 0.347 with the excluded data). Analysis of residuals of the model fitted in Fig. 5 was used to further explore the interaction of source:sink ratio and thermal regime on vine phenology. Temperature effect on development during berry ripening was larger in vines where fruit removal increased source:sink ratio, and increased in the second cycle of fruit load reduction (Fig. 6). The attribution of increasing thermal effect to cycles of fruit load reduction, rather than seasonal conditions, is supported by the lack of seasonal effects on experiments 1 and 3 (P = 0.97).

Experiment 3 allowed for the variety-dependent responses to temperature. Analysis of residuals showed a variety-dependent thermal effect on phenology during berry ripening that ranked Semillon > Cabernet Franc ≈ Chardonnay > Shiraz (Fig. 3b).

3.3. Temperature-driven shifts in phenology moved the timing of subsequent events into relatively cooler conditions

Wheat modelling studies showed that early flowering associated with warming during the last half of the 20th century shifted the post-flowering period into cooler conditions (Sadras and Monzon, 2006). We thus explored the consequences of temperature-driven shifts in two events, beginning of flowering and onset of sugar accumulation, on the temperature immediately after these events. A hypothetical 7 d shift in phenology caused by elevated temperature would shift the timing of subsequent events into relatively cooler conditions (Fig. 7a). The magnitude of the shift in temperature after the event was proportional to the size of the phenological shift and, owing to the seasonal pattern of ambient temperature, stronger in spring than in summer (Figure 7bc).

3.4. Mismatch between warming experiments and time series

Temperature sensitivity of grapevine maturity derived from long-term phenological records is likely to be over-estimated when other sources of variation are not considered (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Tomasi et al., 2011). Comparison of the actual shift in maturity in our experiments with predictions based on reported temperature sensitivity highlights the magnitude of this bias (Table 1). In five out of six cases, the actual shift was 15-44% of the shift predicted from a conservative rate of 6.6 ± 0.92 d $^{o}C^{-1}$. The only case where actual shifts were commensurate with expectations from time series corresponded to the vines where the thermal effect was enhanced by repeated fruit removal that increased the source:sink ratio (experiment 2, 2011-12 in Table 1).

4. Discussion

Climatology is an observational science whereas biology is primarily experimental except at the higher levels of organisation or large time scales (Barnosky et al., 2012). At the interface, where questions are asked about the impacts of climate change on biological systems, a large body of research using long-term records and modelling, supported by theory, makes a strong case for environmentally-driven shifts in plant and animal phenology in diverse natural and agricultural ecosystems. From a biological viewpoint, however, experiments are essential to prove cause-and-effect relationships and to quantify the thermal responses of growth and development. Manipulation of temperature in laboratory settings is straightforward but logistics and cost constrain the modification of thermal regimes of vegetation in the field. Here we used open-top chambers to increase ambient temperature in vineyards, and intensively monitored vine phenology between the break of winter dormancy and maturity.

4.1. Nonlinearity of phenological responses to temperature

We present experimental evidence of nonlinearity in plant phenological responses to temperature (Figures 2 and 5). Comparison of phenological trajectories in plants exposed to elevated temperature and controls under current temperature suggests three basic modes. Divergent trajectories would reflect increasing effects of temperature with advanced development. Parallel trajectories would result from an initial response to temperature that is maintained during a certain phenological range (e.g. horizontal line in Fig. 5). Convergent trajectories would reflect an initial effect that is dampened as phenology progresses. Nonlinearity would therefore emerge from the combination of these three basic modes.

Two complementary explanations are advanced to account for the observed nonlinearity in the phenological response to temperature. First, warming-induced advancement of sensitive phenological events can lead to cooler conditions for subsequent growth and development that partially dampens the effect of warming (Fig. 7). This phenomenon was previously identified in wheat modelling studies where warming-driven early flowering shifted the post-flowering period into cooler conditions, effectively cancelling warming effects between flowering and maturity (Sadras and Monzon, 2006). Conversely, elevated temperature could shift events onto warmer conditions during the part of the season with declining temperatures (Fig 7a), hence reinforcing the effects of warming. Second, a physiological perspective suggests that nonlinearity may partially emerge from the interplay between temperature-driven development and resource-driven growth. Divergent (or parallel) trajectories are expected for phases where temperature-driven development dominates over resource-driven growth. A condition for convergence is that resource availability dampens thermal effects, and this is more likely when resource-driven growth processes are dominant. Using a quantitative scale for phenology during berry ripening and the combination of temperature and source:sink ratios in experiment 3 allowed to evaluate this hypothesis. Temperature effect during the ripening period had a divergent phase, reflected in an increasing thermal effect, which peaked when controls reached 7.6 ^{o}Brix (Fig. 5); this is close to the ~8 ^{o}Brix threshold that signals the onset of rapid sugar accumulation and has been shown to be highly responsive to temperature (Ezzahouani, 2003; Sadras and Petrie, 2011a). Consistent with this finding, the trajectories of sugar concentration in individually-heated bunches of Sultana grapes and those of untreated controls diverged sharply at the onset of rapid increase in sugar concentration and converged afterwards (Radler, 1965). After the peak thermal effect, the trajectories of heated and control treatments converged

leading to relatively small differences in maturity time (Fig. 5, Table 1). Consistent with our hypothesis, resource availability is a stronger driver of sugar accumulation in berries (Bernizzoni et al., 2011; Bindi et al., 2001b; Bravdo et al., 1985; Coombe et al., 1987; Esteban et al., 1999; Freeman et al., 1980; Guidoni et al., 2002; Sadras et al., 2008; Wang et al., 2003) than temperature (Buttrose et al., 1971; Ezzahouani, 2003; Sadras and Petrie, 2011b). Thermal effects on development during berry ripening were significantly larger in vines where fruit removal increased source:sink ratio, and increased in the second cycle of fruit load reduction (Figure 6). This supports the sequence: fruit removal → increased source:sink ratio → relaxed resource constraint → enhanced thermal effect. Interestingly, a similar interaction was reported for cherry tomato whereby thermal effects on fruit sugar content were smaller in plants with higher source:sink ratio (Gautier et al., 2005). Models that only account for thermal effects on phenology are therefore likely to be biased under conditions of varying;

Irrespective of the causes, the nonlinearity of phenology in response to temperature is important for the broader issue of phenological shifts in association with climate change and their ecological and agricultural consequences. Further insight in other plant-based systems can be gained by shifting the emphasis from discrete events such as flowering to the continuous characterisation of phenological trajectories.

4.2. Variety-dependent responses

For development up to veraison in experiment 3, the thermal response was largest in Semillon, smallest in Shiraz and intermediate in Chardonnay and Cabernet Franc. Using different scales and different methods to estimate thermal effects, the same ranking was observed for development during berry ripening. These were likely legitimate variety-dependent differences in phenological responses to temperature, in contrast to studies where varieties are confounded with soils, management and other climate factors. This conclusion is supported by two aspects of our experiments: all four varieties were established in the same soil and managed with the same practices since planting, and thermal regimes were manipulated with small secondary effects (Sadras and Soar, 2009).

4.3. Mismatch between warming experiments and time series

The mismatch between phenological shifts in warming experiments and long-term time series in this paper (Table 1) is qualitatively and quantitatively similar to the mismatch found in the comprehensive meta-analysis of Wolkovich et al. (2012). They identified two sources for the discrepancy: “complex interactions among multiple drivers in the observational data” and experimental artefacts. Both sources are likely to be important, they are not mutually exclusive and their relative importance depends on the particular case study. Long-term acclimation, implicit in time series, was not captured in our study. We are inclined to conclude, however, that thermal sensitivities between 6.6 and 9.8 d $^{o}C^{-1}$ derived from time series over-estimate temperature-driven maturity shifts for three reasons. First, our large-scale, open-top heating systems overcame many of the common secondary effects associated with smaller chambers used in ecological studies, where soil drying and border effects are often significant (Marion et al., 1997; Sadras et al., 2012a; Sadras and Soar, 2009). Moreover, direct comparison of developmental trajectories in chronological and

thermal time scales allowed to confidently excluding large experimental artefacts for the critical ripening and maturity stages. Second, the attribution of cause and effect in time series is not trivial; multiple interpretations and misinterpretations are common (Büntgen and Schweingruber, 2010; Ellwood et al., 2012; Freed and Cann, 2010; Keenan, 2007; Sheehy et al., 2006). In particular, temperature sensitivity of grapevine maturity has been derived from regressions of maturity change vs temperature change, hence neglecting other sources of phenological shift (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Tomasi et al., 2011). Third, phenological studies using time series oversimplify the biology of the plant; this includes snap-shot characterisations (e.g. maturity) that ignore the temporal continuity of the phenotype (West-Eberhard, 2003), and the lack of distinction between temperature-driven developmental processes and resource-driven growth processes.

Interpretation of mismatches between warming experiments and time series needs case-specific appraisal of the sources of error in both observational and experimental data.

Acknowledgements

The insights of one of the referees on the limitations of the E-L phenological scale significantly improved this paper. This work was funded by the Grape and Wine Research and Development Corporation, Department of Agriculture, Fisheries and Forestry and Complementary State NRM Program. Rod Bubner helped to establish the experiments and Treva Hebberman maintained the vineyard.

Table 1. Comparison of temperature-driven shifts in time of maturity of grapevines measured in field trials and the shifts expected from thermal rates derived from time-series analyses. Temperature difference is the actual increase in temperature in heated vines relative to untreated controls during the growing season (1st September to 1st March). Both actual and expected shifts were based on an objective measure of maturity set at sugar concentration in grapes = 21.6 °Brix (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Webb et al., 2012). Reported rates of shift in maturity derived from time series are 6.6 ± 0.92 d $^{o}C^{-1}$ (Petrie and Sadras, 2008b), 8 d $^{o}C^{-1}$ (Tomasi et al., 2011) (no error reported) and 9.8 ± 0.94 d $^{o}C^{-1}$ (Sadras and Petrie, 2011a). Expected shift was calculated as the product between the lower and upper rates in this range and actual temperature differences.

Season	Exp.	Temperature difference (°C)	Actual shift (d)		Expected shift (d)	
			mean	range	lower	upper
2010-11	1	1.5 ± 0.04[a]	1.5	0.3 to 2.6	9.6 ± 1.37	14.3 ± 0.4
	2	0.6 ± 0.23	1.2	0 to 2.3	4.3 ± 1.61	6.4 ± 2.32
	3	1.0 ± 0.16	3.0	-0.6 to 4.3	6.7 ± 1.40	9.9 ± 1.82
2011-12	1	0.9 ± 0.04	-1.4	-2.3 to -0.6	5.8 ± 0.86	8.7 ± 0.93
	2	0.9 ± 0.03	8.3	5.3 to 11.3	5.9 ± 0.85	8.8 ± 0.90
	3	1.0 ± 0.03	2.1	0.6 to 3.3	6.6 ± 0.94	9.9 ± 0.98

[a]standard error

Captions to figures

1. (a-c) Comparison of mean daily temperature in heated and control treatments in three experiments in the Barossa Valley. (d-f) Frequency distribution of temperature difference between heated and control treatments; SD is the standard deviation. Temperature was measured at canopy level. Data from experiment 1 (a, d), 2 (b, e) and 3 (c, f).
2. Effect of temperature on grapevine phenology between stages E-L 4 (first leaf tissue visible) and E-L 35 (veraison). Temperature effect is the time difference between heated and control vines to reach a given phenological stage. Data are means across three experiments and two seasons. Error bars are two standard errors.
3. Variety-dependent effect of temperature on grapevine phenology (a) between stages E-L 4 (first leaf tissue visible) and E-L 35 (veraison) and (b) during berry ripening. In (a) temperature effect is the mean time difference between heated and control vines to reach a phenological stage. In (b) temperature effect is the mean difference in berry sugar concentration between heated and control treatments. SEM is Semillon, CAF is Cabernet Franc, CHR is Chardonnay and SHI is Shiraz. Error bars are two standard errors.
4. Trajectories of berry sugar concentration on chronological (left panels) and thermal time scales (right panels) for control (open symbols) and heated treatments (closed symbols). Error bars are two standard errors. The top-left panel shows analysis of variance for each sampling date; the significance of main sources of variation is $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). F values test the overlap between the curves using the method of Potvin et al. (1990). Data from experiment 3, 2010-11.
5. Effect of temperature on grapevine phenology during berry ripening. Temperature effect is the difference in sugar concentration between heated vines and controls. The solid line is the piecewise linear regression; the vertical line shows the break point. The dotted horizontal line is explained in the text.
6. Temperature effect on vine phenology during berry ripening depended on source:sink ratio. A bilinear model, similar to that in Fig. 5, was fitted to the data for experiment 2, which combined two thermal regimes and two source:sink ratios during two seasonal cycles of fruit load manipulation ($R^2 = 0.41$, $P < 0.0001$). The residuals of the model were (a) larger for vines with higher source : sink ratio, and (b) increased with repeated fruit load reduction. Error bars are two standard errors.
7. Temperature-driven shifts in phenology move the timing of subsequent events into relatively cooler conditions. (a) Shifts in ambient temperature (grey arrows) associated with shifts in phenology (black arrows) at two times during the growing season. Circles are the long-term (1957-2011) daily temperature at Nuriootpa (mean ± standard error). The closed square is the actual time of flowering and the closed triangle is the actual time of onset of rapid sugar accumulation in berries for control treatments (averages across three experiments and two seasons). The open square and open triangle represent 7 d shifts in phenology under elevated temperature. The rate of change in temperature (□T) is higher in spring (October) than in summer (January). (b, c) Actual shifts in ambient temperature associated with measured, temperature-driven shifts in phenology for two key stages: beginning of flowering and onset of rapid increase in sugar concentration in berries. Shift in temperature is the difference between the elevated temperature and control treatments in mean temperature during the five days post-event. Error bars are two standard errors.

Fig. 1

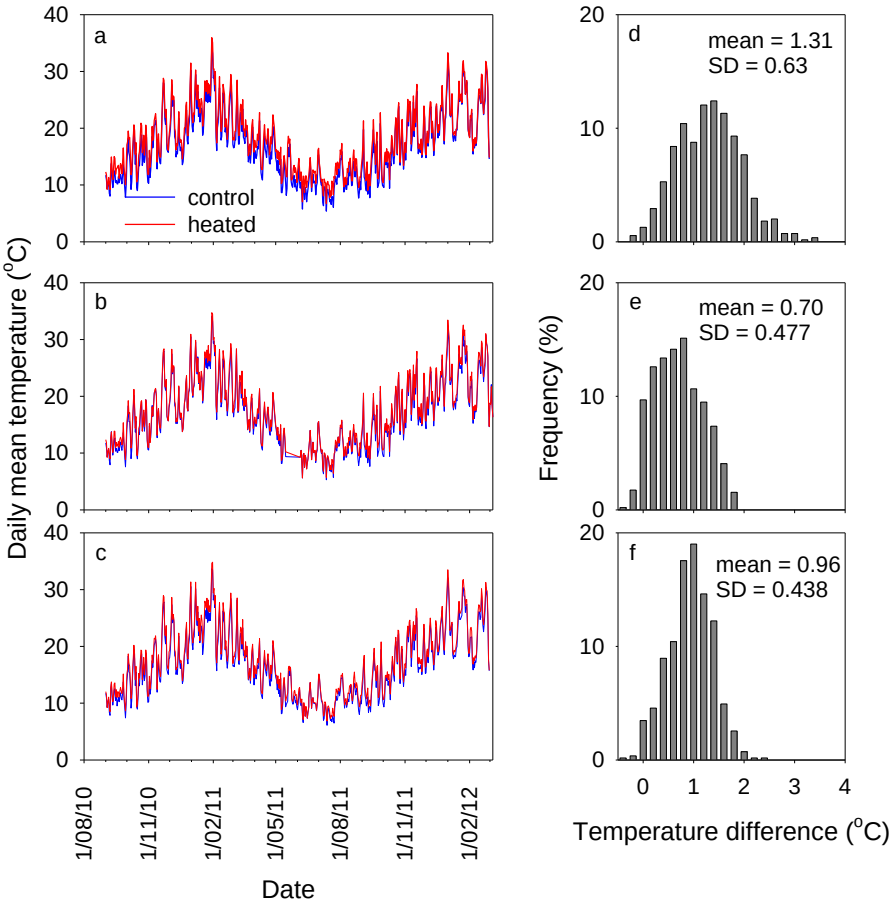

a
b
c
d
e
f
control
heated
mean = 1.31
SD = 0.63
mean = 0.70
SD = 0.477
mean = 0.96
SD = 0.438
Daily mean temperature (°C)
Frequency (%)
Temperature difference (°C)
Date
1/08/10
1/11/10
1/02/11
1/05/11
1/08/11
1/11/11
1/02/12

Fig. 2

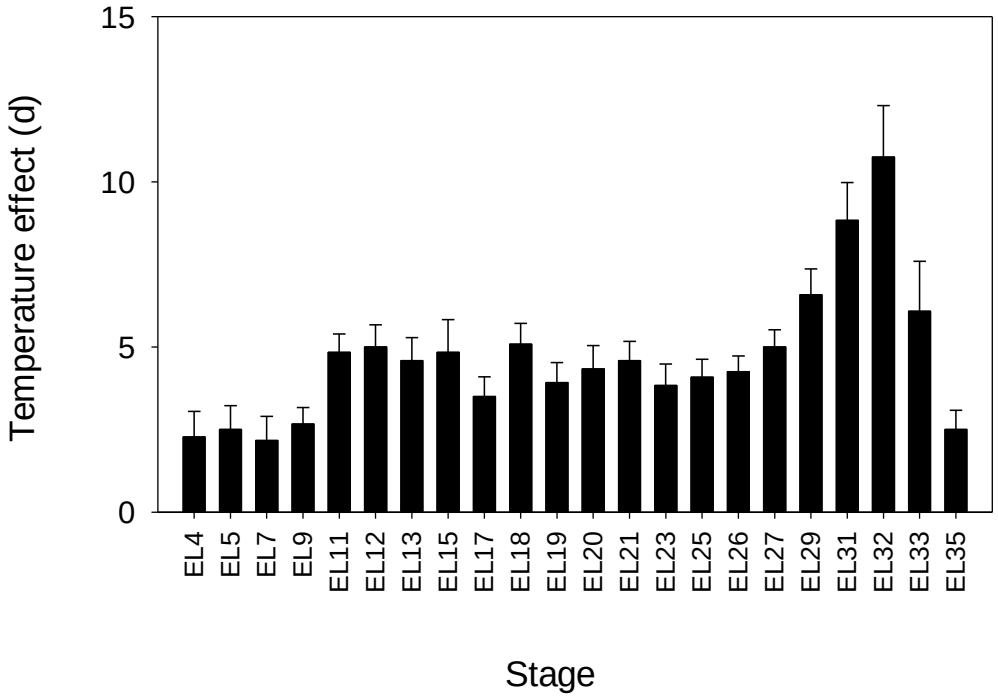

Temperature effect (d)
15
10
5
0
EL4
EL5
EL7
EL9
EL11
EL12
EL13
EL15
EL17
EL18
EL19
EL20
EL21
EL23
EL25
EL26
EL27
EL29
EL31
EL32
EL33
EL35
Stage

Fig. 3

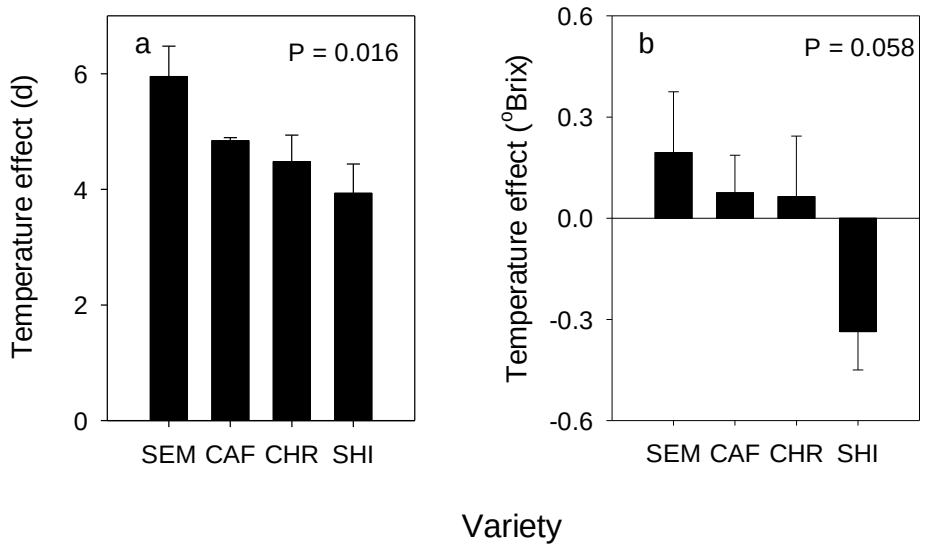

a
P = 0.016
Temperature effect (d)
0
2
4
6
SEM CAF CHR SHI
b
P = 0.058
Temperature effect (°Brix)
-0.6
-0.3
0.0
0.3
0.6
SEM CAF CHR SHI
Variety

Fig. 4

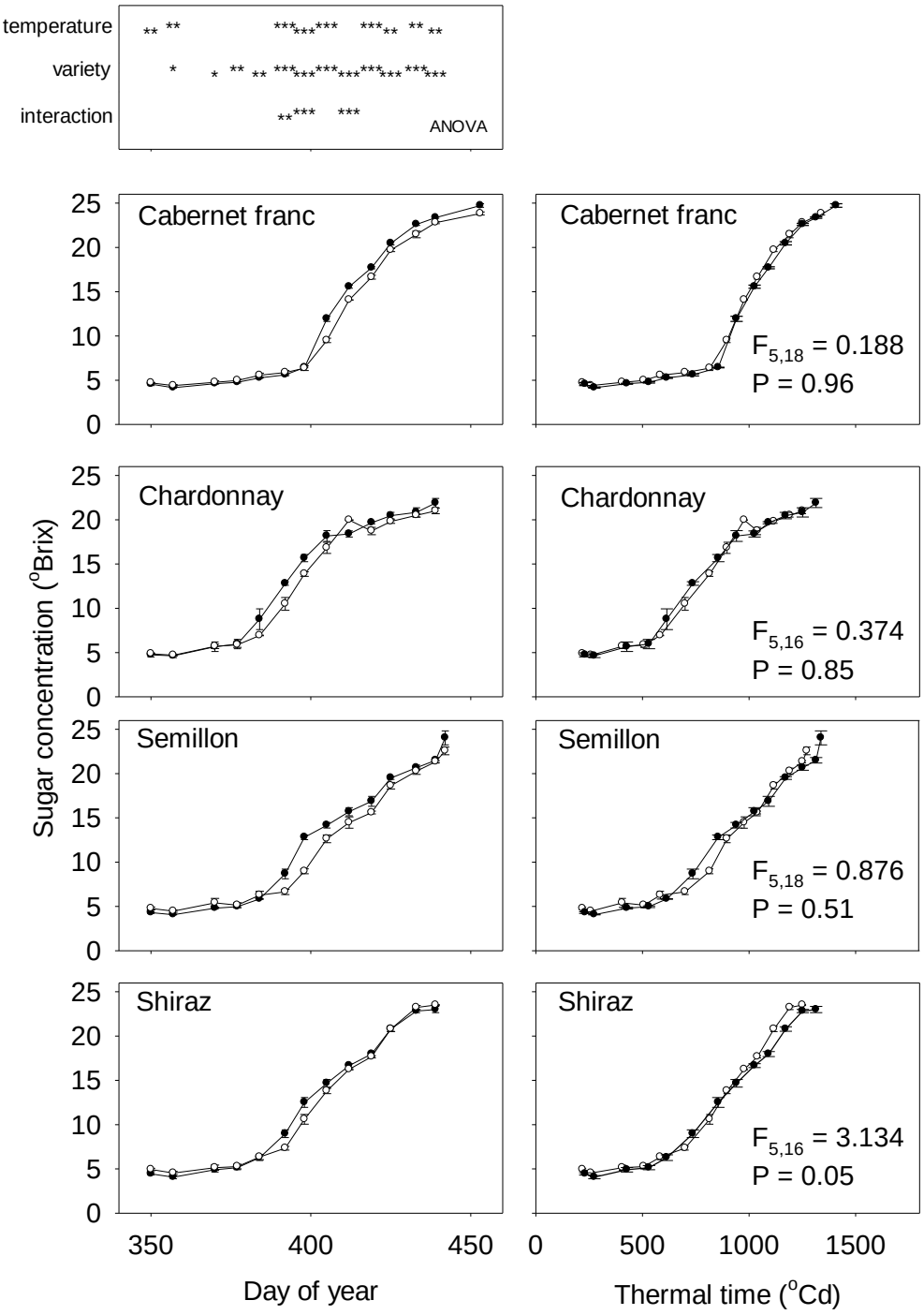

temperature
variety
interaction
ANOVA
Cabernet franc
Chardonnay
Semillon
Shiraz
$F_{5,18}$ = 0.188
P = 0.96
$F_{5,16}$ = 0.374
P = 0.85
$F_{5,18}$ = 0.876
P = 0.51
$F_{5,16}$ = 3.134
P = 0.05
Sugar concentration (°Brix)
Day of year
Thermal time (°Cd)

Fig. 5

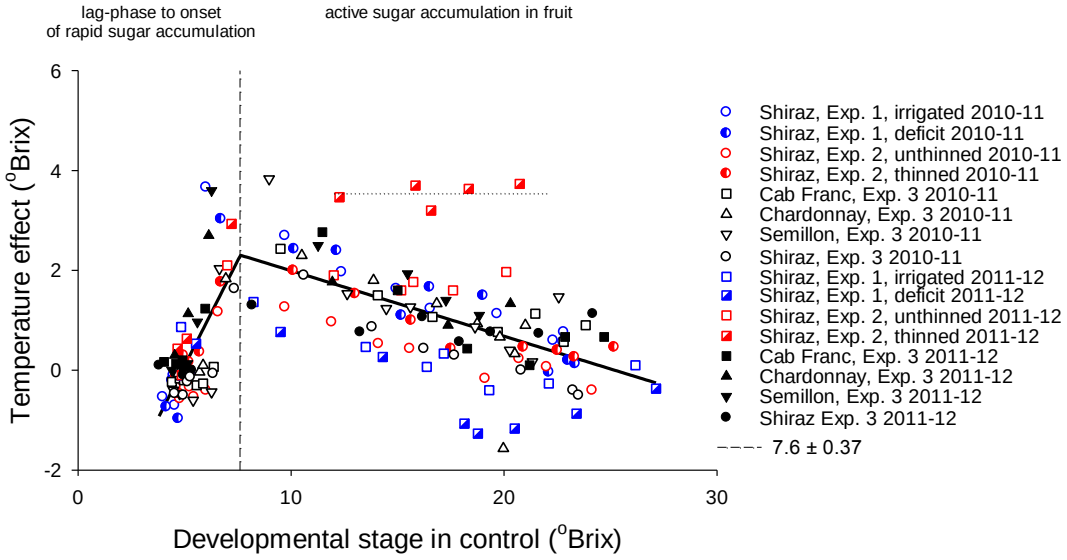

lag-phase to onset
of rapid sugar accumulation
active sugar accumulation in fruit
Temperature effect (°Brix)
Developmental stage in control (°Brix)
6
4
2
0
-2
0
10
20
30
Shiraz, Exp. 1, irrigated 2010-11
Shiraz, Exp. 1, deficit 2010-11
Shiraz, Exp. 2, unthinned 2010-11
Shiraz, Exp. 2, thinned 2010-11
Cab Franc, Exp. 3 2010-11
Chardonnay, Exp. 3 2010-11
Semillon, Exp. 3 2010-11
Shiraz, Exp. 3 2010-11
Shiraz, Exp. 1, irrigated 2011-12
Shiraz, Exp. 1, deficit 2011-12
Shiraz, Exp. 2, unthinned 2011-12
Shiraz, Exp. 2, thinned 2011-12
Cab Franc, Exp. 3 2011-12
Chardonnay, Exp. 3 2011-12
Semillon, Exp. 3 2011-12
Shiraz Exp. 3 2011-12
7.6 ± 0.37

Fig. 6

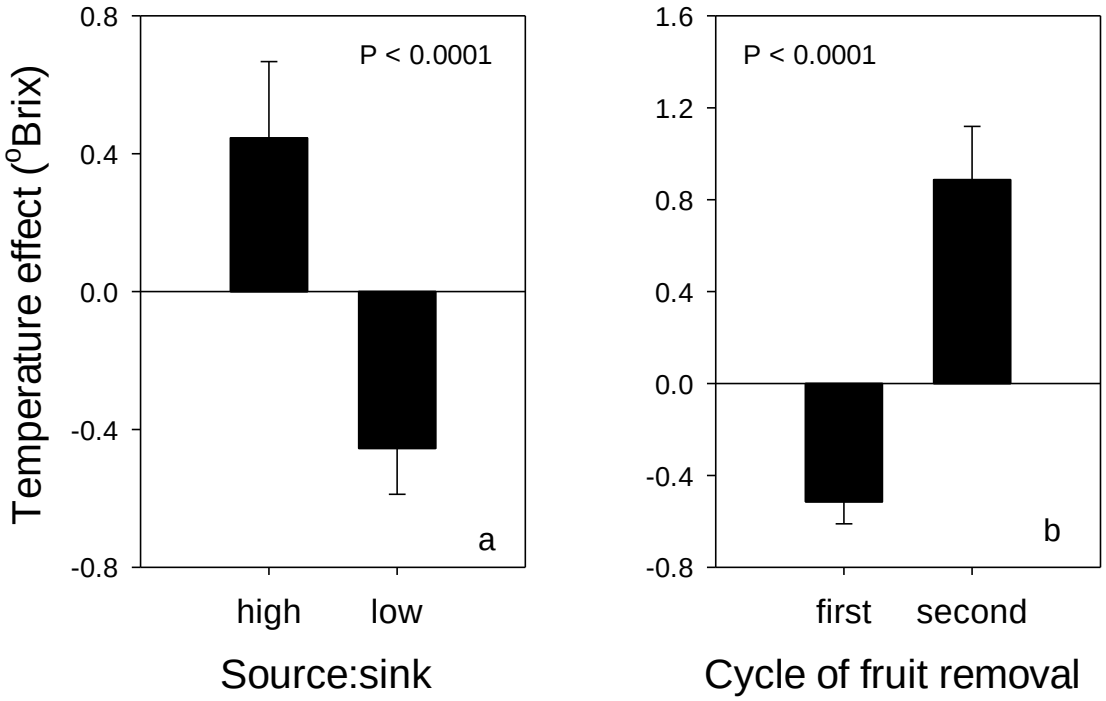
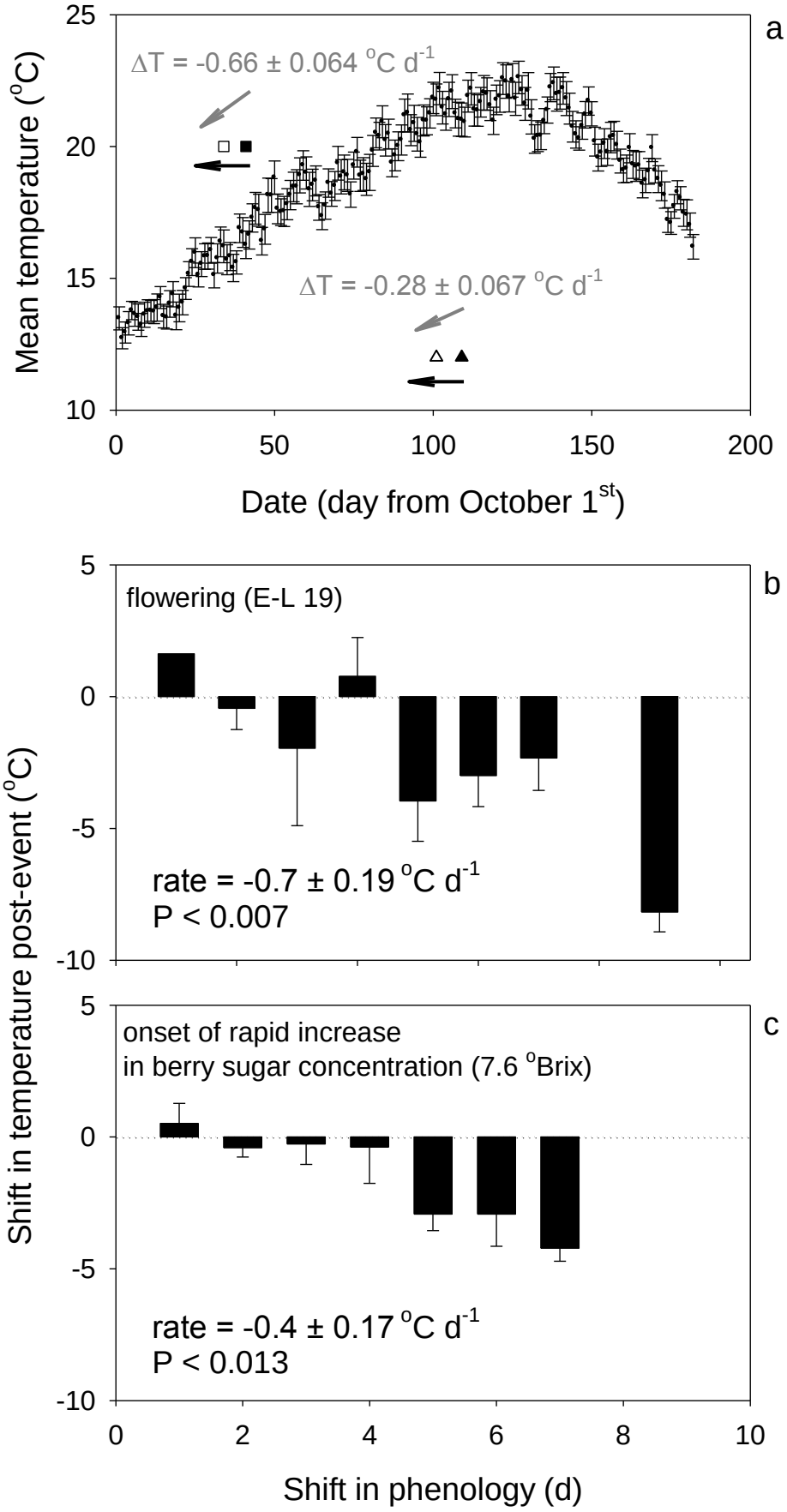
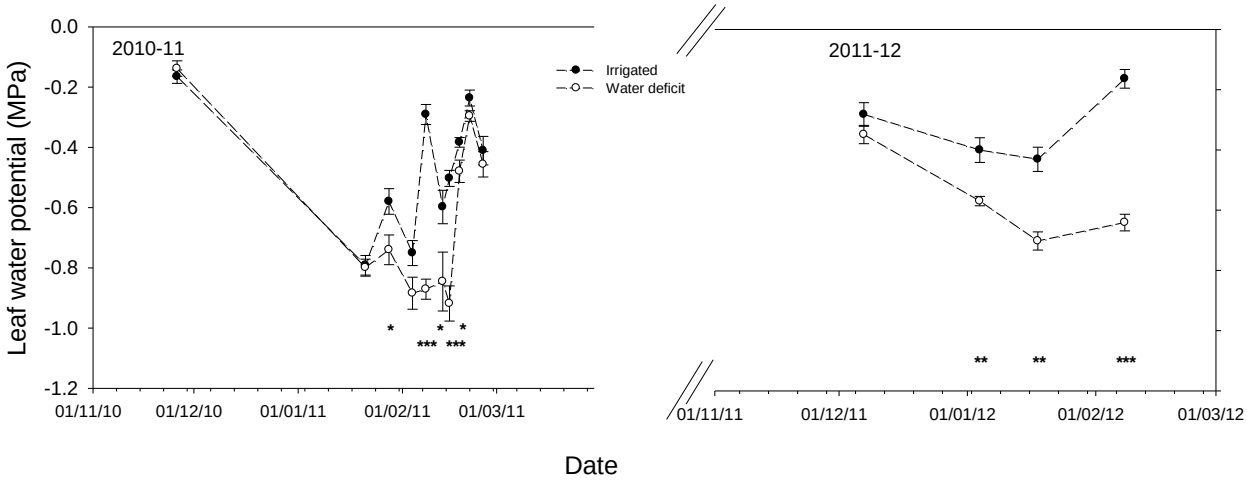

0.8
0.4
0.0
-0.4
-0.8
Temperature effect (°Brix)
P < 0.0001
a
high
low
Source:sink
1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
P < 0.0001
b
first
second
Cycle of fruit removal

Fig. 7


a
ΔT = -0.66 ± 0.064 °C d-1
ΔT = -0.28 ± 0.067 °C d-1
Mean temperature (°C)
Date (day from October 1st)
b
flowering (E-L 19)
rate = -0.7 ± 0.19 °C d-1
P < 0.007
c
onset of rapid increase
in berry sugar concentration (7.6 °Brix)
rate = -0.4 ± 0.17 °C d-1
P < 0.013
Shift in temperature post-event (°C)
Shift in phenology (d)



S**upplementary figure** 1. Effect of irrigation treatments on pre-dawn leaf water potential during berry ripening in experiment 1. Asterisks indicate significant differences between treatments at P < 0.05 (*), P < 0.01 (**) and P<0.0001 (***). Data for 2010-11 were published in Sadras and Moran (2012); here they are reproduced to allow for comparison of the two seasons.

Chapter 3. Asymmetric warming effect on the yield and source:sink ratio of field-grown grapevine

VO Sadras, MA Moran

Abstract

Using large-scale open-top chambers to approximate the thermal regimes of the Barossa Valley in the next decades, we measured yield and vegetative growth of grapevines in three factorial field experiments combining two thermal regimes (elevated temperature *vs* control) with: two water regimes (exp. 1), two source:sink ratios generated by manipulation of fruit load (exp. 2) and four varieties (exp. 3). Water regime, source : sink ratio and varieties are sources of variation typically embedded in climate change studies using time series.

Yield of controls ranged from 1.2 to 10.5 kg vine^{-1}. Warming had asymmetric effects on fruit yield and fruit/pruning weight ratios: yield response to elevated temperature varied from 46% reduction to 177% increase in relation to controls, and the response of fruit/pruning weight ratio varied from 56% reduction to 145% increase. Yield response to temperature was primarily explained by the response of bunch number (r^2=0.92, $P < 0.0001$). The effect of elevated temperature on fruit/pruning weight ratio was closely related to the response of fruit yield (r^2=0.79, $P < 0.0001$) and unrelated to the response of pruning weight ($P = 0.18$). Elevated temperature reduced concentration of starch in trunks but not in roots.

We conclude that the asymmetric responses of yield and fruit/pruning weight ratios resulted from seasonal influences on warming effects, whereby the largest responses of these traits were mediated by the promotion of bunch formation following relatively low temperatures in the preceding season. This highlights the need to assess warming effects in the light of both the background temperature and the distribution of thermally contrasting season in particular locations. Lower source : sink ratio and reduced starch concentration in trunks are two important elements of the carbon balance of the vine with long-term physiological and viticultural implications.

1. **Introduction**

Way and Oren (2010) reviewed the response to temperature of trees in a meta-analysis that allowed for functional groups and biomes, but lacked detail at the level of species. They concluded that elevated temperature enhanced growth in deciduous species more than in evergreen trees and in temperate and boreal species more than in their tropical counterparts. Putative mechanisms of growth enhancement included increased net photosynthesis, from stronger acclimation of respiration relative to gross photosynthesis, and an increased partitioning of resources to leaves at the expense of roots. According to the hypothesis of Way and Oren (2010), warming may reduce grapevine yield in hot environments and enhance yield in cold environments, provided water supply is not compromised.

Our understanding of yield response to temperature in grapevine is fragmented. Yield components including bunches per vine, flowers per bunch, berry set and berry size are all responsive to temperature (Dunn, 2005; Ezzahouani, 2003; Keller et al., 2010; Kliewer, 1977; Petrie and Clingeleffer, 2005). The actual response, however, depends on the range of temperature, yield component and variety. Furthermore, environmental influences on key yield components span two successive seasons and compensation among yield components is widespread. Inflorescence primordia and first order branching of inflorescences takes place during spring in buds that enter dormancy and unfold in the following growing season (Iland et al., 2011). In the study of Ezzahouani (2003), increased daytime temperature did not affect the yield of Danlas grapevine despite a significant reduction in berries per bunch that was fully compensated by larger berries. Flower number per inflorescence decreased but flower size, fruit set, and berry size increased with higher temperature in field experiments where dormant buds and young shoots of Cabernet Sauvignon were exposed to mean temperature between 10 and 19 ^{o}C (Keller et al., 2010). Increasing temperature from 20 to 30 ^{o}C increased the weight of bunch primordia 4-fold in Riesling, but did not change the size of bud primordia in Shiraz (Dunn, 2005). One or two week exposure to elevated daytime temperature at critical phenostages did not alter yield or yield components of irrigated Shiraz in the Barossa Valley (Sadras and Soar, 2009) and irrigated vineyards can return high yields (> 15 t ha^{-1}) in hot regions (Iland, 2005).

Carbohydrate reserves in root and trunk play at least two roles. Early in the growing season, reserves allow for the initial burst of growth (Field et al., 2009). Later in the season, reserves may contribute to buffer berry growth under unfavourable conditions such as low irradiance and defoliation (Iland et al., 2011; Keller et al., 1998). Long-term effects of increased temperature on carbohydrate reserves are unknown. High temperature may extend the period between harvest and leaf fall, hence increasing the potential for reserve accumulation but the increase in respiration with high temperature may accelerate depletion of reserves (Field et al., 2009; Huang et al., 2005; Rogiers et al., 2011). Hence, the effect of temperature on reserves could be negative, neutral or positive depending on the magnitude and direction of temperature effects on individual components of the carbon budget, the pattern of resource allocation and acclimation processes (Atkin et al., 2000; Atkin et al., 2007; Field et al., 2009; Way and Oren, 2010).

We have established three factorial field experiments in the Barossa Valley of Australia to investigate the effect of thermal regime (elevated temperature *vs* control) interacting with water supply, fruit load or variety. Here we present the responses of yield components,

vegetative growth and source:sink ratio. Complementary measurements of starch concentration in trunks and roots are also reported.

2. Methods

2.1. Experiments

Plant material, growing conditions, and experimental design have been described elsewhere (Sadras et al., 2012b; Sadras and Moran, 2012a; Sadras and Moran, 2012b). Briefly, we established three factorial experiments in the Barossa Valley of Australia (34^{o}S, 139^{o} E) where two temperature regimes were combined with two water regimes in experiment 1, two fruit loads in experiment 2 and four varieties in experiment 3. Thermal regimes were untreated controls and elevated temperature achieved using a combination of active and passive heating with the open-top chambers described in Sadras et al. (2012a) for experiment 1 and the passive open-top chambers described in Sadras and Soar (2009) for experiments 2 and 3. The thermal regimes were maintained for two growing seasons (2010-11, 2011-12) and the intervening winter in experiments 1 and 2 and three growing seasons (2009-10, 2010-11, 2011-12) and the two intervening winters in experiment 3.

Experiment 1 used own-rooted Shiraz (clone 1654) planted in 2004; water regimes were: irrigated, and water deficit after veraison; the timing, intensity and magnitude of water deficit were characterised with pre-dawn leaf water potential reported in a companion paper (Sadras and Moran, 2012b). Experiment 2 used Shiraz (clone 1654) on Paulsen 1103 rootstock planted in 2004; fruit loads were: untreated controls and thinned vines, where 2/3 (2011) or ½ (2012) of bunches were removed at phenological stage E-L 32 (Coombe, 1995). Experiment 3 compared own-rooted Cabernet Franc clone Penfold 58 planted in 1988, Chardonnay clone 277 planted in 1995, Semillon clone LRC147 planted in 1989 and Shiraz clone NSW 15 planted in 1997.

In all three experiments, treatments were laid out in a split-plot design with three replicates. In experiment 1, main plot was allocated to temperature treatments and subplots to water regime. In experiment 2 main plot was allocated to temperature and subplots to fruit loads. In experiment 3, main plot was allocated to variety and sub-plots to temperature treatments. Each replicate comprised nine vines, allowing for sampling in seven central vines, two buffer vines within each replicate and at least three further buffer vines between replicates.

2.2. Vine traits

We measured the following traits: fruit yield per vine and its components (bunch number per vine, number of berries per bunch, bunch weight and berry weight), pruning weight per vine and its components (number of shoots per vine, weight per shoot) and concentration of starch in trunk and root during the dormant season.

Weekly berry samples were taken to monitor the dynamics of berry weight and total soluble solids (Sadras and Moran, 2012a). Using the trajectories of total soluble solids, we targeted harvest at ~ 22 ^{o}Brix for Chardonnay and Semillon and ~ 24 ^{o}Brix for Shiraz and Cabernet Franc (Iland et al., 2011). All bunches from the central seven vines in each replicate were harvested and weighted to determine fruit yield per vine. Average bunch weight was calculated from total yield and bunch number. Average number of berries per bunch was calculated from bunch weight and berry weight. After leaf fall early in winter, pruning weights were derived from the same seven vines per replicate which were spur-pruned, leaving between 35 to 40 buds per plant. In 2010-11, rainy winter lead to vigorous vegetative growth, hence the need to trim the canopies of experiments 1 and 2 in summer; using a 1-m rod, we removed shoot lengths in excess of 1 m. In both summer and winter pruning, shoots were counted and fresh weights determined for each replicate. The ratio of fruit weight and pruning weight is widely used in viticultural management and was taken here as a coarse estimate of sink:source ratio (Iland et al., 2011).

In 2010-11, environmental conditions favoured *Botrytis* infection (Smith, 2011); despite regular spraying of fungicides, disease symptoms were evident in experiment 3. We thus separated bunches and bunch sections with visible mould growth from those with no visible mould. The fresh weight ratio between Botrytis affected fruit and total fruit was taken as a measure of disease incidence.

Starch concentration was measured on trunk and root samples taken during one (experiments 1 and 2) or two (experiment 3) seasons during the dormant period in winter (late July). Trunk samples were taken 0.4 m above ground level using an increment borer after removing the bark from the section to be sampled. Roots were excavated and a length of ~5 cm was snipped from the root ball at a depth ~0.4 m. Trunk and root samples were kept at -40º C prior freeze dried. Subsequently, samples were grinded into powder and analysed through MEGAZYME “Total Starch Assay Procedure”, no. K-TSTA (www.megazyme.com) using the protocol described in Edwards et al. {, 2011 #15025}.

We used analysis of variance to assess the effects of main experimental sources of variation and their interactions on measured traits for each experiment. To probe for patterns using the data pooled across experiments, we calculated temperature effect as 100 x (H-C)/C, where H is the trait in the heated treatment and C is the trait in the control.

3. Results

3.1. Thermal regimes and seasonal conditions

In relation to controls, temperature at mid-canopy level in the heated treatment was incremented by 1.61 ^{o}C (SD = 0.630) in experiment 1, 0.70 ^{o}C (SD = 0.477) in experiment 2 and 0.96 ^{o}C (SD = 0.438) in experiment 3 (Sadras and Moran, 2012b). Noticeable seasonal differences in temperature included the cooler spring (September-November) of 2010, which was below the long-term median, in comparison with 2009 and 2011, which were above the median (Fig. 1).

3.2. Yield

3.2.1. Experiment 1: temperature and water regime

The effects of temperature, water regime and their interaction on yield and its components are summarised in Tables 1 (2010-11) and 2 (2011-12). In 2010-11, water deficit reduced total fruit weight by 16-33%; the effects of temperature and water by temperature interaction were not significant. Despite regular fungicide applications, the incidence of Botrytis in controls was 24-34% compared to 3-5% in heated vines. As a consequence, the weight of fruit with no visible symptoms, increased by about 20% in heated vines relative to controls. Healthy bunches in heated vines had 15-17% more berries than controls, and berries were 3-6% heavier. None of these responses was significant, but aggregated to produce 20-24% heavier bunches which accounted for the higher yield in heated vines. In 2011-12, the interaction between thermal and water regimes did not affect yield or its components. In relation to controls, elevated temperature caused a 2.4-fold increase in yield (average across water regimes). This was primarily associated with a 1.9-fold increase in bunch number; bunch weight (28%) and berries per bunch (37%) also increased with elevated temperature. Elevated temperature reduced berry weight by 9%. In relation to fully irrigated controls, water deficit reduced yield by 46% (average across temperature regimes). This was associated with a 31% reduction in bunch weight, a 20% reduction in berries per bunch and a 10% reduction in berry weight; water deficit did not affect bunch number.

3.2.2. Experiment 2: temperature and fruit load

Table 3 shows the effect of temperature, fruit load and their interaction on yield and its components. In both seasons, bunch removal did not cause any significant compensation, i.e. bunch weight, berries per bunch and berry weight were unaffected by fruit load. Hence, yield was reduced in proportion to bunch removal. In 2010-11, elevated temperature reduced bunch weight but this was insufficient to change yield; yield components did not respond to temperature by fruit load interaction. In 2011-12, elevated temperature reduced bunch number but this effect was not reflected on yield, which was unaffected by temperature and temperature by fruit load interaction. Elevated temperature increased bunch weight in the unthinned treatment, and reduced bunch weight in the thinned

treatment (temperature by fruit load interaction: P = 0.03). Temperature increased berries per bunch by 26% in the unthinned treatment, and reduced berries per bunch by 10% in the thinned treatment (temperature by fruit load interaction: P = 0.02).

3.2.3. Experiment 3: temperature and varieties

Table 4 shows the effect of temperature, variety and their interaction on yield and its components. In all three seasons, variety was the main source of variation in yield with consistently high yield for Semillon and low yield in Chardonnay. In 2009-10, yield was unaffected by temperature or temperature x cultivar interaction. In relation to controls, elevated temperature reduced bunch number by an average 10% across varieties, but this was not reflected in yield. The interaction between cultivar and temperature was significant for berry size that increased with elevated temperature in Semillon and was reduced in Chardonnay. In 2010-11, elevated temperature reduced yield by 28% in high-yielding Semillon but not in the other varieties (temperature x variety: P = 0.03). This reduction in yield resulted from the combination of reduced bunch number (18%) and reduced bunch weight (13%), which was in turn accounted for by fewer berries per bunch. Elevated temperature consistently increased berry size in all four varieties. In 2011-12, elevated temperature increased yield in all four varieties (significant temperature effect and lack of cultivar by temperature interaction). Bunch number accounted for this response in Cabernet Franc, Chardonnay and Shiraz (significant effect of both temperature and temperature by variety interaction). The increased yield in Semillon was associated with heavier bunches. Temperature had no effect on berry size.

3.3. Vegetative growth and fruit/pruning weight ratio

3.3.1. Experiment 1: temperature and water regime

Table 5 shows the effect of temperature, water regime and their interaction on vegetative growth. In 2010-11, the summer assessment showed that elevated temperature increased both shoot number and weight per shoot, hence substantially increasing total shoot weight. There was an incipient interaction between temperature and water whereby elevated temperature increased vegetative growth proportionally more under water deficit: shoot number increased 18% in irrigated vines vs 40% in vines under water deficit, weight per shoot increased 52 vs 126%, and total shoot weight increased 18 vs 40%. Winter pruning showed a significant temperature by water interaction where the number of shoots increased slightly in irrigated vines (5%) compared to vines under water deficit (17%). The aggregated summer and winter pruning showed that elevated temperature increased vegetative growth by 9% in irrigated vines and 23% in vines under water deficit.

In 2010-11, the fruit/pruning weight ratio decreased from 2.0-2.2 kg kg^{-1} in controls to 1.5 kg kg^{-1} under elevated temperature (Table 1). In 2011-12 vegetative components were unaffected by temperature and temperature by water interaction. Water deficit reduced weight per cane and total pruning weight by 20% in relation to the fully irrigated treatment. Fruit/pruning weight ratio increased 2.2-fold under elevated temperature; it did not respond to water regime and water by temperature interaction (Table 2).

3.3.2. Experiment 2: temperature and fruit load

Table 6 shows the effect of temperature, fruit load and their interaction on vegetative growth. The summer assessment showed an incipient effect of thinning by temperature interaction on pruning weight that became statistically detectable in the winter pruning: elevated temperature increased both weight per shoot and shoot weight per vine in unthinned vines but not in their thinned counterparts. This interaction was also evident for the aggregated summer and winter pruning weight.

In 2010-11, the fruit/pruning weight ratio was reduced from 1.8-2.3 in the unthinned vines to 1.0-1.1 in their thinned counterparts, and was unaffected by temperature and temperature by fruit load interaction (Table 3). In 2011-12, elevated temperature and bunch removal both reduced fruit/pruning weight ratio, and the effects of these factors were additive (interaction P = 0.67; Table 3).

3.3.3. Experiment 3: temperature and varieties

Table 4 summarises the effects of temperature, variety and their interaction on pruning weight and its components. In 2009-10, elevated temperature increased shoot number, which combined with a modest increase in weight per shoot increased pruning weight by an average 10% across varieties. There were differences among varieties in shoot number, but pruning weight and its components shoot number and weight per shoot were unaffected by the interaction between temperature and cultivar. The lack of response in yield and the increase in pruning weight resulted in significant reductions in fruit/pruning weight ratio of vines at elevated temperature. In 2010-11, elevated temperature did not affect vegetative growth or fruit/pruning weight ratio, which were also unaffected by the interaction between temperature and variety. In 2011-12, elevated temperature did not affect vegetative growth and thus increased fruit/pruning weight ratio as a consequence of the significant yield enhancement.

3.4. Starch concentration in trunk and root

Figure 2 shows the concentration of starch in roots and trunks in the winter of 2011 after one growing season of temperature and water regime treatments in exp. 1. Starch concentration in root was unaffected by any of the experimental sources of variation. Starch concentration in trunk declined substantially under elevated temperature, and was unaffected by water regime or water by temperature interaction.

Figure 3 shows the effect of temperature, fruit load and their interaction on the concentration of starch in roots and trunks in the winter of 2011 after one season of treatments in exp. 2. Starch concentration in root was unaffected by any of the experimental sources of variation. Starch concentration in trunk declined slightly under elevated temperature and increased slightly with bunch thinning; the two effects were additive.

Figure 4 shows the effect of temperature, variety and their interaction on the concentration of starch in roots and trunks in the winters of 2010 and 2011 in exp. 3. Both traits were variety dependent in both seasons ($P < 0.015$). In 2010, starch concentration in root was unaffected by temperature or temperature by variety interaction. Starch concentration in

trunk was affected by all three sources of variation; it was strongly reduced under elevated temperature in Chardonnay and Semillon, with minor reductions in Shiraz and Cabernet Franc. The pattern was reversed in 2011, when starch in trunk was unaffected by temperature or variety x temperature interaction but starch in root was significantly affected by all three sources of variation. Starch concentration in root declined with elevated temperature in all varieties but Cabernet Franc.

3.5. Patterns across experiments

Owing to the large range in the response variables investigated (yield, yield components, pruning weight, and starch concentration), we used a normalised scale to probe for patterns across experiments (section 2.2). Yield response to temperature varied from -46 to 177%; within this range, significant effect of temperature was observed in 6 out of 20 cases, significant interaction of temperature and secondary sources of experimental variation in four cases, and lack of temperature or interaction effect in 10 cases (Fig. 5a). In the six conditions where the response was statistically detectable, yield under elevated temperature increased between 9 and 176% in relation to controls. Bunch number response to temperature ranged from -23 to 102% and accounted for 92% of the variation in yield response to temperature (Fig. 5a); the effect of temperature on bunch number declined with increasing bunch number in the controls (Fig. 5b). Berry number per bunch accounted for a significant part of the residual variation in yield after accounting for bunch number (Fig. 5c). Yield response to temperature was unrelated to the effect of temperature on berry weight ($P = 0.38$).

Pruning weight response to temperature ranged from -23 to 41%; lack of temperature or interaction effect was observed in 16 out of 20 cases, significant interaction in two cases, and significant temperature effect in two cases (Fig. 5d). Weight per shoot accounted for 95% of the variation in pruning weight (Fig. 5d). Temperature effect on pruning weight was weakly related to temperature effect on shoot number ($r^2 = 0.20$, $P = 0.05$).

The effect of temperature on the fruit yield/pruning weight ratio ranged from -56 to 145% and was affected by temperature in 14 out of 20 cases; it was closely related to fruit yield (Fig. 5e) and unrelated to pruning weight ($P = 0.18$). The effect of temperature on this ratio was unaffected by temperature or interactions in 6 out of 20 cases (Fig. 5e).

Figure 6 compares the concentration of starch in control and heated treatments for both root and trunk. Deviations with respect to the $y = x$ line were not significant for starch in roots ($P = 0.32$) whereas significant deviations ($P = 0.0005$) indicated a general reduction of starch in trunks of vines exposed to elevated temperature in relation to controls. Scatter in the regression of Fig. 6b was mostly associated with differences among varieties in experiment 3 (see Fig. 4 for statistics of variety effect); effects of water (Fig. 2) and fruit load (Fig. 3) on trunk starch concentration were statistically undetectable.

4. Discussion

4.1. Experimental conditions in the context of climate change

Temperature at mid-canopy level in the heated treatment was increased by 0.7 to 1.6 ^{o}C in relation to controls (Sadras and Moran, 2012b). This compares with warming ~ 1.5 to 2 ^{o}C by 2050 (baseline 1960-1990 climate) projected with CSIROMK3 model assuming the worst-case scenario for grape growing regions close to the Barossa Valley (Webb et al., 2007). Top soil (0.10 m) in the heated treatment of experiment 1 was 2.2 ^{o}C hotter than in controls during the day and 1.8 ^{o}C hotter during the night (Sadras et al., 2012a). Soil temperature was not measured in experiments 2 and 3, but the passive heating component common to all three experiments suggests a top-soil warming of a similar order of magnitude. Importantly, the open-top chambers allowed for shoots and fruit to be exposed to the natural radiation regime, with minor secondary effects (Sadras et al., 2012a; Sadras and Soar, 2009).

Water regimes in experiment 1 generated large and persistent reductions in both pre-dawn leaf water potential and stomatal conductance (Sadras et al., 2012b; Sadras and Moran, 2012b). Bunch removal in experiment 2 significantly increased source:sink ratio, as assessed with fruit/pruning weight (Table 3), with effects on vine phenology (Sadras and Moran, 2012b) and stomatal conductance (Sadras et al., 2012b). The varieties in experiment 3, i.e. Chardonnay, Semillon, Cabernet Franc and Shiraz, span from maturity group 3 to 5 in the classification of Gladstones (1992). Water regime, source:sink ratio and varieties are embedded sources of variation in time series analyses used to probe for warming effects on grapevine development (Duchêne and Schneider, 2005; Jones and Davis, 2000; Petrie and Sadras, 2008; Webb et al., 2012), hence the importance of these factors in our experimental evaluation of temperature effects on grapevine yield and vegetative growth.

The response of plant growth to warming depends on the background environment, i.e. warming is more likely to favour growth in cool environments and to reduce growth in hot environments (Way and Oren, 2010). The Barossa Valley where we conducted our experiments is an intermediate region with mean January temperature between 21.0 and 22.9 ^{o}C, in comparison to the Riverland, a characteristic hot region with mean January temperature above 23 ^{o}C, and Coonawarra, a cool region with mean January temperature between 19.0 and 20.9 ^{o}C (Dry, 2005). Importantly, the hot tail in the frequency distribution of seasonal temperature in Barossa overlaps with the cool tail in Riverland, and the cool tail in the Barossa overlaps with the hot tail in Coonawarra (Sadras and Petrie, 2011). Hence, interpretation of our findings summarised in the next section, and extrapolation to other environments needs to consider both the background temperature and the distribution of thermally contrasting season in particular locations (Fig. 1).

4.2. Yield

In the first season of treatments (2010-11 for experiments 1 and 2, 2009-10 for experiment 3), temperature effects on yield were statistically undetectable in all three trials whereas the largest temperature effect was recorded in 2011-12 (Tables 1-4). At least two factors contributed to these seasonal differences. First, bunch number is the main source of variation in grapevine yield and this trait is largely defined in the previous season (Dunn et al., 2004), hence the lack of yield response to temperature in the first season of treatments. Consistent with this, the effect of temperature on bunch number accounted for most of the variation in yield response to temperature (Fig. 5a). Second, the effects of warming on plant growth and yield depend on the background temperature (section 4.1). The spring was cool in 2010 compared with 2009 and 2011 (Fig. 1). During this period, current season inflorescences and flowers develop and fruit set is established, and the inflorescence primordia of next season are formed (Iland et al., 2011). Hence, warming substantively enhanced the formation of inflorescence primordia during the relatively cool 2010-11, as reflected on the number of bunches and total yield in two out of three experiments in 2011-12 (Tables 1-4, Fig. 5a). The increase in bunch number associated with warming declined with increasing bunch number in controls (Fig. 5b).

The yield response to temperature was asymmetric: yield reductions were smaller and largely not significant compared to the larger and often significant yield enhancement (open vs closed symbols in Fig. 5a). This effect of temperature was largely accounted for by bunch number, with a smaller contribution of berries per bunch (inset Fig. 5a). The number of berries per bunch is a function of the number of flowers per inflorescence and the proportion of flowers setting fruit (Collins and Dry, 2009); flower number responds to temperature during the current and previous season, and fruit set can be affected by temperature during the current season (Dunn, 2005; Dunn and Martin, 2000; Friend and Trought, 2007). In the cool Marlborough region of New Zealand, for example, delayed pruning shifts reproduction to warmer conditions and improves fertilisation of flowers and berry set (Friend and Trought, 2007).

In experiment 2, water deficit reduced fruit yield in both seasons (Tables 1 and 2). The reduction was ~0.8 kg vine^{-1} in the first season, and ~2.0 kg vine^{-1} in the second season, where the repeated water deficit reduced yield components more severely, except for bunch number. Petrie et al. (2004) found statistically significant reduction in bunch number and yield of Shiraz in response to water deficit in the previous season whereas Intrigliolo and Castel (2010) found no carry over effect of water regime on bud fertility of Tempranillo. Indeed, the carry over effect can be negative, neutral or positive depending on variety, timing, intensity and duration of water deficit (Williams and Matthews, 1990). Consistently over the two experimental seasons, the interaction between temperature and water regime did not affect yield and its components. Research in Mediterranean environments where temperature and water deficit increase in parallel from spring to summer suggests strong interactions between these factors (Chaves et al., 2010) but factorial experiments are needed for direct evaluation of this interaction (Sadras et al., 2012b).

In 2010-11, environmental conditions favoured *Botrytis* bunch rot in the Barossa Valley (Smith, 2011). Fungicide treatments effectively controlled the disease in experiments 1 and 2, but symptoms were evident in experiment 3. High relative humidity (> 90%) and an optimum thermal range of 15 to 20^{o}C favour germination of *Botrytis spp* conidia and infection (Bulit and Dubos, 1988). Elevated temperature and a slight reduction in relative

humidity (Sadras et al., 2012a) possibly contributed to reduced bunch rot in our heated treatment (Table 1), thus highlighting shifts in plant-pathogen interactions likely to emerge under warmer climates (Seem, 2004).

4.2. Vegetative growth and fruit/pruning weight ratio

Weight per shoot, rather than shoot number, explained the effect of temperature on pruning weight (Fig. 5d). Measurements in experiments 1 and 2 showed that elevated temperature enhanced both stomatal conductance and leaf light saturated photosynthesis in relation to controls (Sadras et al., 2012b). These leaf-level responses might have contributed to the increased yield in several combinations of water regimes and varieties (Fig. 5a) and to the enhanced vegetative growth in experiment 1 in 2010-11 (Table 5), and unthinned vines in experiment 2 in 2010-11 (Table 6). However, potential gains in carbon assimilation at the vine level could have been counteracted by a number of factors, including higher respiration with elevated temperature (Atkin et al., 2010; Campbell et al., 2007). Indeed, leaf-level responses do not necessarily scale up to the crop level (Pettigrew et al., 1989; Sinclair et al., 2004).

The range of temperature effect on pruning weight was small compared with the range of response of fruit yield (Fig. 5a vs 5d). Hence, fruit yield was the main factor underlying the effect of temperature on fruit/pruning weight ratio (Fig. 5e). Fruit/pruning weight ratio was the trait with more frequent response to temperature (closed symbols in Fig. 5e) and, similar to yield, its response was asymmetrical: from 57% reduction to 145% increase. The increase in fruit/pruning weight ratio with warming is viticulturally important and physiologically interesting. Empirical ranges of this ratio are used to guide management practices such as pruning and training system to achieve specific viticultural targets (p. 272-4, Iland et al., 2011). An increase in sink:source ratio might alter the long-term carbon balance of the vine, for example, by reducing the rate of accumulation of reserves during the period when fruit growth coincides with storage of reserves (Bates et al., 2002; Zapata et al., 2004). Consistent with this proposition, elevated temperature reduced and fruit removal increased starch concentration in trunks (Figs. 3b and Fig. 6b). In addition to the increased sink:source ratio, local effects including enhanced respiration may have contributed to the reduction of starch concentration in trunks.

In contrast to the thermal response of starch in trunks, starch concentration in roots was largely unaffected by ambient temperature (Fig. 6a). Significant reductions in root carbohydrate reserves associated with increased respiration are common in experiments where the temperature of the soil and root system are manipulated (Field et al., 2009; Huang et al., 2005; Rogiers et al., 2011). In our experimental system, top-soil (0.1 m) temperature increased in a similar order of magnitude than ambient temperature (about 2^{o}C or less), but the warming at 0.4 m where roots were sampled was possibly minor. Factorial experiments are required to separate local (i.e. soil temperature) from changes in root reserves mediated by source:sink ratio.

5. Conclusion

Using open-top chambers to approximate the thermal regimes of the Barossa Valley in the next decades, we found that warming had asymmetric effects on fruit yield and fruit/pruning ratio. The asymmetry resulted from seasonal influences on warming effects, whereby the largest responses of these traits were mediated by the promotion of bunch formation following relatively low temperatures in the preceding season. This highlights the need to assess warming effects in the light of both the background temperature and the distribution of thermally contrasting season in particular locations. Reduction in both source:sink ratio and starch concentration in trunks are two important elements of the carbon balance of the vine with long-term physiological and viticultural implications.

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Table 1. Yield and yield components of Shiraz vines grown in a factorial experiment combining two temperatures and two water regimes in the Barossa Valley, season 2010-11 (experiment 1). Biological yield and fruit/shoot ratio include all fruit, whereas viticultural yield and components excludes bunches with visual symptoms of Botrytis damage. Botrytis incidence is the fresh weight ratio between damaged and total fruit. Values are mean ± standard error; *P* indicates effect of temperature, water regime and interaction.

Temperature	Water regime	Biological Yield (kg vine^{-1})	Fruit/shoot (kg kg^{-1})	Botrytis incidence	Viticultural Yield (kg vine^{-1})	Bunch No (vine^{-1})	Bunch wt (g bunch^{-1})	Berry No (bunch^{-1})	Berry wt (g berry^{-1})
control	irrigated	8.9 ± 0.42	2.2 ± 0.09	0.34 ± 0.076	5.8 ± 0.37	78 ± 2.2	75 ± 6.8	49 ± 7.5	1.56 ± 0.092
heated		7.4 ± 0.59	1.5 ± 0.04	0.05 ± 0.052	7.0 ± 0.42	78 ± 2.0	90 ± 5.8	56 ± 5.1	1.61 ± 0.046
control	deficit	6.7 ± 0.56	2.0 ± 0.07	0.24 ± 0.029	5.0 ± 0.23	74 ± 1.1	68 ± 2.7	47 ± 1.1	1.45 ± 0.058
heated		6.4 ± 0.01	1.5 ± 0.18	0.03 ± 0.018	6.2 ± 0.10	73 ± 0.7	85 ± 2.2	55 ± 2.8	1.54 ± 0.037
P-temperature		*0.15*	*0.0003*	*0.003*	*0.01*	*0.76*	*0.02*	*0.19*	*0.32*
P-water		*0.02*	*0.76*	*0.336*	*0.05*	*0.03*	*0.32*	*0.80*	*0.25*
P-interaction		*0.29*	*0.34*	*0.514*	*0.99*	*0.93*	*0.89*	*0.95*	*0.76*

Table 2. Yield and yield components of Shiraz vines grown in a factorial experiment combining two temperatures and two water regimes in the Barossa Valley, season 2011-12 (experiment 1). Values are mean ± standard error; *P* indicates effect of temperature, water regime and interaction.

Temperature	Water regime	Yield (kg vine^{-1})	Bunch No (vine^{-1})	Bunch wt (g bunch^{-1})	Berry No (bunch^{-1})	Berry wt (g berry^{-1})	Fruit/shoot (kg kg^{-1})
control	irrigated	5.0 ± 0.76	48 ± 4.5	102.4 ± 7.12	66 ± 4.3	1.55 ± 0.012	1.41 ± 0.126
heated		10.2 ± 1.28	85 ± 5.1	118.7 ± 7.70	82 ± 4.8	1.45 ± 0.008	2.74 ± 0.179
control	deficit	3.0 ± 0.63	41 ± 7.2	71.6 ± 3.03	50 ± 1.8	1.44 ± 0.012	1.04 ± 0.180
heated		8.2 ± 0.55	82 ± 6.3	99.6 ± 2.30	75 ± 1.3	1.32 ± 0.011	2.55 ± 0.146
P-temperature		*0.0003*	*0.0002*	*0.004*	*0.0003*	*<0.0001*	*<0.0001*
P-water		*0.05*	*0.42*	*0.002*	*0.01*	*<0.0001*	*0.12*
P-interaction		*0.99*	*0.70*	*0.32*	*0.19*	*0.54*	*0.59*

Table 3 Yield components of Shiraz vines grown in a factorial experiment with two temperatures and two fruit loads over two seasons in the Barossa Valley (experiment 2). Values are mean ± standard error; *P* indicates effect of temperature, fruit load and interaction.

Season	Temperature	Fruit load	Yield (kg vine^{-1})	Bunch No (vine^{-1})	Bunch weight (g bunch^{-1})	Berry No (bunch^{-1})	Berry weight (g berry^{-1})	Fruit/shoot (kg kg^{-1})
2010-11	control	unthinned	7.4 ± 0.41	60 ± 4.9	124 ± 5.1	87 ± 2.5	1.43 ± 0.037	2.27 ± 0.086
	heated		8.1 ± 0.26	70 ± 0.4	116 ± 4.6	79 ± 3.7	1.48 ± 0.042	1.77 ± 0.096
	control	thinned	3.9 ± 0.29	30 ± 3.1	134 ± 8.2	93 ± 7.2	1.44 ± 0.030	1.04 ± 0.113
	heated		3.9 ± 0.52	36 ± 6.0	114 ± 3.5	84 ± 3.1	1.37 ± 0.047	1.14 ± 0.267
	P - temperature		*0.36*	*0.12*	*0.04*	*0.09*	*0.78*	*0.24*
	P- fruit load		*0.0001*	*0.0001*	*0.49*	*0.24*	*0.24*	*0.0004*
	P- interaction		*0.40*	*0.63*	*0.30*	*0.80*	*0.13*	*0.09*
2011-12	control	unthinned	6.3 ± 1.26	65 ± 4.3	95 ± 14.2	63 ± 3.8	1.48 ± 0.147	3.38 ± 0.288
	heated		6.8 ± 0.41	54 ± 2.7	126 ± 7.6	80 ± 3.2	1.57 ± 0.058	2.90 ± 0.195
	control	thinned	5.0 ± 0.69	39 ± 2.1	127 ± 11.9	76 ± 6.4	1.67 ± 0.052	2.21 ± 0.342
	heated		3.1 ± 0.64	30 ± 3.6	100 ± 8.5	68 ± 2.6	1.46 ± 0.076	1.50 ± 0.187
	P - temperature		*0.40*	*0.01*	*0.85*	*0.30*	*0.51*	*0.05*
	P- fruit load		*0.02*	*0.0001*	*0.80*	*0.98*	*0.65*	*0.001*
	P- interaction		*0.17*	*0.74*	*0.03*	*0.02*	*0.14*	*0.67*

Table 4 Vegetative growth and yield components of grapevines in a factorial experiment combining four cultivars and two temperature regimes during three consecutive seasons in the Barossa Valley (experiment 3). Values are mean ± standard error; *P* indicates effect of temperature, cultivar and interaction.

Season	Cultivar	Temp.	Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Yield (kg vine^{-1})	Bunch No (vine^{-1})	Bunch wt (g bunch^{-1})	Berry No (bunch^{-1})	Berry wt (g berry^{-1})	Fruit/shoot (kg kg^{-1})
2009-10	Cab Franc	control	57 ± 2.9	14.6 ± 2.42	0.84 ± 0.185	2.7 ± 0.37	78 ± 3.3	46 ± 7.3	62 ± 5.7	0.73 ± 0.048	3.4 ± 0.26
		heated	69 ± 1.3	13.6 ± 1.94	0.92 ± 0.120	2.0 ± 0.19	69 ± 2.1	42 ± 1.5	56 ± 0.5	0.75 ± 0.020	2.2 ± 0.12
	Chardonnay	control	52 ± 3.9	14.7 ± 0.68	0.76 ± 0.033	2.3 ± 0.49	85 ± 12.6	39 ± 0.4	65 ± 2.4	0.60 ± 0.023	2.8 ± 0.55
		heated	60 ± 1.4	16.0 ± 2.02	0.96 ± 0.124	1.2 ± 0.33	75 ± 5.3	25 ± 1.2	46 ± 0.9	0.55 ± 0.033	1.2 ± 0.19
	Semillon	control	52 ± 1.1	16.4 ± 1.47	0.85 ± 0.098	5.9 ± 0.57	110 ± 5.8	66 ± 4.8	97 ± 3.2	0.67 ± 0.028	7.0 ± 0.21
		heated	54 ± 0.6	18.3 ± 2.04	0.99 ± 0.111	6.2 ± 0.82	100 ± 2.4	81 ± 15.4	98 ± 16.5	0.82 ± 0.043	6.2 ± 0.28
	Shiraz	control	47 ± 1.4	15.4 ± 1.34	0.72 ± 0.050	3.5 ± 0.12	82 ± 2.7	55 ± 2.1	83 ± 4.9	0.66 ± 0.038	4.9 ± 0.21
		heated	50 ± 2.1	17.3 ± 1.37	0.87 ± 0.098	3.1 ± 0.01	77 ± 0.3	50 ± 1.3	78 ± 4.1	0.65 ± 0.018	3.7 ± 0.39
	P-temp.		*0.001*	*0.42*	*0.09*	*0.15*	*0.05*	*0.71*	*0.15*	*0.35*	*0.0001*
	P-cultivar		*0.0001*	*0.31*	*0.72*	*0.0001*	*0.0002*	*0.0001*	*0.0001*	*0.002*	*0.0001*
	P-interaction		*0.13*	*0.81*	*0.97*	*0.50*	*0.96*	*0.18*	*0.52*	*0.04*	*0.56*
2010-11	Cab Franc	control	70 ± 0.7	29.2 ± 5.39	2.1 ± 0.39	4.5 ± 0.66	77 ± 5.0	58 ± 6.7	58 ± 5.0	1.00 ± 0.032	2.2 ± 0.25
		heated	68 ± 1.7	31.5 ± 3.76	2.1 ± 0.22	4.7 ± 0.60	73 ± 5.8	64 ± 3.3	57 ± 1.7	1.11 ± 0.030	2.2 ± 0.23
	Chardonnay	control	49 ± 1.1	18.6 ± 2.42	0.9 ± 0.14	2.6 ± 0.10	49 ± 2.7	54 ± 1.0	67 ± 1.4	0.81 ± 0.027	3.0 ± 0.63
		heated	52± 2.2	13.5 ± 2.95	0.7 ± 0.18	2.3 ± 0.46	46 ± 4.4	49 ± 5.7	57 ± 2.7	0.87 ± 0.080	3.4 ± 0.44
	Semillon	control	52 ± 0.8	38.4 ± 7.57	2.0 ± 0.41	10.5 ± 0.86	66 ± 4.4	160 ± 9.7	104 ± 11.0	1.56 ± 0.110	5.7 ± 1.12
		heated	51 ± 0.7	40.4 ± 4.60	2.1 ± 0.26	7.5 ± 0.66	54 ± 3.8	139 ± 6.3	86 ± 5.4	1.62 ± 0.036	3.7 ± 0.46
	Shiraz	control	51 ± 0.4	33.1 ± 3.11	1.7 ± 0.17	3.5 ± 0.35	51 ± 2.1	68 ± 4.2	57 ± 2.1	1.20 ± 0.064	2.1 ±0.19
		heated	51 ± 1.8	32.5 ± 4.69	1.6 ± 0.25	3.6 ± 0.20	49 ± 3.0	74 ± 3.7	56 ± 0.5	1.32 ± 0.055	2.3 ± 0.37
	P-temp.		*0.75*	*0.91*	*0.88*	*0.07*	*0.09*	*0.44*	*0.05*	*0.05*	*0.37*
	P-cultivar		*<0.0001*	*0.0009*	*0.0007*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0008*
	P-interaction		*0.42*	*0.84*	*0.94*	*0.03*	*0.59*	*0.10*	*0.27*	*0.94*	*0.16*

Table 4 continued

Season	Cultivar	Temp.	Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Yield (kg vine^{-1})	Bunch No (vine^{-1})	Bunch wt (g bunch^{-1})	Berry No (bunch^{-1})	Berry wt (g berry^{-1})	Fruit/shoot (kg kg^{-1})
2011-12	Cab Franc	control	55 ± 4.2	39.4 ± 7.24	2.2 ± 0.51	2.1 ± 0.92	44 ± 12.3	41 ± 7.7	39 ± 5.8	1.05 ± 0.037	0.9 ± 0.19
		heated	57 ± 2.5	41.4 ± 3.63	2.4 ± 0.30	4.5 ± 0.11	80 ± 1.7	56 ± 0.0	53 ± 0.4	1.06 ± 0.008	1.9 ± 0.23
	Chardonnay	control	33 ± 0.7	31.9 ± 0.12	1.0 ± 0.02	1.2 ± 0.06	34 ± 3.5	38 ± 6.0	38 ± 4.3	1.00 ± 0.045	1.3 ± 0.09
		heated	38 ± 2.6	23.7 ± 4.15	0.9 ± 0.20	2.2 ± 0.42	56 ± 5.3	37 ± 4.8	39 ± 3.1	0.96 ± 0.117	2.6 ± 0.33
	Semillon	control	43 ± 1.6	44.6 ± 4.71	1.9 ± 0.25	10.3 ± 1.52	72 ± 4.0	143 ± 14.4	91 ± 12.6	1.58 ± 0.070	5.4 ± 0.10
		heated	43 ± 1.1	35.9 ± 3.48	1.6 ± 0.18	11.3 ± 1.00	71 ± 1.6	160 ± 11.0	102 ± 8.6	1.56 ± 0.024	7.4 ± 0.93
	Shiraz	control	43 ± 1.4	42.5 ± 1.46	1.8 ± 0.08	4.4 ± 0.42	50 ± 5.8	88 ± 4.8	61 ± 1.8	1.44 ± 0.045	2.4 ± 0.15
		heated	43 ± 1.5	35.4 ± 3.90	1.5 ± 0.21	5.1 ± 0.74	60 ± 5.1	83 ± 5.6	63 ± 3.7	1.32 ± 0.030	3.3 ± 0.09
	P-temp.		*0.29*	*0.08*	*0.42*	*0.05*	*0.001*	*0.28*	*0.15*	*0.32*	*0.0001*
	P-cultivar		*< 0.0001*	*0.02*	*0.001*	*<0.0001*	*0.006*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
	P-interaction		*0.74*	*0.52*	*0.72*	*0.72*	*0.04*	*0.45*	*0.69*	*0.70*	*0.47*

Table 5. Vegetative growth of Shiraz vines grown in a factorial experiment combining two temperatures and two water regimes during two consecutive seasons in the Barossa Valley (experiment 1). Values are means ± standard error; P indicates effect of temperature, cultivar and interaction. Summer pruning was required due to vigorous canopies resulting from rainy winter in 2010-11; it includes canes and leaves. Winter pruning includes only canes.

Season	Temperature	Water	Summer pruning			Winter pruning			Total	
			Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Shoot No (vine^{-1})	Shoot wt (kg vine^{-1})
2010-11	control	irrigated	24 ± 2.1	51.9 ± 2.57	1.2 ± 0.17	60 ± 0.5	47.9 ± 2.87	2.9 ± 0.18	84 ± 2.4	4.11 ± 0.331
	heated		28 ± 2.1	67.5 ± 1.77	1.9 ± 0.12	63 ± 0.9	50.7 ± 4.65	3.2 ± 0.32	91 ± 3.0	5.07 ± 0.376
	control	deficit	21 ± 0.3	42.3 ± 3.85	0.9 ± 0.09	56 ± 0.8	42.1 ± 1.74	2.4 ± 0.17	77 ± 1.1	3.24 ± 0.169
	heated		29 ± 2.4	68.8 ± 0.95	2.0 ± 0.14	66 ± 1.4	46.1 ± 1.74	3.0 ± 0.45	94 ± 0.8	4.58 ± 0.446
	P-temperature		*0.01*	*0.0001*	*0.0004*	*0.0003*	*0.31*	*0.06*	*0.001*	*0.003*
	P-water supply		*0.50*	*0.19*	*0.32*	*0.50*	*0.19*	*0.17*	*0.43*	*0.17*
	P-interaction		*0.34*	*0.09*	*0.14*	*0.01*	*0.79*	*0.44*	*0.08*	*0.24*
2011-12	control	irrigated				47 ± 0.3	74.2 ± 4.68	3.5 ± 0.25		
	heated					46 ± 0.6	78.9 ± 6.51	3.7 ± 0.27		
	control	deficit				46 ± 0.9	60.6 ± 3.67	2.8 ± 0.20		
	heated					47 ± 1.9	68.3 ± 2.20	3.2 ± 0.10		
	P-temperature					*0.83*	*0.21*	*0.20*		
	P-water supply					*0.89*	*0.03*	*0.03*		
	P-interaction					*0.73*	*0.74*	*0.66*		

Table 6. Vegetative growth of Shiraz vines in a factorial experiment combining two temperatures and two fruit loads during two consecutive seasons in the Barossa Valley (experiment 2). Values are means ± standard error; P indicates effect of temperature, cultivar and interaction. Summer pruning was required in 2010-11 due to vigorous canopies resulting from rainy winter; it includes canes and leaves. Winter pruning includes only canes.

Season	Temperature	Fruit load	Summer pruning			Winter pruning			Summer + winter pruning	
			Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Shoot No (vine^{-1})	Weight per shoot (g)	Shoot wt (kg vine^{-1})	Shoot No (vine^{-1})	Shoot wt (kg vine^{-1})
2010-11	control	unthinned	30 ± 2.5	45.2 ± 3.61	1.4 ± 0.21	61 ± 1.9	31.0 ± 0.49	1.9 ± 0.08	91 ± 4.2	3.3 ± 0.29
	heated	unthinned	30 ± 0.7	74.4 ± 4.78	2.2 ± 0.19	63 ± 0.6	37.7 ± 2.21	2.4 ± 0.14	93 ± 0.9	4.6 ± 0.32
	control	thinned	33 ± 3.1	50.0 ± 4.53	1.7 ± 0.31	61 ± 2.1	34.8 ± 0.79	2.1 ± 0.09	94 ± 4.9	3.8 ± 0.40
	heated	thinned	25 ± 2.6	61.2 ± 3.04	1.5 ± 0.22	63 ± 2.1	33.2 ± 1.78	2.1 ± 0.12	87 ± 4.1	3.6 ± 0.34
	P- temperature		*0.09*	*0.20*	*0.001*	*0.20*	*0.07*	*0.13*	*0.07*	*0.14*
	P-fruit load		*0.71*	*0.45*	*0.33*	*0.45*	*0.81*	*0.83*	*0.81*	*0.55*
	P-interaction		*0.12*	*0.07*	*0.06*	*0.07*	*0.04*	*0.03*	*0.04*	*0.05*
2011-12	control	unthinned				44 ± 2.9	42.1 ± 5.74	1.9 ± 0.33		
	heated	unthinned				44 ± 1.4	53.0 ± 4.61	2.3 ± 0.13		
	control	thinned				46 ± 1.8	50.4 ± 4.06	2.3 ± 0.27		
	heated	thinned				43 ± 2.5	46.4 ± 2.49	2.0 ± 0.20		
	P- temperature					*0.68*	*0.46*	*0.70*		
	P-fruit load					*0.92*	*0.84*	*0.83*		
	P-interaction					*0.44*	*0.13*	*0.15*		

Captions to figures

1. Monthly mean temperature during the experiments (circles and triangles) and long-term records. The lines are, from top to bottom, the 90^{th}, 75^{th}, 50^{th}, 25^{th} and 10^{th} decile for the period 1957-2011 at Nuriootpa, Australia.
2. Starch concentration in root and trunk of Shiraz grapevines in experiment 1 combining two thermal regimes and two water regimes, irrigated control and water deficit. Error bars are one standard error of the mean. *P* values from analysis of variance are also shown.
3. Starch concentration in root and trunk of Shiraz grapevines in experiment 2 combining two thermal regimes and two fruit loads, unthinned control (closed bar) and thinned (open bar). Error bars are one standard error of the mean. *P* values from analysis of variance are also shown.
4. Starch concentration in root and trunk of grapevines in experiment 3 combining four varieties and two thermal regimes, control (closed bar) and elevated temperature (open bar). Varieties are Cabernet Franc (CAF), Chardonnay (CHR), Semillon (SEM) and Shiraz (SHI). Error bars are one standard error of the mean. *P* values from analysis of variance are also shown.
5. Effects of temperature on vine traits for a pooled data set including 20 cases resulting from the combinations of three experiments over 2-3 seasons in the Barossa Valley of Australia. (a) Relationship between temperature effect on yield and temperature effect on bunch number. (c) Residuals of the regression in (a) as a function of temperature effect on berry number per bunch. (d) Relationship between temperature effect on pruning weight per vine and average shoot weight. (e) Relationship between fruit/pruning weight ratio and fruit yield. Temperature effect is 100 x (H-C)/C, where H is the trait in the heated treatment, and C is the trait in the control. In (a, d, e) symbols indicate lack of temperature or interaction effect ($P > 0.05$), significant interaction between temperature and additional sources of experimental variation ($P \leq 0.05$) and significant effect of temperature ($P \leq 0.05$), but no interaction ($P > 0.05$).
6. Comparison of starch concentration between control and heated treatments pooled across experiments and seasons for (a) root and (b) trunk. Error bars are two standard errors of the mean. *P* values indicate significance of deviations from the y = x line (*t*-test, null hypothesis: deviations = 0). The fitted regression for trunk (thick line) highlights the significant deviation with respect to y = x.

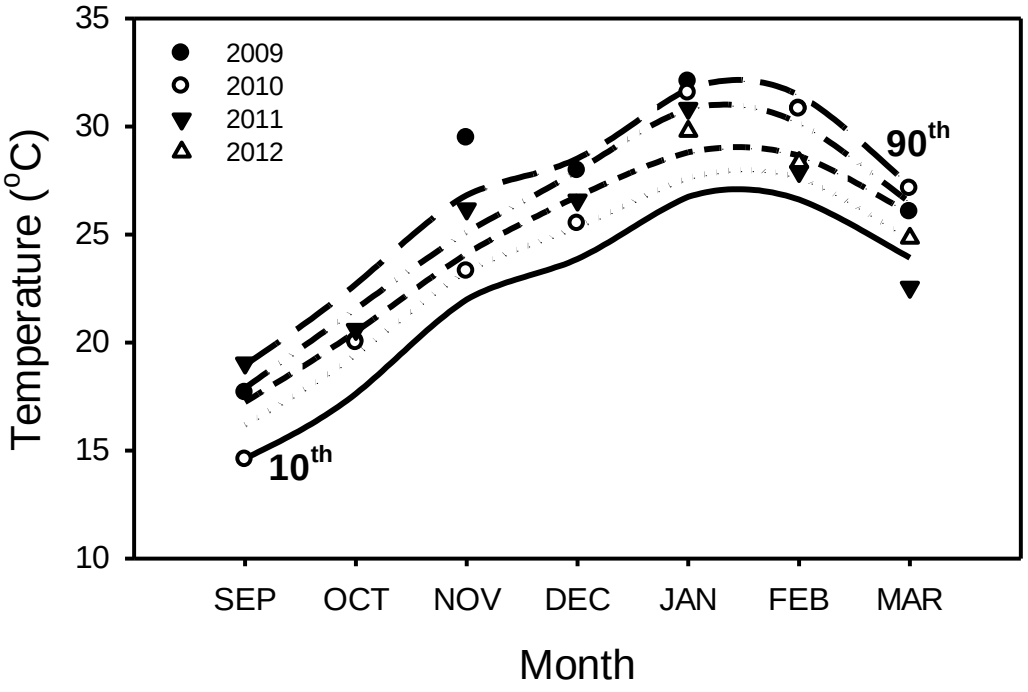
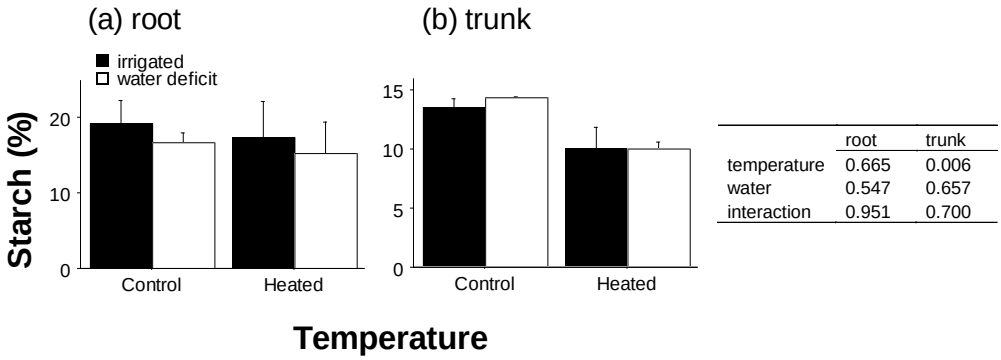
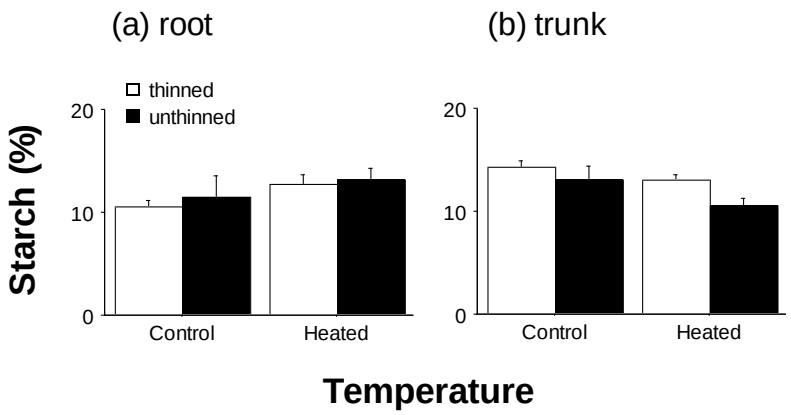


Figure 1



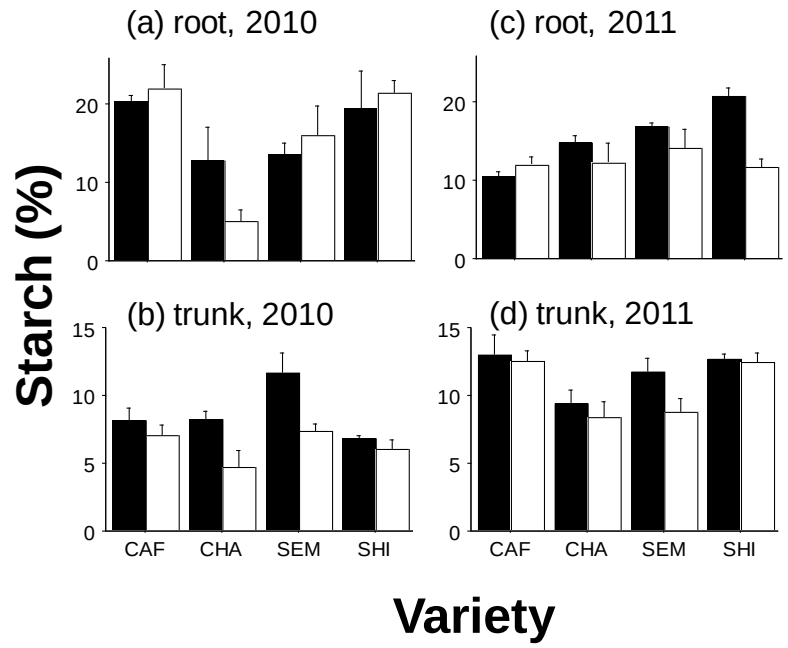
	root	trunk
temperature	0.665	0.006
water	0.547	0.657
interaction	0.951	0.700

Figure 2



	root	trunk
temperature	0.18	0.06
fruit load	0.62	0.07
interaction	0.86	0.46

Figure 3



		2010	2011
root	temperature	0.851	0.007
	variety	0.004	0.015
	interaction	0.347	0.020
trunk	temperature	0.001	0.130
	variety	0.011	0.003
	interaction	0.020	0.537

Figure 4

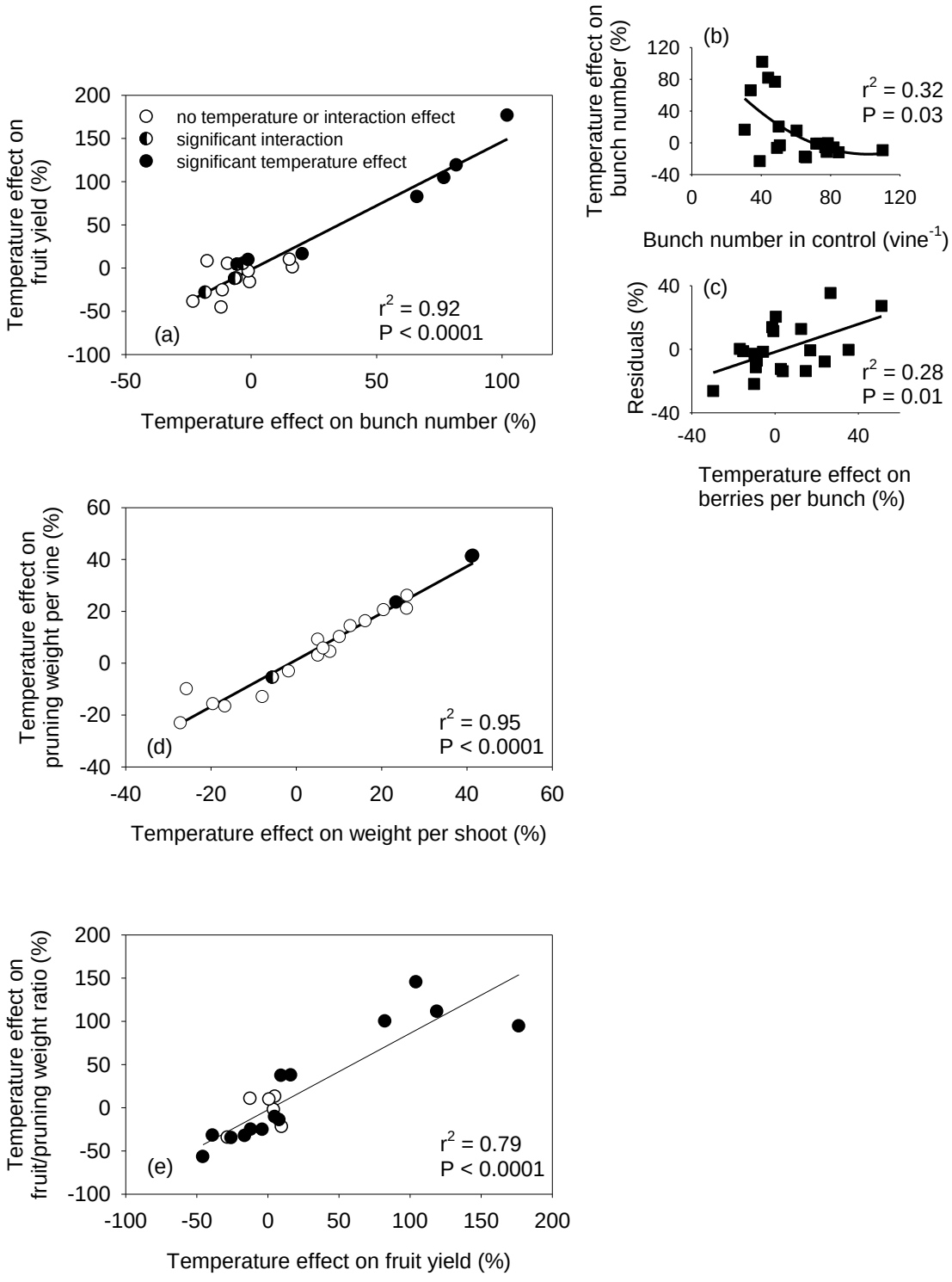

(a)
no temperature or interaction effect
significant interaction
significant temperature effect
r2 = 0.92
P < 0.0001
Temperature effect on fruit yield (%)
Temperature effect on bunch number (%)
(b)
r2 = 0.32
P = 0.03
Temperature effect on bunch number (%)
Bunch number in control (vine-1)
(c)
r2 = 0.28
P = 0.01
Residuals (%)
Temperature effect on berries per bunch (%)
(d)
r2 = 0.95
P < 0.0001
Temperature effect on pruning weight per vine (%)
Temperature effect on weight per shoot (%)
(e)
r2 = 0.79
P < 0.0001
Temperature effect on fruit/pruning weight ratio (%)
Temperature effect on fruit yield (%)

Figure 5

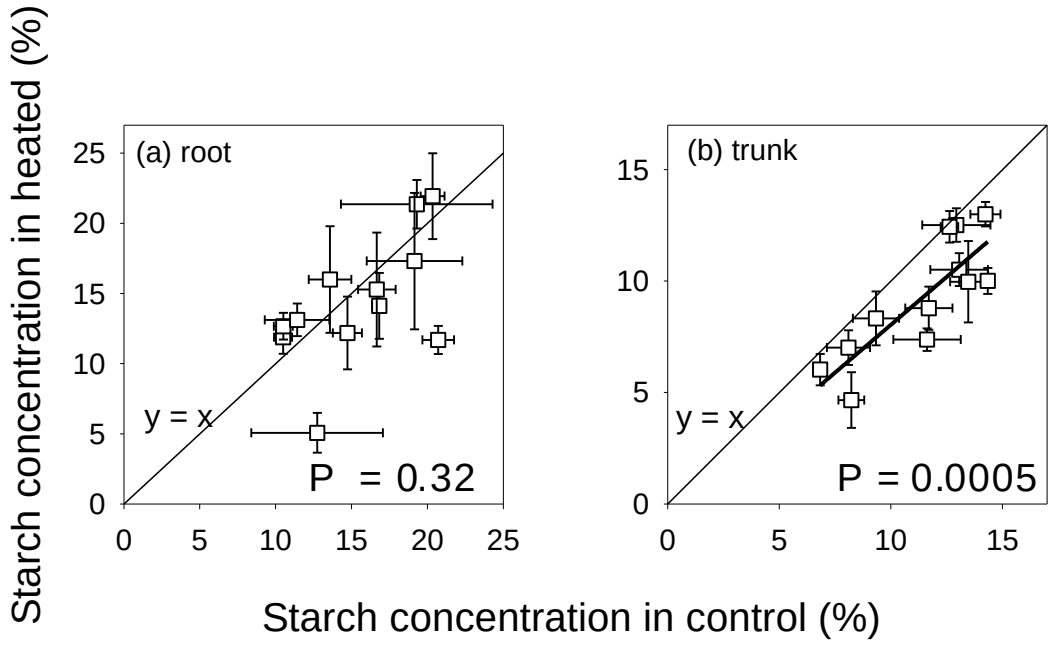

Starch concentration in heated (%)
(a) root
y = x
P = 0.32
(b) trunk
y = x
P = 0.0005
Starch concentration in control (%)

Figure 6

Chapter 4. Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc

VO Sadras, MA Moran

Abstract

We tested the hypothesis that elevated temperature decouples anthocyanins and total soluble solids (TSS) in developing berries and explored the effects of fruit load and water supply on the putative decoupling. Two thermal regimes (elevated temperature, control) where combined with varieties (Shiraz, Cabernet Franc), fruit loads (bunch removal, control) or water regimes (irrigated, water deficit). The relationship between anthocyanins and TSS had a lag phase where TSS increased with no change in anthocyanins and an approximately linear phase where both traits increased in parallel for TSS > 11.4 ^{o}Brix. Mean residuals in the linear phase were 0.065 mg g^{-1} in controls compared to -0.064 mg g^{-1} in the heated treatment hence demonstrating the thermal decoupling of anthocyanins and TSS across varieties fruit loads and water regimes ($P<0.0001$). Delayed onset of anthocyanin accumulation at elevated temperature explained the decoupling. Water deficit increased the anthocyanin : TSS ratio. We show direct evidence that elevated temperature decoupled anthocyanins and sugars in berries by a relative shift in onset rather than rate of accumulation of these berry components. Warming trends are likely to disrupt the anthocyanin : sugar ratio in berries with consequences for colour : alcohol balance in red wines. Water deficit established shortly before veraison could partially restore these ratios.

Introduction

Against the backdrop of global warming, thermal decoupling results from differential effects of temperature on the components of biological systems from molecular to ecosystem scales (Büntgen and Schweingruber, 2010; Edwards and Richardson, 2004; Englund et al., 2011). Indirect evidence supports the decoupling of important berry traits and wine attributes in parallel to warming trends. In France between 1957 and 1997, Jones and Davies (2000) found increasing sugar:acid ratio for Merlot and Cabernet Sauvignon. In Australia between 1993 and 2006, the rate of change in harvest date for Chardonnay was commensurate with the rate of change in maturity, hence berry sugar concentration at harvest remained stable whereas the advancement of harvest for Cabernet Sauvignon and Shiraz only partially offset the advancements in maturity, hence the increase in the concentration of berry sugar at harvest, up to ~ 0.3°Brix $year^{-1}$ (Petrie and Sadras, 2008b). The increase in sugar concentration at harvest corresponded to an increase in potential alcohol of 0.14-0.17% $year^{-1}$ (Petrie and Sadras, 2008b) which agrees with time-trends in independent studies (Godden and Gishen, 2005). We speculate that the increase in alcohol documented for Cabernet Sauvignon and Shiraz, but not for Chardonnay, has been partially due to the temperature-driven decoupling of anthocyanins and sugars in berries of red wine varieties; if accumulation of sugars is more responsive to temperature than accumulation of anthocyanins, delayed harvest to allow for higher concentration of anthocyanins would be associated with higher sugar concentration and potential alcohol.

Temperature effects have been widely investigated individually for anthocyanins and sugars (Bergqvist et al., 2001; Buttrose et al., 1971; Haselgrove et al., 2000; Mori et al., 2007; Mori et al., 2005a; Mori et al., 2005b; Sadras and Petrie, 2011a; Sadras and Petrie, 2011b; Spayd et al., 2002; Tarara et al., 2008a; Tarara et al., 2008b; Yamane et al., 2006). The genetic and physiological links between anthocyanins and sugars have attracted some interest (Hiratsuka et al., 2001; Koshita et al., 2011; Solfanelli et al., 2005; Vitrac et al., 2000). However, even in studies where both traits have been measured, no attempt has been made to directly test the hypothesis of a thermal decoupling of sugars and anthocyanins. Likewise, studies on the effect of source:sink ratio and water supply have focused on anthocyanins and sugars individually (Aaberge; Esteban et al., 2001; Girona et al., 2009; Guidoni et al., 2002; Keller et al., 2008; Kliewer, 1970; Kliewer and Dokoozlian, 2005; Nuzzo and Matthews, 2006; Orts et al., 2005; Petrie and Clingeleffer, 2006; Roby et al., 2004; Santesteban et al., 2007); few attempted to explicitly account for the degree of coupling between these traits (Keller et al., 2005; Sadras et al., 2007).

Here we present direct evidence of a temperature-driven decoupling of sugars and anthocyanins in berries of Shiraz and Cabernet Franc in experiments where temperature has been manipulated under realistic vineyard conditions in the temperate environment of the Barossa Valley. We also explore the modulation of the putative thermal decoupling by fruit load and water supply in Shiraz.

Method

The vines were established on a red brown earth at the South Australian Research and Development Institute, Nuriootpa (34 ^{o}S, 139 ^{o}E) in the Barossa Valley of South Australia. Gladstones (1992) and Dry and Coombe (2005) described the climate and viticultural practices of the region. Vines are arranged with a row by vine spacing of 3.50 x 2.25m (experiment 1) or 3.0 x 2.25m (experiments 2 and 3), cordon-trained to a single-wire trellis and have been spur pruned. Vines have been managed with supplementary irrigation since planting (experiment 1: single 4 l h^{-1} dripper per vine; experiments 2 and 3: double 2.2 l h^{-1} drippers per vine). We established three 2^2 factorial experiments where temperature regimes were combined with varieties (exp. 1), fruit loads (exp. 2) or water regimes (exp. 3). In all three experiments, treatments were laid out in a split-plot design with three replicates. In experiment 1, main plot was assigned to variety and subplot to temperature regime. In experiment 2, main plot was assigned to thermal regime and subplot to fruit load. In experiment 3, main plot was assigned to thermal regime and subplot to water regime. In all experiments each replicate comprised nine vines, allowing for sampling in seven central vines, two buffer vines within each replicate and a three further buffer vines between replicates.

Experiment 1

Single rows of own-rooted Cabernet Franc (clone Penfold 58), planted in 1988, and own-rooted Shiraz (clone NSW 15), planted in 1997, were managed similarly since establishment in the same block. The two varieties were exposed to two temperature regimes, untreated controls and elevated daytime temperature, which were established on 21 October 2009. Daytime temperature was elevated using passive, open-top chambers which have negligible effects on both minimum temperature and relative humidity during the growing season. Full details of the heating system, its construction, performance and limitations are in Sadras and Soar (2009).

Experiment 2

Shiraz (clone 1654) on Paulsen 1103 rootstock planted in 2004 was used in a factorial experiment combining two fruit loads and two temperature regimes. Fruit loads were: thinned vines, where 2/3 of bunches were removed before veraison (6 January 2011) and untreated controls. The temperature regimes, which were established on 18 November 2010, were: control with background ambient temperature, and elevated daytime temperature using open-top chambers as in Experiment 1.

Experiment 3

Own-rooted Shiraz (clone 1654) planted in 2004 was used in a factorial experiment combining two temperature regimes and two water regimes. Temperature regimes,

initiated on 7 October 2010, were: control with background ambient temperature, and a heated treatment. The heating system combines passive heating on clear days and active heating on overcast days and during the night, and it allows for regulation of the aperture to release excess heat as necessary. This system tracks the daily and seasonal time courses of temperature and vapour pressure deficit, lacks significant secondary effects on vine canopies which are essentially exposed to the normal radiation climate, and provides a steadier increase in temperature than the passive system (Fig. 1). Full details of the heating system, its construction, performance and limitations are in Sadras et al. (2011). Water regimes were: controls, which were watered weekly between January 24th and March 7th, 2011 (7 weeks @ 35 l vine^{-1} week^{-1}), and water deficit which was irrigated twice on 21st and 28th February (2 weeks @ 35 l vine^{-1} week^{-1}).

Measurements

In each experiment, temperature was measured continuously and recorded at 15 minute intervals using shielded sensors that were located in the centre of each replicate at mid-canopy level (TinyTag Ultra2 loggers, Hastings Dataloggers, Port Macquarie, Australia).

Phenological stage in the E-L system (Coombe, 1995) was monitored weekly on three tagged vines per replicate until veraison. To evaluate the magnitude and timing of stress in the water treatments of experiment 3, we monitored pre-dawn leaf water potential regularly; four fully expanded leaves per replicate were measured using the procedures of Hsiao (1990).

Berries were sampled in two consecutive seasons in experiment 1, and on one season in experiments 2 and 3. Berries were sampled and sugars were measured as described in Sadras et al. (2008). Briefly, weekly samples of 100 berries per replicate were taken by cutting with scissors through the pedicel as close as possible to their point of attachment. Samples were taken from the seven central vines in each row from bottom, mid and top sections of randomly chosen bunches. Berries were collected into polyethylene bags, placed immediately into a container cooled with ice in the field. Total soluble solids were measured on the same day of collection in 80 berries; the remaining 20 berries were stored at -40 ^{o}C for anthocyanin analysis. Berries in the collection bag were crushed with a manual press and the free-run juice was decanted into a 50-mL centrifuge tube and spun at 2000 x *g* for 10 minutes. Total soluble solids, largely sugars (Coombe and Iland, 2005), were measured on the clarified juice sample using an RFM710 digital refractometer (Bellingham and Stanley Ltd, Kent, UK). We used the method described in Iland et al. (2000) to measure anthocyanins in homogenates (60s at 24,000 rpm) from the remaining samples.

Statistical analyses

The concentration of compounds in berries depends on size-dependent and size-independent mechanisms which can be disentangled with allometric analysis (Sadras

and McCarthy, 2007; Sadras et al., 2007). We therefore used an allometric approach relating concentration of anthocyanins (A) and concentration of total soluble solids (TSS) which removes effects of berry size (Coleman and McConnaughay, 1995; Coleman et al., 1994; Gedroc, 1996; McConnaughay and Coleman, 1999; Niklas, 1994). The following two-phase model was fitted:

Phase 1:	$A = a + b\ TSS$	if $TSS \leq TSSx$	eq. 1a
Phase 2:	$A = a' + b'\ TSS$	if $TSS > TSSx$	eq. 1b

where *a* and *a'* are intercepts and *b* and *b'* are slopes of linear regressions and TSSx is a threshold that marks the shift from Phase 1 to Phase 2. The threshold was objectively determined using the piece-wise routine of SigmaPlot (version 11.0, Systat Software Inc.). Intercepts and slopes were derived from Model II regression to account for errors in both A and TSS (Niklas, 1994). The significance of a quadratic term was used to test for departures from linearity in eq. 1b. Analysis of each individual experiment, thermal regime and secondary factor (variety, fruit load, water regime) showed no departure from linearity in 11 out of 12 cases; the exception was Shiraz control in Exp. 1, where Phase 2 slightly departed from linearity (quadratic term significant at $P < 0.03$). We would therefore refer to Phase 2 as the linear phase hereafter.

We used analysis of residuals to test the effects of temperature and the other experimental sources of variation specific to each experiment (Berg and Grace, 2011; Ericsson et al., 2009; Freed and Cann, 2010; Mayo et al., 2009; Monzon et al., 2006; Sadras et al., 2003; Tang et al., 2011). Eq. 1 was fitted to the data and the effect of treatments on residuals was tested with analysis of variance. The null hypothesis is that the residuals of heated vs controls (all experiments), Cabernet Franc vs Shiraz (exp. 1), thinned vs unthinned (Exp. 2) and irrigated vs water deficit (exp. 3) are statistically undistinguishable.

Results

Thermal regimes and phenological patterns

Figure 1 compares the thermal regimes of control and heated treatments and outlines vine phenological patterns. The passive heating system in experiments 1 and 2 elevated maximum temperature with small effects on minimum temperature, whereas the active heating in Experiment 3 increased both maximum and minimum temperature, and maintained a more steady difference in relation to controls. The rainy winter and spring 2010 led to high canopy vigour of Shiraz in experiments 2 and 3, which were trimmed early in January 2011. Fig. 1e highlights the diminished effectiveness of the passive heating system under the shade of large canopies, and

how trimming restored the heating capacity of the system. As recommended from a physiological perspective (Hall and Sadras, 2009), both heating systems had small effects on relative humidity, hence increased vapour pressure deficit (Caviglia et al., 2011; Sadras and Soar, 2009).

In experiment 1, 2010, elevated temperature had minor effects on the timing of full bloom (E-L stage 23) and advanced veraison (E-L stage 35) by about a week in both Cabernet Franc and Shiraz. The difference in timing of veraison between treatments persisted at maturity (E-L stage 38) for Shiraz but not for Cabernet Franc (Fig. 1). In experiment 1, 2011, elevated temperature had minor or no effects on the timing of full bloom, veraison and harvest. In experiment 2, elevated temperature advanced full bloom by 3 days and veraison by 10 days but both treatments reached harvest ripeness simultaneously. In experiment 3, elevated temperature advanced full bloom by five days but this effect was transient and both treatments reached veraison and harvest simultaneously.

Water treatments in experiment 3

Temperature treatments did not affect pre-dawn leaf water potential; the data pooled across temperature regimes showed significant differences in pre-dawn leaf water potential between irrigated and water deficit treatments started to develop shortly before veraison and lasted for three weeks (Fig. 2).

Relationship between the dynamics of anthocyanins and total soluble solids

The pooled data from all experiments supported a two-phase relationship with a lag phase, where total soluble solids increased with largely undetectable change in anthocyanins, and an approximately linear phase were both traits increased in parallel (Fig. 3a). A piecewise regression fitted to the pooled data returned an onset for the linear phase at 11.4 ± 0.68 ^{o}Brix (red line in Fig. 3a). Using this threshold, we restricted the data set to the linear phase, fitted a linear regression (Fig. 3b) and calculated the residuals for the elevated temperature and control treatments (inset of Fig. 3b). The negative mean residual for the heated treatment in comparison to the positive residual for controls indicated a lower concentration of anthocyanins at the same concentration of soluble solids in the heated treatment. This demonstrated the thermal decoupling of anthocyanins and sugars across seasons, varieties, fruit loads and water regimes ($P < 0.0001$). Using a quadratic function to account for a slight departure from linearity in controls we reached the same conclusion, as the mean residual was 0.080 ± 0.025 mg g^{-1} for controls and -0.057± 0.015 mg g^{-1} for heated treatment ($P < 0.0001$).

The factorial nature of our experiments allowed for the questions: are there differences in anthocyanin : sugar proportion between Shiraz and Cabernet Franc (exp. 1), between high and low fruit ratio (exp. 2) and between irrigated and water deficit treatments (exp. 3)? Linear regressions for the second phase of each experiment and analysis of residuals indicated that the anthocyanin : sugar

proportion was higher in Shiraz than in Cabernet Franc, unaltered by fruit load, and higher under water deficit than in the fully irrigated treatment (Fig. 4).

Onset and rate of linear phase

The decoupling of anthocyanins and sugars in response to elevated temperature demonstrated for the pooled data could be related to a delayed onset of linear phase, a lower rate during this phase or a combination of both. To explore these mechanisms, we calculated onset and rate using linear regressions for each combination of treatments and experiments. In seven out of eight cases, elevated temperature delayed the onset of the linear stage and this effect was significant in four cases (Figure 5, Table 1). The magnitude of this effect ranged from 7 to 30%. The effect of temperature on rates was smaller, less consistent and statistically undetectable in all cases (Figure 5 and Table 1).

In experiment 2, the linear rate increased from 0.084 mg g^{-1} $^{o}Brix^{-1}$ in the unthinned treatment to 0.091 mg g^{-1} $^{o}Brix^{-1}$ in the thinned treatment under elevated temperature, but thinning did not affect rates in non-heated controls (Table 1). In experiment 3, the rate increased from 0.075-0.077 mg g^{-1} $^{o}Brix^{-1}$ in the irrigated treatment to 0.082-0.087 mg g^{-1} $^{o}Brix^{-1}$ under water deficit (Table 1).

Discussion

Our experiments demonstrated that elevated temperature can decouple anthocyanins and sugars in berries of Shiraz and Cabernet Franc in a temperate environment, and that this effect was mediated by delayed onset in the linear stage of the relationship between anthocyanins and sugars with undetectable changes in rates (Figures 3-5, Table 1). Our treatments involved realistic field conditions, moderate temperature differences between treatments (Fig. 1), and a focus on the dynamics of berry traits in comparison to many studies where heated and control treatments involved larger differences, up to 10 ^{o}C, controlled environments or a snapshot of mature berries (see Introduction). Furthermore, comparison of our results with published data is not straightforward because there have been no attempts to formally test the thermal decoupling of anthocyanins and sugars. We thus re-analysed key papers to allow for comparisons (Fig. 6).

Spayd et al. (2002) established elegant experiments to separate the effects of radiation and temperature on Merlot; Fig. 6a shows that heating reduced and cooling increased the anthocyanin : sugar ratio of mature grapes in relation to controls. In the experiment of Yamane et al. (2006), increasing temperature from 20 to 30 ^{o}C reduced anthocyanin : sugar ratio in mature Aqui Queen grapes when 2-week treatments were applied after but not before veraison (Fig. 6b). The picture that emerges is therefore one of a consistent decoupling effect of elevated temperature during berry ripening. Rather than changes in the relative rate of response of net

anthocyanin and sugar build up, our experiments highlighted the relative shift in the onset of the linear phase for both traits (Table 1, Figure 5). Consistently, indirect evidence from commercial vineyards indicates that early maturity associated with elevated temperature is mediated by early onset rather than higher rate of sugar accumulation in Shiraz and Cabernet Sauvignon (Sadras and Petrie, 2011a; Sadras and Petrie, 2011b). The thermal disruption of the anthocyanin : sugar relationship was slightly larger in experiment 3, where both day and night temperature were increased, than in experiments 1 and 2 where we only increased daytime temperature. The lack of a factorial combination of day and night temperature in our study precludes unequivocal conclusions, but the larger thermal disruptions in the experiment where we manipulated day and night temperature is consistent with the observed reduction in anthocyanin concentration in response to elevated night temperature (Mori et al., 2005a).

Vineyard practices and oenological techniques can partially counteract the low anthocyanin: sugar ratio associated with elevated temperature; manipulations in the winery are out of the scope of this paper. Delayed harvest to allow for further colour development has the undesirable effect of increasing potential alcohol (Godden and Gishen, 2005; Petrie and Sadras, 2008b) and increased risk of incomplete primary fermentation (Bisson, 1999). Water deficit favoured higher anthocyanins : sugar ratios of Shiraz in our experiment (Fig. 4). Likewise, water deficit increased anthocyanins : sugar ratios of Cabernet Sauvignon in the Riverland of Australia (Sadras et al., 2007) and the Columbia Valley of USA (Keller et al., 2008). The consistency of this effect indicates that restricted water supply during berry development, a common practice to achieve quality fruit (e.g.Pellegrino et al., 2006), can contribute to partially restore anthocyanin : sugar ratios disrupted by high temperature. Two aspects of irrigation management need to be considered. First, the timing of water deficit has to account for the thermal shift in the onset of the linear phase in the anthocyanin : sugar relationship. Whereas the effect of temperature on anthocyanin : sugar relationship seems to be mediated by changes in onset, rate-mediated changes driven by water deficit cannot be excluded. Our trial suggests that water deficit shortly before veraison could be effective, but more refined studies on timing of water deficit are required. Second, a trade-off exists between the putative benefit of water deficit that favours the colour : sugar proportion and the maintenance of water supply required for evaporative cooling and tolerance to extreme temperatures in vineyards (Soar et al., 2009). Dealing with this trade-off thus requires proper quantification of the two distinct aspects associated with warming trends: the increase in background temperature, as simulated experimentally in this study, and the incidence of heat waves.

We found no effect of increased source : sink ratio on the degree of thermal decoupling of anthocyanins and sugars in Shiraz (Fig. 4). The literature shows all three possible responses: the anthocyanin:sugar relationship increased, decreased or was unchanged by manipulation of source : sink ratio (Fig. 6c-e). This diversity of responses is partially accounted for by the non-linear relationship between total soluble solids and source:sink ratio (Kliewer and Dokoozlian, 2005). Hence, manipulation of source:sink ratio to partially restore anthocyanin : sugar ratios disrupted by high temperature does not seem straightforward.

In summary, indirect evidence supported the hypothetical thermal decoupling of anthocyanins and sugars in red wine varieties. Here we presented direct proof of this phenomenon under realistic field conditions in the Barossa Valley, a temperate region. The decoupling is more likely to be caused by a delayed onset in the accumulation of anthocyanins, rather than relative changes in rates. Water deficit shortly before veraison could partially restore the anthocyanin : sugar ratio disrupted by elevated temperature.

Captions to figures

1. *(a-c)* Temperature in controls and *(d-f)* temperature difference between heated and control treatments. Solid lines are maximum temperatures and dashed lines are minimum temperatures. Temperature was measured at mid-canopy level in each of three replicates. Experiments 1 and 2 used passive heating systems that increase day time temperature in clear days, and experiment 3 used a combination of passive and active heating that increased day and night temperature. Arrow-heads indicate time of full bloom (E-L = 23), veraison (E-L = 35) and harvest (E-L = 38); closed symbols are for control and open symbols for heated treatments. In experiments 2 and 3, canopies were trimmed at the dates indicated in (*e)* and (*f)*.

2. Irrigation regime and pre-dawn leaf water potential of irrigated and water deficit treatments in experiment 3. Vertical bar indicates veraison (E-L stage 35), open arrow-heads indicate irrigation events in irrigated controls, closed arrow-heads indicate irrigation events in water deficit treatment and diamonds indicate rainfall events (> 20 mm). Each irrigation event applied 35 l per vine. Error bars are two standard errors of the mean and asterisks indicate difference between water regimes at $P<0.05$ (*) and $P<0.0001$ (***).

3. (a) The relationship between anthocyanins and total soluble solids in berries of Shiraz and Cabernet Franc involved a lag phase, where total soluble solids increased with no detectable changes in anthocyanins, and an approximately linear phase where both traits increased in parallel. The red line is the piecewise regression with a break point at 11.4 ± 0.068 ^{o}Brix. (b) Linear phase relationship between anthocyanins and total soluble solids highlighting control and heated treatments across three experiments. The line is the fitted regression. Inset compares the regression residuals for control and heated treatments (mean and 1 standard error).

4. *(left panels)* Linear phase relationship between anthocyanins and total soluble solids in and *(right panels)* residuals of regressions (mean and 1 standard error) comparing cultivars in experiment 1, fruit loads in experiment 2 and water regimes in experiment 3. Note the difference in scales for anthocyanins and residuals.

5. Effect of elevated temperature on the relationship between concentration of anthocyanins and total soluble solids in the linear phase. Lines are least square regressions ($0.95 \leq r \leq 0.99$; $P < 0.0001$). Rate (slope) and onset (x-intercept) of the regressions are in Table 1.

6. Re-analysis of published data on the effect of (a-b) temperature and (c-e) fruit load on the degree of coupling between anthocyanins and sugars in grapes. (a) Heating reduced and cooling increased the anthocyanin : sugar ratio of mature Merlot grapes in Washington, USA. Open symbols are low and closed symbols are high radiation. Numbers are % change relative to controls. (b) Increasing temperature from 20 to 30 ^{o}C reduced anthocyanin : sugar ratio in

mature Aqui Queen (*Vitis labrusca x V. vinifera*) grapes when 2-week treatments were applied after (stages II-IV) but not before veraison (stage I). Numbers are % change relative to controls. (c) Fruit thinning did not alter the anthocyanin : sugar relationship of Cabernet Sauvignon in north-western Victoria, Australia. (d) Fruit thinning reduced the anthocyanin : sugar ratio in 2003, but did not change the ratio in 2004 and 2005 in berries of Cabernet Sauvignon in the Riverland, Australia. Numbers are slopes ± s.e. of fitted regressions. (e) Fruit thinning increased the anthocyanin : sugar relationship of Nebbiolo in Piedmont, Italy. Note differences in the expressions of anthocyanins (e.g. mass per unit weight, mass per unit skin area) and sugars. Adapted from (a) (Spayd et al., 2002), (b) (Yamane et al., 2006), (c) (Petrie and Clingeleffer, 2006), (d) (Sadras et al., 2007), (e) (Guidoni et al., 2002).

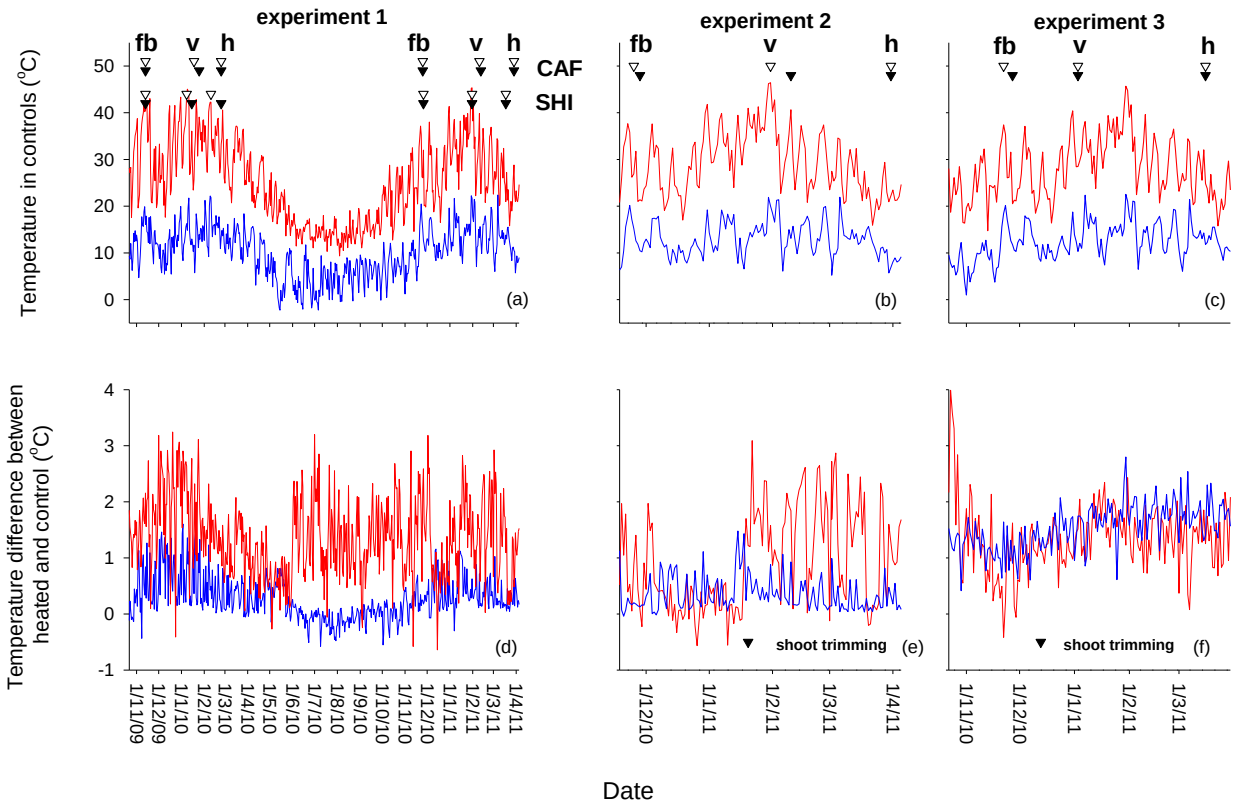

experiment 1
experiment 2
experiment 3
fb
v
h
CAF
SHI
Temperature in controls (°C)
Temperature difference between heated and control (°C)
(a)
(b)
(c)
(d)
(e)
(f)
shoot trimming
Date

Fig. 7

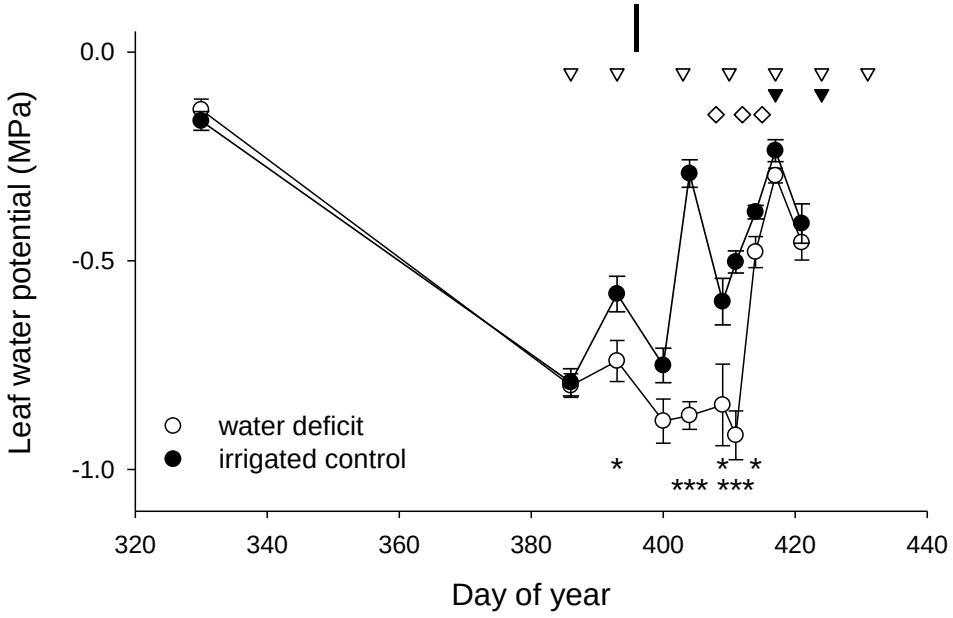

0.0
-0.5
-1.0
Leaf water potential (MPa)
water deficit
irrigated control
*
*
*
***
***
320
340
360
380
400
420
440
Day of year

Fig. 2

Fig. 3

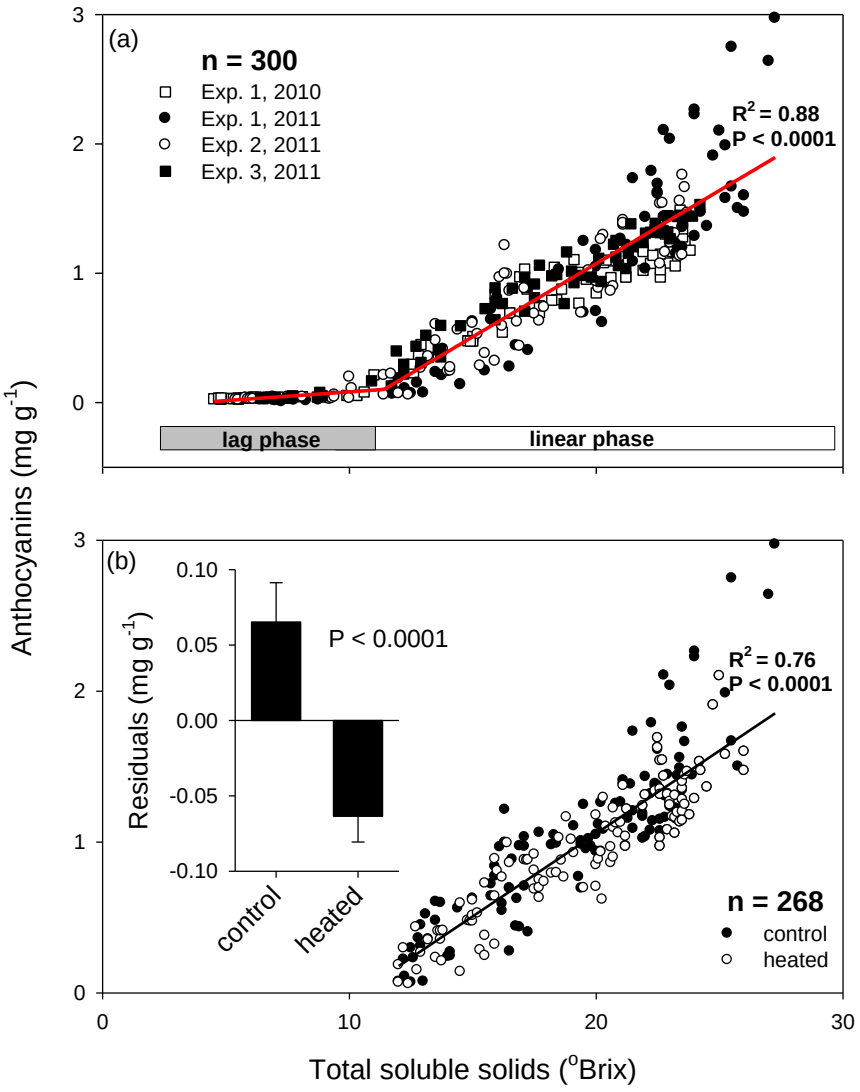

(a)
n = 300
Exp. 1, 2010
Exp. 1, 2011
Exp. 2, 2011
Exp. 3, 2011
R2 = 0.88
P < 0.0001
lag phase
linear phase
(b)
P < 0.0001
Residuals (mg g-1)
control
heated
R2 = 0.76
P < 0.0001
n = 268
control
heated
Anthocyanins (mg g-1)
Total soluble solids (°Brix)

Fig. 4

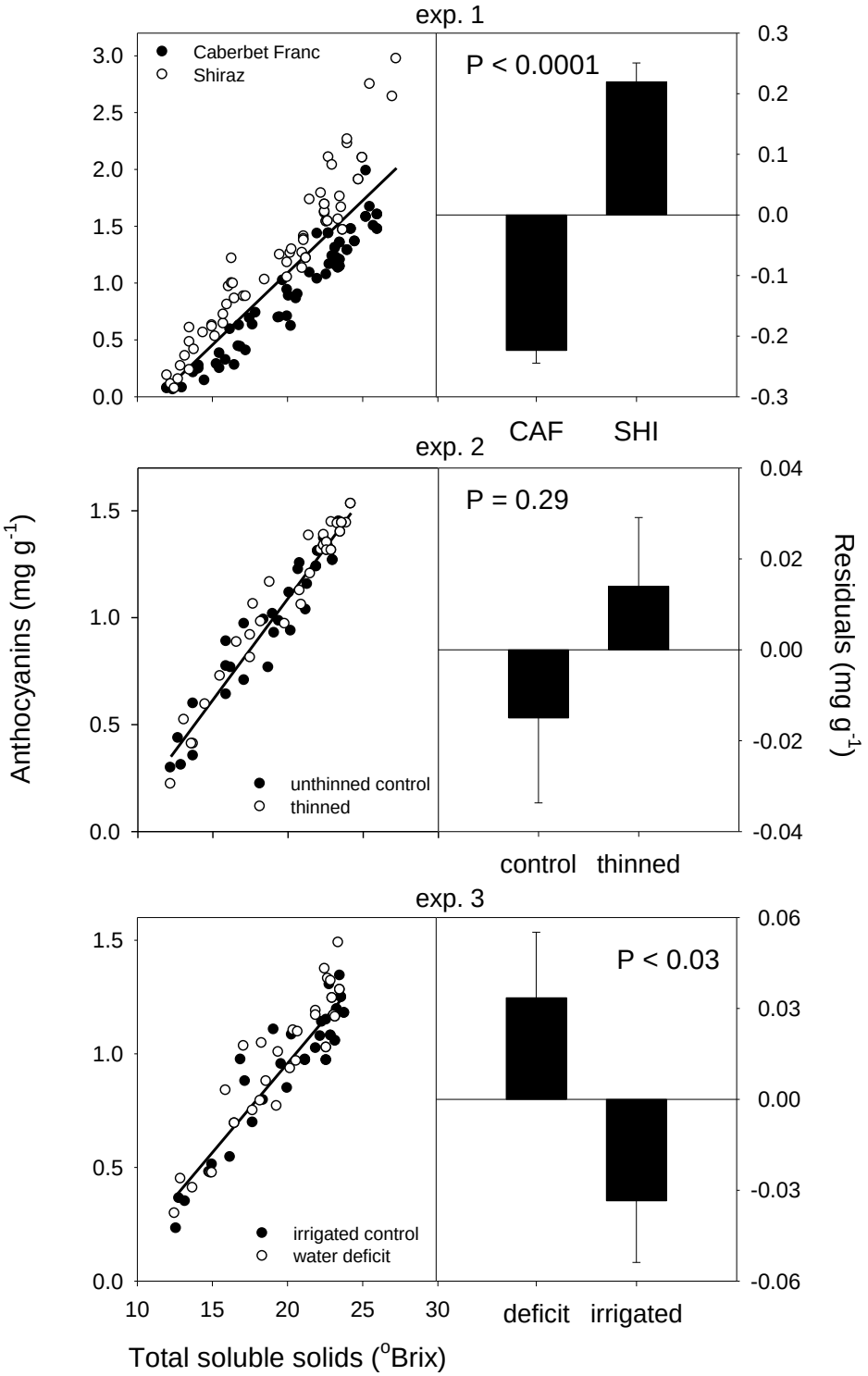

exp. 1
Caberbet Franc
Shiraz
P < 0.0001
CAF
SHI
exp. 2
P = 0.29
unthinned control
thinned
control
thinned
exp. 3
P < 0.03
irrigated control
water deficit
deficit
irrigated
Anthocyanins (mg g-1)
Residuals (mg g-1)
Total soluble solids (°Brix)

Fig. 5

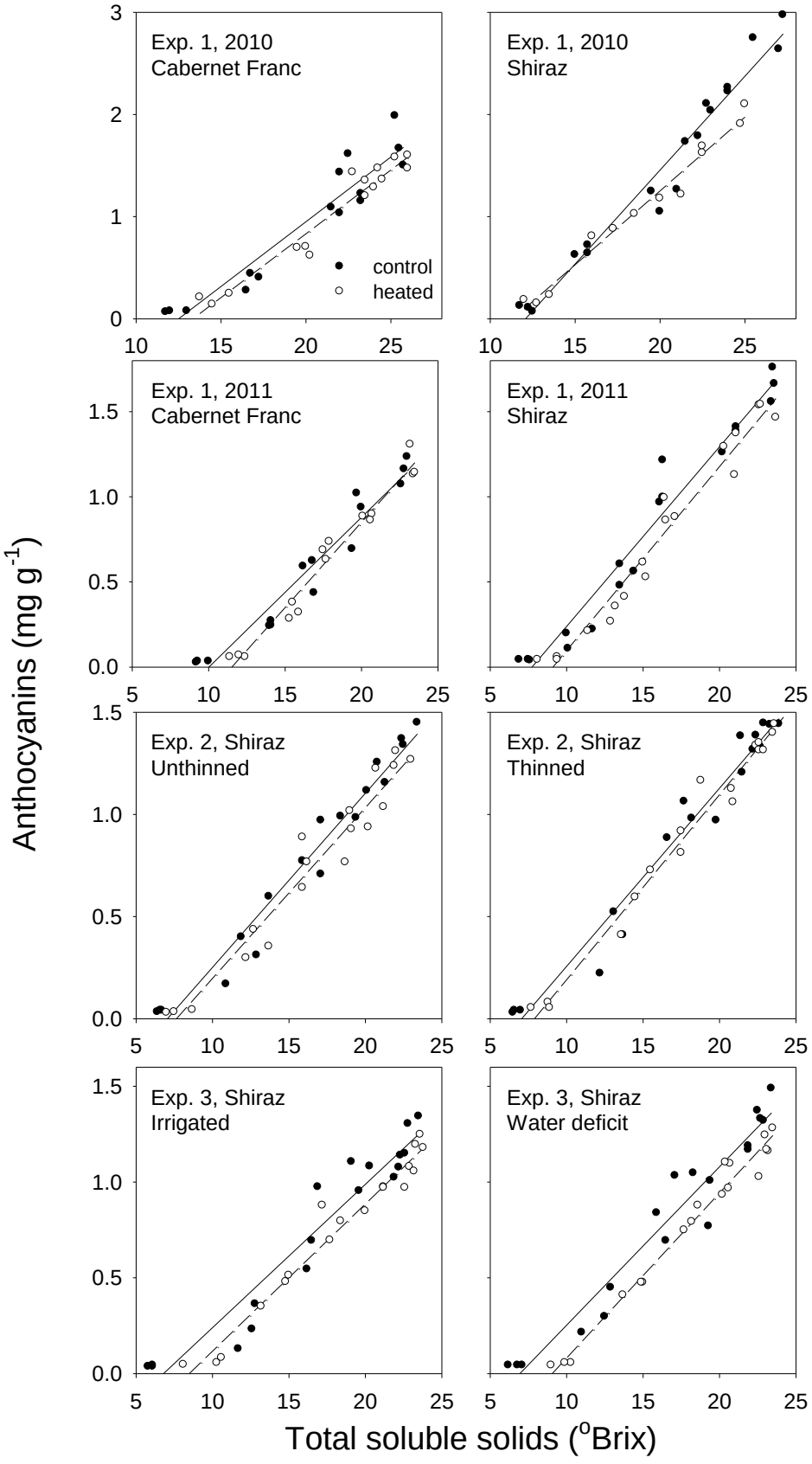
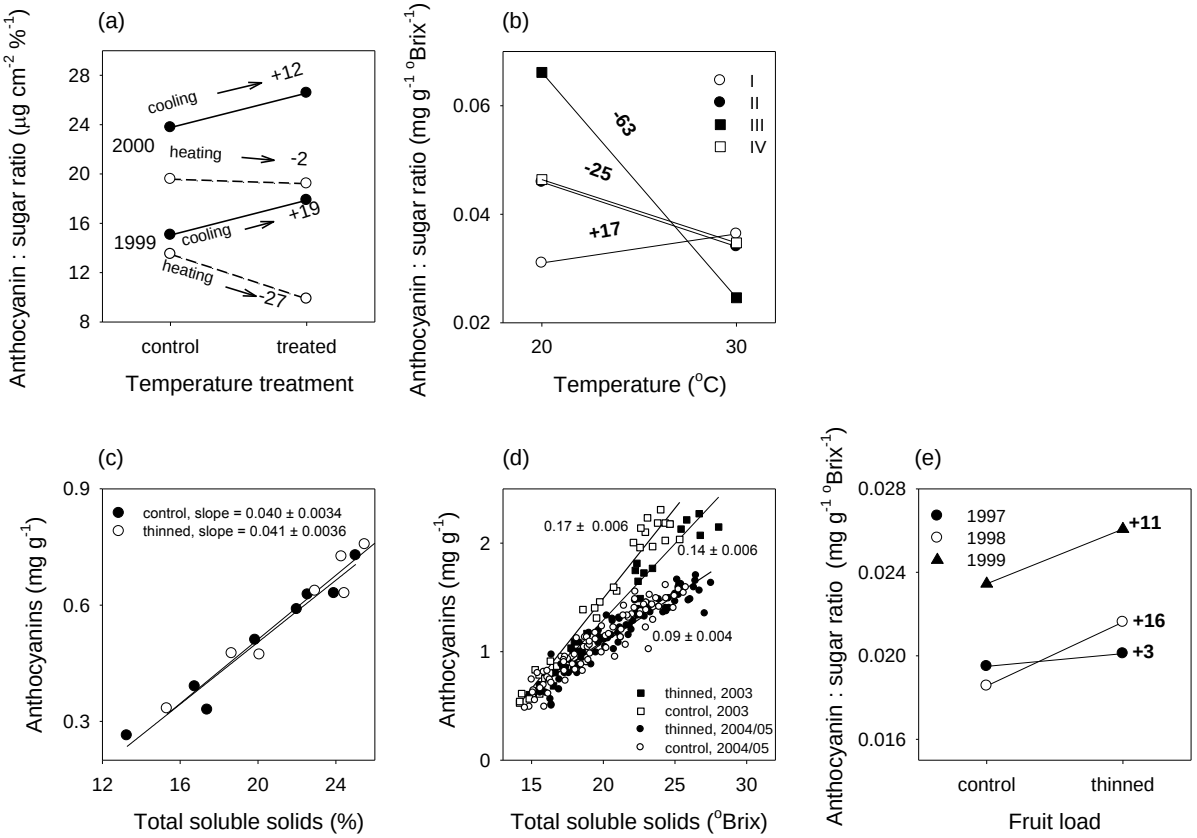

Exp. 1, 2010
Cabernet Franc
control
heated
Exp. 1, 2010
Shiraz
Exp. 1, 2011
Cabernet Franc
Exp. 1, 2011
Shiraz
Exp. 2, Shiraz
Unthinned
Exp. 2, Shiraz
Thinned
Exp. 3, Shiraz
Irrigated
Exp. 3, Shiraz
Water deficit
Anthocyanins (mg g-1)
Total soluble solids (oBrix)

Fig. 6


(a)
Anthocyanin : sugar ratio (μg cm-2 %-1)
28
24
20
16
12
8
2000
cooling
+12
heating
-2
1999
cooling
+19
heating
-27
control
treated
Temperature treatment
(b)
Anthocyanin : sugar ratio (mg g-1 °Brix-1)
0.06
0.04
0.02
I
II
III
IV
-63
-25
+17
20
30
Temperature (°C)
(c)
Anthocyanins (mg g-1)
0.9
0.6
0.3
control, slope = 0.040 ± 0.0034
thinned, slope = 0.041 ± 0.0036
12
16
20
24
Total soluble solids (%)
(d)
Anthocyanins (mg g-1)
2
1
0
0.17 ± 0.006
0.14 ± 0.006
0.09 ± 0.004
thinned, 2003
control, 2003
thinned, 2004/05
control, 2004/05
15
20
25
30
Total soluble solids (°Brix)
(e)
Anthocyanin : sugar ratio (mg g-1 °Brix-1)
0.028
0.024
0.020
0.016
1997
1998
1999
+11
+16
+3
control
thinned
Fruit load

Chapter 5. Effects of elevated temperature in grapevine: berry sensory traits

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Abstract

Thermal decoupling results from the differential effect of temperature on different components of biological systems from molecular to ecosystem scales. We tested the hypotheses that elevated temperature decouples berry sensory traits and that this effect is variety dependent. We assessed 20 sensory traits in berries from a factorial field experiment combining two temperature regimes (elevated temperature, control) and four varieties (Cabernet Franc, Chardonnay, Semillon, Shiraz) over two thermally contrasting seasons in the Barossa Valley. The thermal response had two elements: offset, which accounts for the average advancement (or delay) across traits, and decoupling, which measures the scatter in the response of the different traits. Large decoupling was generally associated with large offset (e.g. Cabernet Franc) but large decoupling was also observed with minor offset (e.g. Semillon). Two types of decoupling were identified: decoupling mostly driven by differential response between seed and other berry components and decoupling with a dominant variation in traits within berry parts.

In conclusion, elevated temperature decoupled berry sensory traits and this effect was variety and season dependent. Awareness of the magnitude and type of decoupling would likely contribute to viticultural and winemaking adaptations in warmer regimes.

Introduction

Assessment of berry traits for oenological purposes includes complementary chemical and sensory traits. Berry chemical profiles allow for quantitative and repeatable characterisation of fruit traits. As tools for decision making, however, analytical techniques are often expensive and time-consuming, hence the interest in developing cheaper and faster methods for real-time assessment of these traits (Le Moigne et al., 2008a; Shah et al., 2010). More importantly, chemical profiles of berries are often fragmented and correlations with sensory wine attributes are not necessarily strong (Blackman et al., 2010; Ristic et al., 2010). Chemical characterisation can therefore be complemented with sensory assessment that integrates important fruit attributes (Winter et al., 2009). The main drawbacks of sensory assessments are the significant person-to-person variation, person-by-trait and trait-by-trait (e.g. visual-by-taste) interactions which stem from the complexity of wine attributes compounded by the variability of human smell and taste sensitivity (Atanasova et al., 2005; Parr et al., 2004; Pretorious et al., 2004; van Beilen et al., 2011; Verdu Jover et al., 2004). Rigorous protocols seek to reduce these sources of variation (Lohitnavy et al., 2010; Winter et al., 2009).

In the context of warming trends, the focus of this paper is the effect of elevated temperature on berry sensory traits. The effects of temperature on berry and wine attributes have been investigated with indirect approaches that compare seasons or regions (Cozzolino et al., 2011; Duchêne and Schneider, 2005; Grifoni et al., 2006; Jones and Davis, 2000; Soar et al., 2008). This indirect approach is valuable but is bound to be inconclusive because temperature is confounded with other climatic factors, and in many cases with management practices and soils. Owing to the difficulty and cost involved in manipulation of temperature in the field, direct comparisons where temperature is manipulated experimentally has favoured controlled environments (Mori et al., 2007; Mori et al., 2005a; Wen et al., 2008). Tarara et al. (2000) and Soar et al. (2009) discussed the limitations of controlled environments in temperature studies of grapevine.

Thermal decoupling results from the differential effect of temperature on different components of biological systems from molecular to ecosystem scales (Büntgen and Schweingruber, 2010; Edwards and Richardson, 2004; Englund et al., 2011; Sheldon et al., 2011). Thermal decoupling of berry traits is a likely contributor to unbalanced wine (Petrie and Sadras, 2008b; Sadras and Moran, 2012). Direct measurements in field-grown Shiraz and Cabernet Franc demonstrated that elevated temperature can decouple anthocyanins and sugars in berries, with consequences for the colour-alcohol balance of wine (Sadras and Moran, 2012).

Here we test the hypotheses that elevated temperature decouples berry sensory traits in grapevine, and that this effect is variety dependent. We present results from a factorial field experiment combining two temperature regimes (elevated temperature, control) and four varieties (Cabernet Franc, Chardonnay, Semillon, Shiraz) over two thermally contrasting seasons in the Barossa Valley.

Method

This paper reports aspects of a larger study that has been partially described elsewhere (Sadras and Moran, 2012). To avoid multiple cross references and facilitate interpretation, we duplicate some elements reported before (varieties, husbandry, experimental design, heating systems) and develop with more detail the aspects of methods that are particular to this paper.

Vines, site and experimental design

The vines were established on a red brown earth at the South Australian Research and Development Institute, Nuriootpa (34 ^{o}S, 139 ^{o}E) in the Barossa Valley of South Australia. Gladstones (1992) and Dry and Coombe (2005) described the climate and viticultural practices of the region. Vines are arranged with a row by vine spacing of 3.50 x 2.25m, cordon-trained to a single-wire trellis, have been spur pruned and managed with supplementary irrigation since planting using a single 4 L h^{-1} dripper per vine.

A factorial experiment combining four varieties and two temperature regimes was established on October 2009. Own-rooted varieties were Cabernet Franc clone Penfold 58 planted in 1988, Chardonnay clone 277 planted in 1995, Semillon clone LRC147 planted in 1989 and Shiraz clone NSW 15 planted in 1997. Unlike management in commercial vineyards that accounts for specific varietal requirements, all varieties are in the same block and have been managed similarly since planting. The temperature regimes were a control with background ambient temperature, and a treatment in which daytime temperature was elevated using open-top chambers (Table 1). Temperature was measured continuously and recorded at 15 minute intervals using shielded sensors that were located in the centre of each replicate at mid-canopy level (TinyTag Ultra2 loggers, Hastings Dataloggers, Port Macquarie, Australia). Sadras and Soar (2009) present details of the heating system, its construction, performance and limitations.

Treatments were laid out in a split-plot design with three replicates. Main plot was allocated to variety and sub-plots to temperature treatments. Each replicate comprised nine vines, allowing for two buffer vines and sampling in seven central vines.

Tracking sugar accumulation

Sampling date for sensory assessment was specific for each variety and thermal regime in order to achieve a similar sugar concentration in berries of heated and control treatments. To meet this objective, we monitored sugar concentration as follows. Weekly samples of 100 berries per replicate were taken by cutting with scissors through the pedicel as close as possible to their point of attachment. Samples were taken from the seven central vines in each row from bottom, mid and top sections of randomly chosen bunches. Berries were collected into polyethylene

bags and placed immediately into a container cooled with ice in the field. Berries in the collection bag were crushed with a manual press and the free-run juice was decanted into a 50-mL centrifuge tube and spun at 2000 x *g* for 10 minutes. Total soluble solids were measured on the clarified juice sample using an RFM710 digital refractometer (Bellingham and Stanley Ltd, Kent, UK). We targeted sampling for sensory assessments at ~ 22 ^{o}Brix for Chardonnay and Semillon and ~ 24 ^{o}Brix for Shiraz and Cabernet Franc (Iland et al., 2011).

Sensory assessment

Assessments were conducted by trained panels using the protocol of Winter et al. (2009). Training comprised four steps. First, we prepared solutions of sugar (0 to 80 g L^{-1} range in 10 g L^{-1} steps) and tartaric acid (3.5 g L^{-1}, dilution range 10 to 100% in 10% steps) in water or commercial grape juice. Panellists tasted the solutions in random order and sorted them according to sweetness or acidity. Second, we combined sugars and acids firstly into water and then into grape juice and tests were conducted until we had confidence that panellists had the capacity to grade sugar and acid concentration in these solutions. Third, panellists practised the grading of astringency and tannic intensity in seeds and skins of berries. Fourth, a pilot test was conducted by testing berries against the 20 traits of Winter et al. (2009), and discussions were held leading to an agreed interpretation of the protocol and grading system by the members of the panel. After this training process, the berry sensory assessment was performed on five berries per replicate using the attributes summarised in Table 2, and scales from 1 = unripe to 4 = fully ripe (Winter et al. 2009). Assessment of each treatment therefore was based on 15 berries (3 replicates x 5 berries per replicate), which compares with the recommended sample size of 6-8 berries (p. 28, Winter et al., 2009). Berries were collected using the sampling approach described for total soluble solids (above) and stored in a fridge for assessment on the following day. Berries were served in a randomised order in paper cups; water and a pectin rinse (1g L^{-1}) were provided as palate cleansers during evaluation. A small panel of three scientists tested the sampling and assessment protocols in 2010. A larger panel of 12 members conducted the assessment in 2011; these panellists were selected based on interest and availability and were paid for their assessment.

A Shapiro-Wilk normality test (P = 0.05) indicated that the sensory traits matched the pattern expected if the data were drawn from a population with a normal distribution in 81% of cases (Sigmaplot v. 11.0, SystaSoftware Inc.). We therefore preformed analysis of variance and regression analysis using the untransformed data. Analysis of variance was used to account for all sources of variation on each berry trait, i.e. variety, temperature, panellist and their interactions. A complementary analysis of variance probed for temperature effects on berry traits for each variety. We quantified the degree of decoupling in two steps. First, we fitted least squares regressions between scores for heated and control treatments with a forced slope = 1. The intercept of this regression represents the average offset whereby heated berries were advanced (intercept > 0) or delayed (intercept < 0) in relation to

controls. Second, we calculated the root mean square error (Kobayashi and Salam, 2000):

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(M_i - E_i)^2}$$

were n (=20) is the number of traits for each variety and season, M_i is the measured score and E_i is the score derived from the fitted model. Scatter around the fitted line, reflected in RMSE, was used to quantify decoupling.

Results

Thermal regimes

Table 1 summarises the thermal regimes during the experiment. Maximum temperature was higher in 2009-10 than in 2010-11; particularly important for this study, maximum temperature during the key ripening period of February and March was ~5 ^{o}C higher in the first season. Relative to controls, the heated system elevated maximum temperature by 1.6 ^{o}C in 2009-10 and by 1.4 ^{o}C in 2010-11, with smaller effect on minimum temperature. This compares with a projected increase in mean temperature of 2^{o}C for Nuriootpa by 2050 under the scenarios and modelling conditions of Jones et al. (2005).

Sugar concentration at the time of sensory assessment

The effect of elevated temperature on the time-course of total soluble solids (TSS) showed three stages, as illustrated in Fig. 1a. During the lag-phase (TSS ~ 5 ^{o}Brix), temperature had no detectable effect on total soluble sugars. The trajectories of heated and control treatments diverged during the phase between 5 and 8 ^{o}Brix in controls. In the third stage (> 8 ^{o}Brix in controls), the trajectories of both treatments converged, leading to small differences in the timing of targeted TSS for harvest. Nonetheless, we aimed at similar ($P > 0.05$) sugar concentration in heated and control berries at the time of sensory assessment. By staggering sampling date (from 0 to 14 days) this was achieved in 6 out of 8 cases (Fig. 1bc). The exceptions were berries in heated treatments that had higher total soluble solids than controls for Chardonnay in 2009-10 and for Cabernet Franc in 2010-11. In 2009-10, control berries had a higher concentration of sugars than their heated counterparts in Shiraz; the difference was statistically marginal ($P = 0.063$) but sufficiently large to alter the sensory assessment, as discussed below.

Sensory traits in 2009-10

Scores for sensory traits varied among panellists ($P < 0.0001$) and there was a significant interaction between panellist and trait ($P < 0.0001$). There was no detectable panellist x treatment interaction ($P > 0.54$) or panellist x treatment x trait interaction ($P > 0.84$) indicating an unbiased assessment of the differences between heated and control treatments.

Table 2 summarises the analysis of variance accounting for variety, temperature and temperature x variety interaction and Fig. 2 shows the mean scores with a complementary analysis of variance. Varieties differed in 21 out of 22 traits ($P < 0.05$); the exception was pulp acidity. Analysis of variance for individual varieties detected effects of temperature for six traits in Chardonnay and for 3-4 traits in the other varieties (Fig. 2).

Berry colour was affected by temperature and by variety x temperature interaction; this was primarily driven by the more advanced colour in heated Chardonnay. Berry softness and stalk removal were unaffected by treatments.

Two skin traits responded to the interaction: fruity aromas, which were more developed in heated berries of Semillon and Chardonnay, and disintegration, which was advanced by high temperature in Chardonnay and delayed in Shiraz. Elevated temperature also reduced the score for skin astringency in Shiraz.

Two pulp traits responded to the interaction: juiciness which was more developed in heated berries of Semillon and Chardonnay and fruity aromas, which were more developed under high temperature in Chardonnay and less developed under high temperature in Shiraz.

Elevated temperature enhanced both colour and flavours in seeds; these significant effects were mostly driven by the strong responses of Cabernet Franc and Chardonnay. Moreover, for Cabernet Franc all seed traits were consistently more advanced under elevated temperature than in controls, with four (crushability, colour, flavours, tannic intensity) out of five reaching statistical significance. The strong increase in seed tannic intensity in Cabernet Franc, and the lack of responses in the other varieties was reflected in a significant variety x temperature interaction.

Sensory traits in 2010-11

Scores for sensory traits varied among panellists ($P < 0.0001$) and there was a significant interaction between panellist and trait ($P < 0.0001$). There was no detectable panellist x treatment interaction ($P > 0.43$) or panellist x treatment x trait interaction ($P > 0.90$) indicating an unbiased assessment of the differences between heated and control treatments.

Table 2 summarises the analysis of variance accounting for variety, temperature and temperature x variety interaction and Fig. 3 shows the mean scores with a complementary analysis of variance. Varieties differed in 12 out of 22 traits ($P < 0.05$). Analysis of variance for individual varieties detected effects of temperature for

five traits in Chardonnay, three in Semillon, two in Cabernet Franc, and none in Shiraz (Fig. 3).

Berry colour was affected by temperature and variety x temperature interaction; this was primarily driven by the delayed development of colour in heated Semillon. Elevated temperature advanced the development of skin astringency in Chardonnay and delayed skin disintegration in Cabernet Franc and Semillon.

Analysis of variance of pulp traits for individual varieties showed that elevated temperature advanced fruity aromas in Chardonnay and delayed the development of sweetness in Semillon. None of these effects were strong enough to be detected in the full analysis of variance. Elevated temperature delayed pulp detachment in Cabernet Franc, and this was reflected in the variety x temperature interaction.

Elevated temperature advanced seed colour, mostly in Semillon, and advanced the development of seed in Chardonnay, which showed significantly higher scores for crushability, tannic intensity and astringency.

Quantifying thermal offset and decoupling

The previous analysis dealt with the effects of temperature on individual traits. The hypothesis of thermal decoupling requires comparison of the relative responses of all traits. Figure 4 compares the score for each trait in the heated treatment against the score in controls. There are two aspects to this analysis: offset and decoupling. The offset indicates whether heated berries were on average advanced (intercept > 0) or delayed (intercept < 0) in relation to controls. The scatter, quantified with RMSE, measures the magnitude of decoupling attributable to the thermal regime. The offset ranged from ~0 to 0.33, and was larger in the hotter 2009-10 season. RMSE ranged between 0.23 and 0.32 in 2009-10, and between 0.12 and 0.19 in 2010-11, reinforcing the conclusion that decoupling was larger in the hotter 2009-10 season ($P < 0.0001$).

For Cabernet Franc in 2009-10, there was a strong offset and thermal decoupling, which was associated with the enhanced ripening of seed in comparison to the other berry traits (inset Fig. 4a). In the milder 2010-11 (Fig. 4e), the overall response to temperature was small (intercept ~ 0; small RMSE).

For Chardonnay in both seasons, elevated temperature consistently increased the score of most traits (intercept > 0). The decoupling was larger in 2009-10 than in 2010-11, i.e. RMSE 0.32 vs. 0.18. In 2010, differences among berry parts were not evident, but temperature decoupled substantially traits within berry parts, e.g. flavours and tannic intensity in seed (arrow in Fig. 4b).

For Semillon in both seasons, the offset in berry sensory traits in response to temperature was very small compared to the other varieties (intercept ~ 0). As in all other varieties, the decoupling was larger in 2009-10 than in 2010-11. In 2009-10 for example, temperature strongly decoupled pulp sweetness and juiciness. Despite the smaller effect on the milder 2010-11 season, we detected a significant decoupling

associated with the advanced development of seed relative to the other berry components (inset Fig. 4g).

For Shiraz in 2009-10, temperature significantly delayed the development of sensory attributes in berries (intercept < 0). This is largely attributable to the difference in total soluble solids at the time of assessment, which was 26.6 ^{o}Brix in controls compared to 24.1 ^{o}Brix in the heated treatment (Fig. 1). The more advanced ripening in controls likely cancelled the effect of temperature and accounted for the negative offset depicted in Fig. 4d. In 2010-11, the thermal offset (intercept ~ 0) and decoupling were both small (Fig. 4h).

Discussion

Effects of temperature on the biology of grapevines and chemical and sensory attributes of fruit and wine can be investigated with indirect methods, where seasons or regions are compared, or with direct methods where temperature is experimentally manipulated. Owing to confounding factors, indirect methods are useful but inconclusive (Soar et al., 2008). Likewise, experimental artefacts in controlled environments lead to useful but generally inconclusive results (Düring, 1980; Passioura, 2002; Passioura, 2006; Sadras et al., 1993; Soar et al., 2009; Tarara et al., 2000). Some studies measured chemical traits of berries in response to temperature in the field (Spayd et al., 2002; Tarara et al., 2008a; Tarara et al., 2008b) and many indirect studies inferred responses of sensory and chemical wine attributes to temperature (Cozzolino et al., 2011) (Duchêne and Schneider, 2005; Grifoni et al., 2006; Jones and Davis, 2000; Soar et al., 2008). This is the first report of berry sensory properties measured in experiments where temperature has been manipulated under realistic vineyard conditions.

Limitations to this study

The results of this study need to be considered in the light of three main limitations related to the heating system, timing of sampling and sensory assessment.

The heating system used in these experiments has been fully evaluated and its limitations discussed in detail (Sadras and Soar, 2009). It tracks the diurnal and seasonal dynamics of temperature and maintains relative humidity, hence realistically increasing vapour pressure deficit. The heating system increased maximum temperature with minor effects on minimum temperature (Table 1). This is unrealistic in relation to realised warming trends between 1910 and 2000 in our region, where minimum temperature has increased proportionally more than maximum temperature (McInnes et al., 2002). Furthermore, the composition of seed and fruit are often responsive to night temperature (Aguirrezábal et al., 2009; Kliewer and Torres, 1972; Mori et al., 2005a; Mori et al., 2005b).

Berry traits are highly dynamic (Coombe and Iland, 2005; Keller, 2010b). The timing of sampling for sensory traits is therefore important, as more or less developed

berries would have different proportions of key compounds and different relations among sensory traits. We set a common concentration of sugars in heated and control berries as the criterion for sampling time for two reasons. First, sugar concentration is the primary trait used to decide the timing of commercial harvest (Pearce and Coombe, 2005; Sadras and Petrie, 2012). Second, sugars are not only responsible for the sweet taste of berries but also interfere with the perception of sourness, bitterness and astringency (Keller, 2010b). Based on weekly measurements of total soluble solids, we achieved the aim of statistically similar sugar concentration in berries of heated and control treatments in most cases (Fig. 1). The larger differences in sugar concentration between treatments were detected in 2009-10 for Chardonnay and Shiraz. For Chardonnay, the higher sugar concentration in the heated treatment at the time of assessment lead to the over-estimation of the offset between heated and control treatments (Fig. 4b). For Shiraz, the 2.5 ^{o}Brix difference in sugar concentration between treatments likely contributed to the apparent delay in development of sensory traits in heated berries (Fig. 4d). In these two (out of eight) cases therefore, the effect of temperature on sensory traits was partially confounded with differences attributable to sugar concentration. Another aspect of sample timing is that the scale of Winter et al. (2004) rates berry traits from unripe to ripe. It indicates differences in berry composition and maturity but it does not necessarily follow that the desired composition for a particular style of wine is the most mature category. However, categorising berry sensory traits into the unripe-to-ripe scale is valid for evaluating decoupling patterns.

Scoring sensory traits involve complex interactions between variable fruit traits resulting from genotype, environment and genotype x environment interaction, and variable human smell and taste sensitivity that depends on a myriad of biological and cultural factors. Thus, a significant “panellist effect” has been found whenever this source of variation was statistically tested (Baiano et al., 2010; Chapman et al., 2004; Chapman et al., 2005; Le Moigne et al., 2008a; Le Moigne et al., 2008b; Tardaguila et al., 2008). We also detected a significant panellist effect in scoring sensory traits. However, there was a statistically undetectable interaction between panellist and temperature regime in both seasons, which suggests lack of bias in the assessment of differences attributable to temperature (Baiano et al., 2010). The effects of temperature on berry traits were much larger in 2009-10 than in 2010-11 (Figure 2 vs 3; Figure 4). This could be partially related to the small panel used in 2009-10. However, the substantially hotter background temperature during the ripening period in 2009-10 was the more likely source of differences between seasons (Table 1); the same increment of maximum temperature over a baseline of 30-35 ^{o}C in February and March 2010 possibly had a larger effect than the same increment over a baseline of 24-30 ^{o}C in 2011 (Schultz, 2000; Sheldon et al., 2011; Webb, 2006). Four observations further support the conclusion that assessments in 2009-10 were reliable despite the small panel: (i) the consistent delay in ripe characters in Shiraz associated with the differences in sugar concentration, (ii) the consistent advancement of ripe traits in Chardonnay, which was reproduced in 2010-11 with a larger panel, (iii) the strong and significant decoupling of seed and pulp/skin characters in Cabernet Franc, which was observed again for Semillon in 2010-11, and (iv) the close match between the signature traits of sensory maturity described

by Winter et al. (2009) for Chardonnay and Shiraz, and the significant effects of temperature shown in Fig. 2; this is further discussed below.

Effects of temperature in the profile of berry traits: offset and decoupling

Intercepts of least square regressions (with slope = 1) between heated and control treatments measure the temperature-driven offset and scatter around this model measures decoupling (Fig. 4). The null hypothesis that elevated temperature offsets the pattern of development of sensory traits (intercept ≠ 0) with no alteration of relative scores (RMSE ~ 0) is a useful starting point for the analysis.

Elevated temperature caused large offset and decoupling in Cabernet Franc in the hot 2009-10 season, but not in the milder 2010-11. Interestingly, a large part of the thermal decoupling in 2009-10 was associated with a strong differential response of seed and other berry components. The decoupling of seed components and sensory traits in pulp and skin is partially attributable to the asynchrony in the development of these components (Coombe and Iland, 2005). However, the seed vs rest-of-berry decoupling was found in only two of eight cases, indicating that other variety-dependent causes are important in addition to developmental asynchrony. Irrespective of the cause, seed in a more advanced stage of development relative to pulp and skin has winemaking implications discussed in next section.

In 2009-10, elevated temperature caused large offset and decoupling in Chardonnay. Both the higher background temperature and a slightly higher sugar concentration at the time of assessment in the heated treatment contributed to the differences between seasons. In contrast to Cabernet Franc in 2009-10, decoupling in Chardonnay was not associated with different thermal response among berry parts, but rather to decoupling of traits within the same berry component; note for example the large decoupling between flavours and tannic intensity in seed (arrow in Fig. 4b). In 2010-11, there was a significant offset of berry traits but the scatter was moderate, suggesting some capacity to maintain the balance of berry components that is essential for the adaptation of this variety to a very broad thermal range (Dry et al., 2005; Stevenson, 2005). The notion that Chardonnay shifts its sensory profile in response to temperature but is able to maintain the balance among traits was further tested using the signature traits identified by Winter et al. (2009). From experiments and surveys, they identified a subset of traits characteristic of optimum maturity in Chardonnay: skin colour with no trace of green, pulp that is not very attached to skin, very fluid, sweet, fruity rather than herbaceous and slightly acidic, skin with almost no acidity and fruity rather than herbaceous, and seed with no green traces. In close agreement with this profile, we found that these traits were significantly responsive to temperature particularly in 2009-10 (Fig. 2). Temperature advanced these traits but they remained fairly coupled in both seasons (insets in Fig. 4bf).

Semillon had a unique response to temperature: there was a small offset even in the hot 2009-10 season and there was significant scatter even in the mild 2010-11 season. Decoupling of Semillon traits in 2010-11 was associated with a differential response of seed relative to other berry components (Fig. 4g). The asynchrony in the

development of seed and other berry parts maybe one component of this response, as proposed for Cabernet Franc. Owing to differences in winemaking techniques for red and white wines, however, the oenological consequences of the seed vs rest-of-berry decoupling in Semillon are different to those for Cabernet Franc, as outlined in the next section.

The negative thermal offset for Shiraz in 2009-10 (Fig. 4d) was the likely result of differences in sugar concentration as discussed in the previous section. The sensory signature of maturity in Shiraz has the following elements: easy stalk removal, sweet and fruity pulp, crumbly skin and brown and cracked seed (Winter et al., 2009). Most of these were statistically more advanced in controls than in the heated treatment, hence reinforcing the conclusion that the higher sugar concentration in controls reflected a difference in development that overcome the putative effects of temperature (Fig. 2). In 2010-11, under conditions that caused significant offset in Chardonnay and marked decoupling in Semillon, Shiraz showed a much more stable sensory profile, which is consistent with the local adaptation of Shiraz to the Barossa Valley (Fuller and Walsh, 1999).

Viticultural and oenological implications

Management practices to counteract thermal effects on the balance of berry sensory attributes could seek to reduce energy load in the canopy, shift the timing of critical developmental stages towards cooler conditions late in the season, or a combination of both. Canopy architecture and bunch exposure, as modified by training and pruning system, has direct implications on the energy balance of canopy and fruit (Iland et al., 2011; Prieto, 2011; Smart and Sinclair, 1976b). Application of auxins before veraison delayed ripening of Shiraz in the cool environment of Adelaide Hills (35° S, elevation 400 m) (Bottcher et al., 2011). Delayed pruning may also shift phenological development towards cooler conditions late in the season (Friend and Trought, 2007; Martin and Dunn, 2000; Pearce and Coombe, 2005). All of these practices are likely to involve tradeoffs that require local resolution; late pruning for example can have a long-term effect on carbohydrate reserves.

Here we use three experimental findings to highlight the implications of: (i) decoupling associated with unbalanced development of seed and rest-of-berry in a red wine variety (Cabernet Franc 2009-10), (ii) decoupling associated with unbalanced development of seed and rest-of-berry in a white wine variety (Semillon 2010-11) and (iii) decoupling associated with unbalanced development within berry parts (Chardonnay 2009-10).

The decoupling driven by differences in ripening between seed and rest-of-berry components has different implications for red and white wines. Consider the decoupling between seed and other berry parts in Cabernet Franc 2009-10 (Fig. 4a) and the implications of different degree of development of seed and skin, the main sources of tannins. First, the concentration and degree of polymerization of tannins vary with tissue of origin (Downey et al., 2003). Second, the contribution of tannins to

sensory properties also depends on the source: tannins from skin mostly contribute astringency and tannins from seed also contribute bitterness (Kennedy et al., 2006). Third, phenolic extractability between veraison and harvest decreases for both, seed and skin, as phenolics are gradually structured in larger molecules and associated with cell wall material (Hanlin et al., 2010). However, skin tannins are extracted earlier in fermentation than tannins present in the hard seed coat (Iland et al., 2011; Kennedy, 2008). Decoupling of seed and skin ripeness could have therefore potential effects on the balance between bitterness and astringency of red wines (Kennedy et al., 2006). In this sense, a relative advance in seed ripening may represent a desirable increase in the proportion of skin-derived tannins in the wine and a general improvement in the tannin quality (Kassara and Kennedy, 2011; Kennedy, 2008; Ristic et al., 2010). Conversely, other components influencing tannin perception, such as sugar, acid, ethanol, anthocyanins and polysaccharides, may be negatively affected by the seed vs rest-of-berry decoupling (Kennedy et al., 2006). Awareness of this displacement may suggest viticultural and oenological practices to avoid otherwise unbalanced wines such as shift in harvest time, differential berry crushing intensity, enzyme use and adjustment in maceration time and conditions (Kennedy, 2008; Sacchi et al., 2005).

Owing to differences in winemaking techniques between red and white wines, the decoupling driven by differential ripening of seed and other berry components has different oenological consequences for cases such as Semillon in 2010-11 (Fig. 4g). The common winemaking approach for white wines involves an early separation of berry components; the contact among skin, pulp and seed is confined to the process of crushing and pressing (Boulton et al., 1998). This represents a natural lower risk of releasing undesirable compounds from seeds and skin to the must when the decoupling among tissues had taken place (Ferreira et al., 1995). A putative delay in the ripening of skin traits may represent a wine enrichment with undesirable herbaceous characters, when pre-fermentative maceration techniques are used to increase the aromatic varietal profile of white wines (Ferreira et al., 1995; Gómez-Míguez et al., 2007; Selli et al., 2003). In this case, some palliative measures aimed at quality pressing must include gentle extraction and pumping methods, avoiding crushing the solid parts of the clusters, permitting self-filtering of juices in the press itself and limiting hydrolytic enzyme activity (Boulton et al., 1998; Darias-Martın et al., 2004; Jackson, 2008).

Chardonnay 2009-10 represents a third example, where the decoupling was mostly associated with variation within berry parts (Fig 4b). Despite the tighter coupling for the subset of critical maturity descriptors primarily associated with pulp and skin maturity (Winter et al., 2009), some traits remained decoupled (inset in Fig. 4b). On the one hand, heated berries had higher intensity and more advanced development of pulp fruit aromas, in contrast to control berries. On the other hand, the heated berries had a higher content (lower score) of pulp herbaceous aromas. This shows how temperature could operate decoupling the development of two characters closely related to the aromatic profile of quality Chardonnay wines.

Conclusion

Sheldon et al. (2011) used the term “community disassembly” to emphasise the thermal decoupling of interactions among key species with consequences for the structure and function of ecosystems. Here we showed that elevated temperature can disassemble sensory traits in berries with direct oenological implications. The responses were variety and season dependent, with larger effects in hot than in mild seasons. The thermal response of berry sensory traits had two elements: offset and decoupling. Large decoupling was generally associated with large offset but characteristically large decoupling was observed with minor offset in Semillon. Two types of decoupling were identified depending on the dominance of variation among or within berry parts. Awareness of the magnitude and type of decoupling would likely contribute to viticultural and winemaking adaptations in warmer regimes.

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Table 2. Canopy-level maximum (Tmax) and minimum (Tmin) temperature in controls and difference between heated and control treatments. Values are means ± standard errors (^{o}C). This is a summary of the more detailed description of thermal regimes presented elsewhere (Sadras et al., 2012a).

Season	Month	Control		Difference	
		Tmax	Tmin	Tmax	Tmin
2009-10	Oct	28.5 ± 1.82	10.8 ± 0.87	1.4 ± 0.09	0.1 ± 0.07
	Nov	31.7 ± 1.48	13.3 ± 0.68	1.5 ± 0.10	0.5 ± 0.08
	Dec	31.0 ± 1.26	11.4 ± 0.67	2.2 ± 0.15	0.8 ± 0.08
	Jan	35.1 ± 1.06	13.6 ± 0.59	1.9 ± 0.13	0.7 ± 0.09
	Feb	34.5 ± 0.86	15.5 ± 0.63	1.4 ± 0.07	0.4 ± 0.06
	Mar	30.1 ± 0.86	12.6 ± 0.51	1.2 ± 0.08	0.3 ± 0.04
2010-11	Oct	21.7 ± 0.80	7.3 ± 0.50	1.5 ± 0.11	0.0 ± 0.04
	Nov	25.6 ± 1.02	10.1 ± 0.80	1.5 ± 0.14	0.2 ± 0.04
	Dec	27.4 ± 1.14	12.2 ± 0.53	0.9 ± 0.14	0.4 ± 0.07
	Jan	34.5 ± 0.89	14.7 ± 0.62	1.6 ± 0.11	0.5 ± 0.06
	Feb	30.4 ± 1.08	14.6 ± 0.68	1.5 ± 0.12	0.4 ± 0.04
	Mar	24.6 ± 0.84	12.2 ± 0.57	1.2 ± 0.14	0.3 ± 0.04

Table 3. P values from analysis of variance assessing the effect of variety, temperature and their interaction on sensory properties of berries during two seasons at the Barossa Valley.

Part	Trait	2009-10			2010-11		
		variety	temp.	interaction	variety	temp.	interaction
Berry	colour	0.0001	0.0076	0.0001	0.0001	0.7895	0.0452
	softness	0.0001	0.1206	0.9843	0.0623	0.7149	0.5003
	stalk removal	0.0001	0.7814	0.2038	0.0001	0.7278	0.7797
Skin	acidity	0.0098	0.9700	0.6530	0.0053	0.3905	0.2956
	astringency	0.0138	0.4844	0.5566	0.1409	0.0501	0.0761
	disintegration	0.0003	0.4702	0.0328	0.0001	0.0017	0.2948
	fruity aromas	0.0001	0.1419	0.0477	0.1916	0.8056	0.4773
	herbaceous aromas	0.0001	0.5112	0.4037	0.1021	0.3361	0.8052
	tannic intensity	0.0121	0.5957	0.9618	0.3334	0.2724	0.1961
Pulp	acidity	0.8178	0.8456	0.7878	0.0002	0.3462	0.9824
	detachment	0.0001	0.5236	0.0923	0.1129	0.3578	0.0585
	fruity aromas	0.0001	0.8436	0.0075	0.0007	0.1566	0.1367
	herbaceous aromas	0.0008	0.9438	0.3735	0.1033	0.2075	0.7205
	juiciness	0.0021	0.5026	0.0389	0.0198	0.7533	0.7681
	sweetness	0.0154	0.1094	0.8313	0.4849	0.2614	0.0137
Seed	astringency	0.0061	0.4061	0.4732	0.0295	0.8598	0.2109
	colour	0.0001	0.0091	0.0800	0.0015	0.0412	0.2368
	crushability	0.0001	0.2552	0.3470	0.0037	0.2301	0.3678
	flavours	0.0272	0.0487	0.2077	0.0276	0.1392	0.8977
	tannic intensity	0.0001	0.5605	0.0357	0.0030	0.1084	0.3082

Captions to figures

1. (a) Example of seasonal dynamics of total soluble solids in response to temperature (Chardonnay 2011). (b, c) Total soluble solids at the time of sampling for sensory assessment of berries during two seasons in the Barossa Valley. Varieties are Cabernet Franc (CAF), Chardonnay (CHR), Semillon (SEM) and Shiraz (SHI). Error bars are two (a) or one (b,c) standard error of the mean. Asterisks (b, c) indicate difference between temperature treatments at $P < 0.05$.

2. Sensory profiles of berries of four varieties under elevated temperature (closed symbols) and controls (open symbols) in 2009-10. The graph in the centre shows that scale of increasing ripeness from 0 at the origin to 4 at the outermost circle. Asterisks indicate significant effect of temperature at *** $P < 0.001$, ** $P < 0.05$ and * $P < 0.10$.

3. Sensory profiles of berries of four varieties under elevated temperature (closed symbols) and controls (open symbols) in 2010-11. The graph in the centre shows that scale of increasing ripeness from 0 at the origin to 4 at the outermost circle. Asterisks indicate significant effect of temperature at *** $P < 0.001$, ** $P < 0.05$ and * $P < 0.10$.

4. Comparison of scores for sensory traits in control and heated treatments in four varieties over two seasons in the Barossa Valley. The solid lines are least squares regressions with slope = 1 and intercept = *a*. The dashed lines are y = x. RMSE is the root mean square error (eq. 1). Insets in (a) and (g) are the residuals (+ s.e.) for berry (black), pulp (white), seed (red) and skin (blue). Insets in (b) and (f) show the ten trademark traits for sensory maturity in Chardonnay according to Winter et al. (2009). The arrow in (b) highlights the decoupling between flavours and tannic intensity in seed.

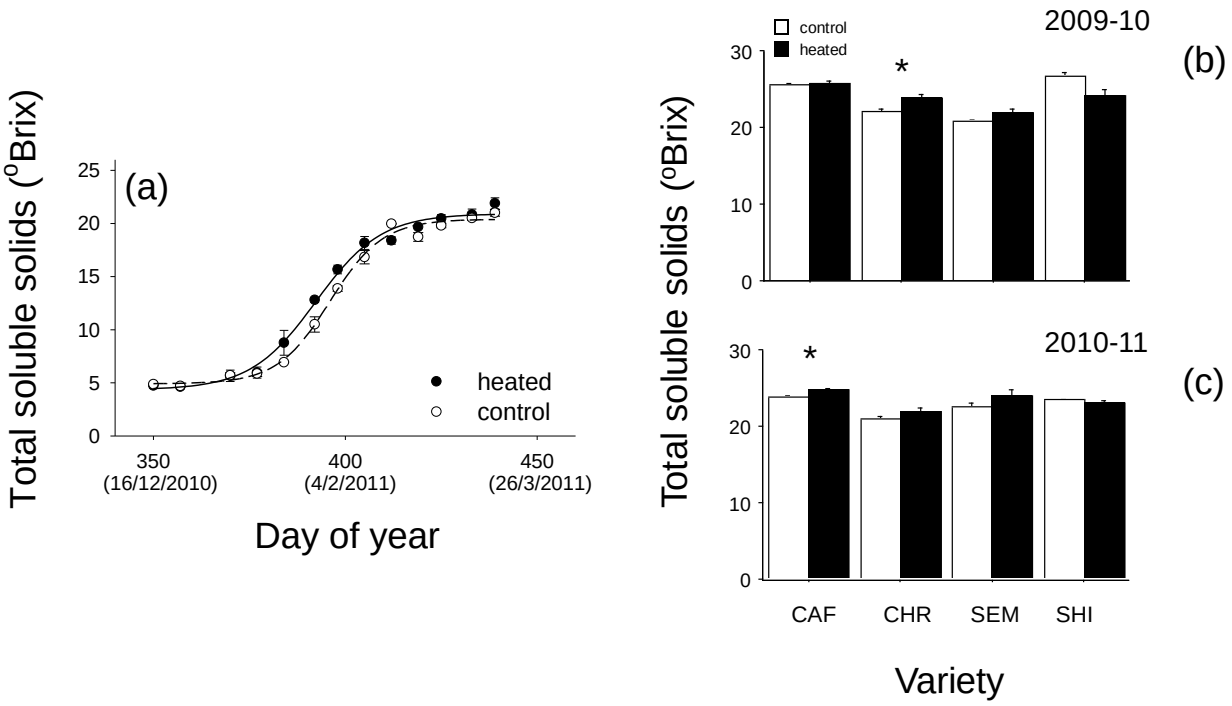

(a)
Total soluble solids (°Brix)
25
20
15
10
5
0
350
(16/12/2010)
400
(4/2/2011)
450
(26/3/2011)
Day of year
heated
control
2009-10
(b)
control
heated
30
20
10
0
*
2010-11
(c)
30
20
10
0
*
CAF
CHR
SEM
SHI
Variety

Fig. 1

Fig. 2

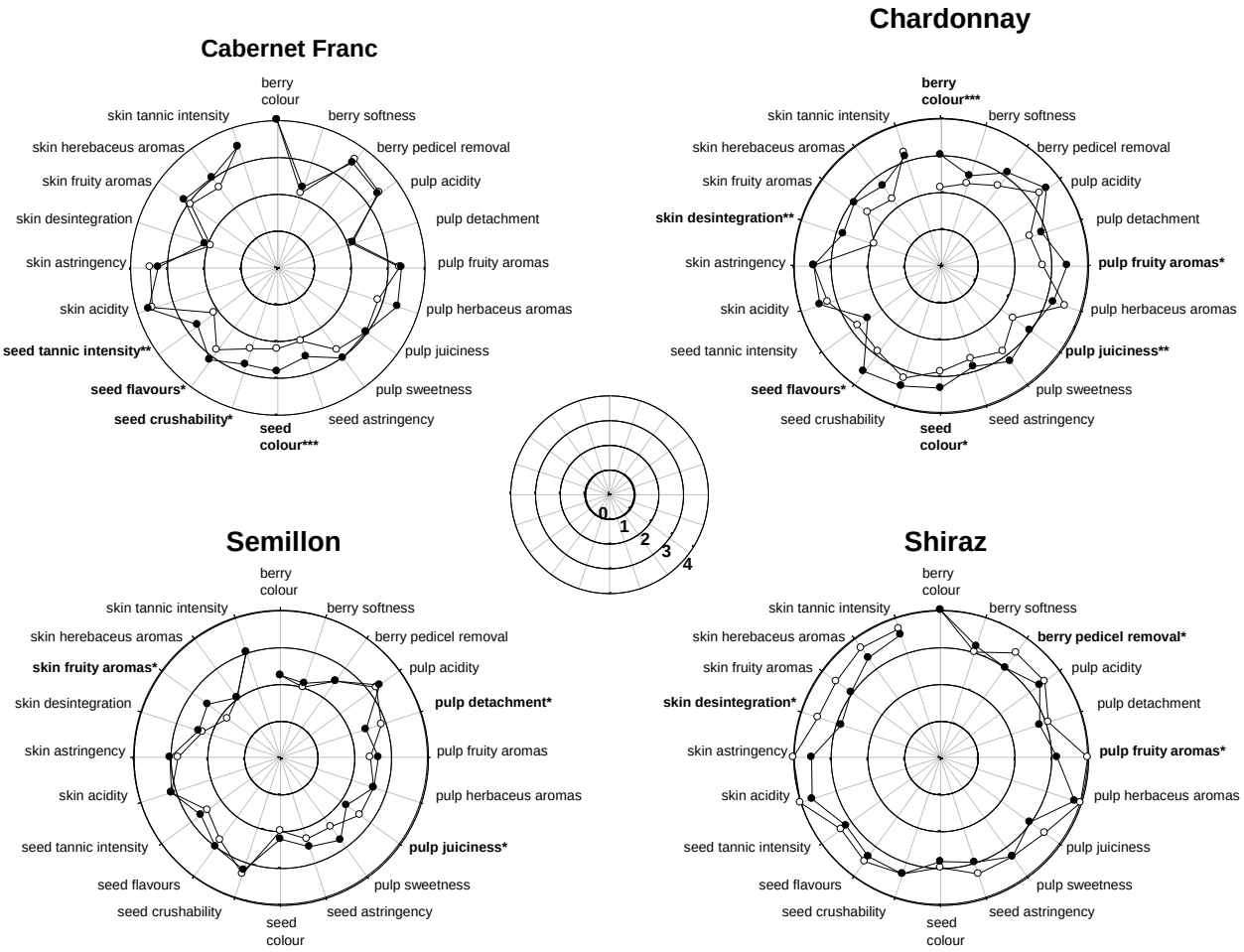

Cabernet Franc
Chardonnay
Semillon
Shiraz
berry colour
berry softness
berry pedicel removal
pulp acidity
pulp detachment
pulp fruity aromas
pulp herbaceus aromas
pulp juiciness
pulp sweetness
seed astringency
seed colour
seed crushability
seed flavours
seed tannic intensity
skin acidity
skin astringency
skin desintegration
skin fruity aromas
skin herebaceus aromas
skin tannic intensity
0
1
2
3
4

Fig. 3

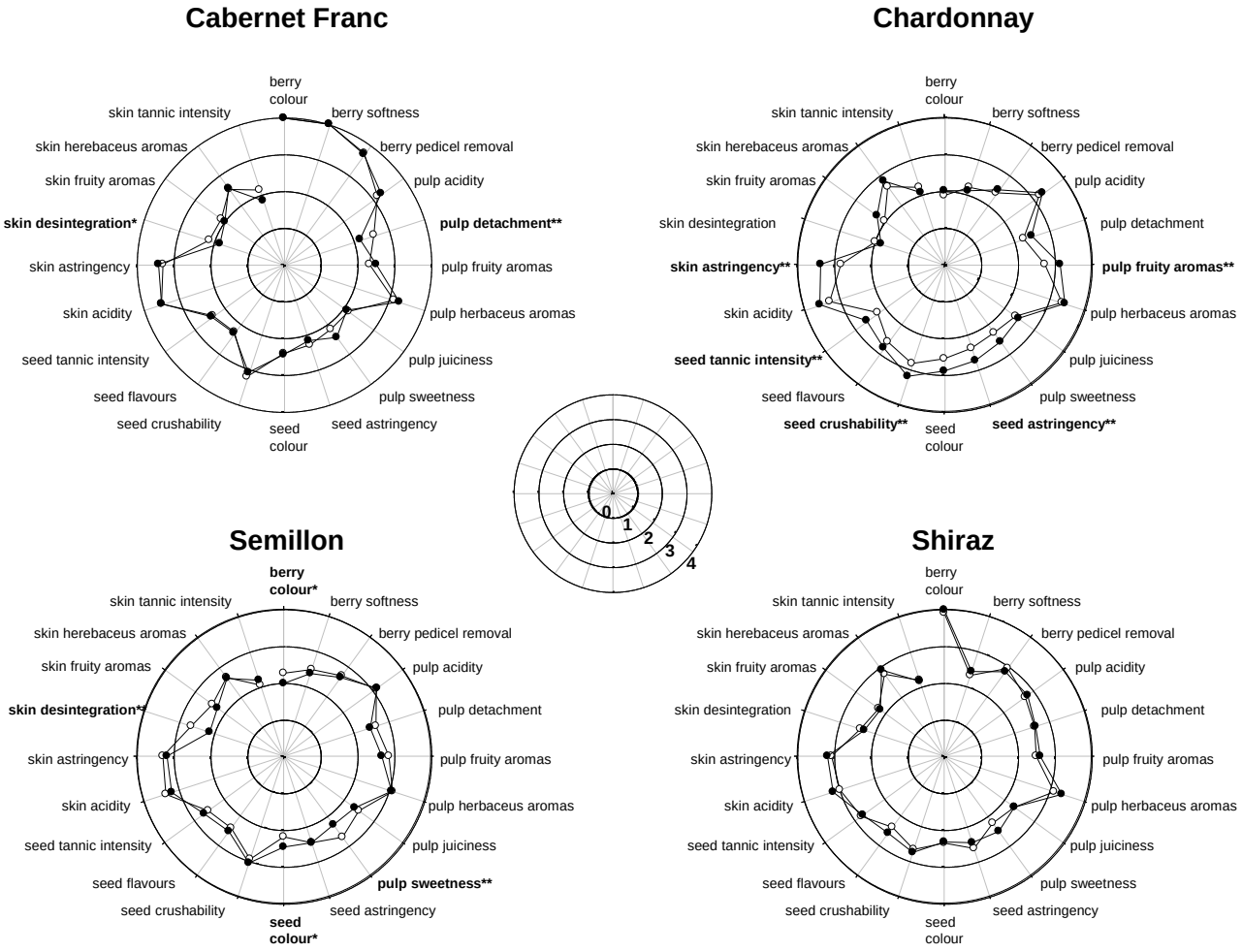
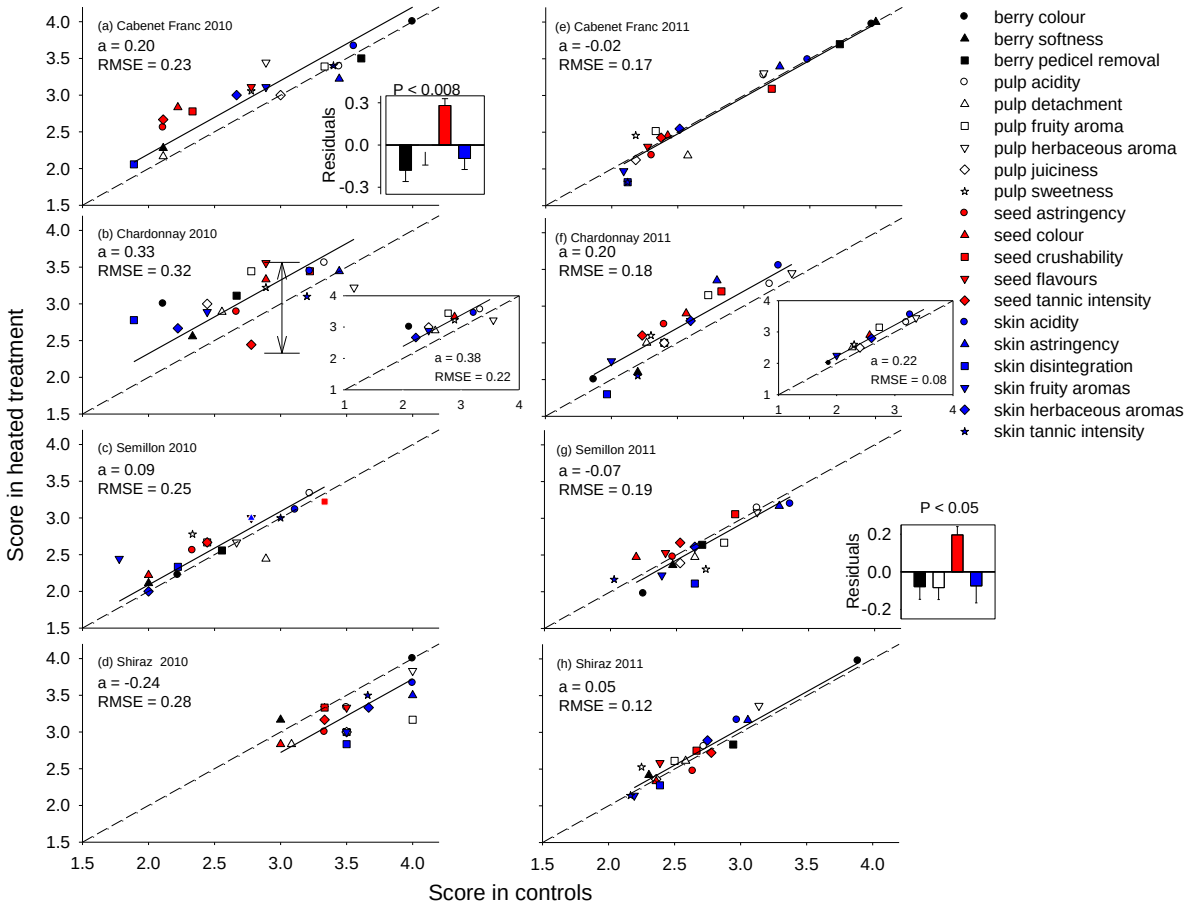

Cabernet Franc
Chardonnay
Semillon
Shiraz
berry colour
berry softness
berry pedicel removal
pulp acidity
pulp detachment**
pulp fruity aromas
pulp herbaceus aromas
pulp juiciness
pulp sweetness
seed astringency
seed colour
seed crushability
seed flavours
seed tannic intensity
skin acidity
skin astringency
skin desintegration*
skin fruity aromas
skin herebaceus aromas
skin tannic intensity
skin astringency**
pulp fruity aromas**
seed tannic intensity**
seed crushability**
seed astringency**
skin desintegration
berry colour*
pulp sweetness**
seed colour*
0
1
2
3
4

Fig. 4


(a) Cabenet Franc 2010
a = 0.20
RMSE = 0.23
P < 0.008
Residuals
(b) Chardonnay 2010
a = 0.33
RMSE = 0.32
a = 0.38
RMSE = 0.22
(c) Semillon 2010
a = 0.09
RMSE = 0.25
(d) Shiraz 2010
a = -0.24
RMSE = 0.28
(e) Cabenet Franc 2011
a = -0.02
RMSE = 0.17
(f) Chardonnay 2011
a = 0.20
RMSE = 0.18
a = 0.22
RMSE = 0.08
(g) Semillon 2011
a = -0.07
RMSE = 0.19
P < 0.05
Residuals
(h) Shiraz 2011
a = 0.05
RMSE = 0.12
Score in heated treatment
Score in controls
berry colour
berry softness
berry pedicel removal
pulp acidity
pulp detachment
pulp fruity aroma
pulp herbaceous aroma
pulp juiciness
pulp sweetness
seed astringency
seed colour
seed crushability
seed flavours
seed tannic intensity
skin acidity
skin astringency
skin disintegration
skin fruity aromas
skin herbaceous aromas
skin tannic intensity

Chapter 6. Effects of elevated temperature in grapevine: Juice pH, titratable acidity and wine sensory attributes

VO Sadras, PR Petrie, MA Moran

Abstract

The effects of vineyard temperature on wine attributes are largely inferred from indirect comparisons between thermally contrasting regions or vintages. Here we directly assessed wine attributes in trials where manipulation of temperature in the field minimised confounded effects typical of indirect comparisons. Cabernet Franc, Chardonnay, Semillon and Shiraz vines were exposed to two thermal regimes (elevated temperature, control) over two growing seasons. Juice titratable acidity (TA) and pH, and wine sensory attributes were assessed. Three types of responses to elevated temperature were found for juice TA and pH: (i) pH increased and TA decreased (Cabernet Franc, Chardonnay), (ii) both traits were unresponsive (Shiraz) and (iii) pH increased but acidity was unresponsive (Semillon). Elevated temperature consistently reduced green and citrus aromas and enhanced rich mouth feel and tropical flavours in Semillon. Thermal effects on sensory traits of Shiraz and Cabernet Franc were strongly season dependent. Responses of juice TA and pH to elevated vineyard temperature in Shiraz and Semillon did not conform to expectations from indirect comparisons. Owing to confounded environmental factors, the effect of temperature on wine attributes inferred from regional or seasonal comparisons needs to be interpreted with caution. Direct evaluation of the effect of elevated vineyard temperature on wine attributes can help developing winemaking adaptations for warmer futures.

Introduction

Wine attributes vary seasonally and regionally. In viticulture, the term ‘vintage’ refers to seasonal effects and ‘terroir’ captures location effects, primarily mediated by soil and climate (Stevenson, 2005; Trubek et al., 2010). Biologically, both vintage and terroir reflect phenotypic plasticity, i.e. environmentally contingent trait expression (Bradshaw, 1965; DeWitt and Scheiner, 2004; Piersma and van Gils, 2011; Pigliucci, 2001; Woltereck, 1909). Temperature, the focus of this paper, is a major source of both seasonal and regional variation in fruit quality traits with direct consequences for wines. For example in the competition Chardonnay-du-Monde 2009, wines from cool Canadian regions and wines from warm Australian regions were in the top ten among hundreds of entries worldwide (http://www.chardonnay-du-monde.com). This is the outcome of both the phenotypic plasticity of vines in response to temperature and other environmental factors (Calò et al., 1975; Sadras et al., 2009), and the technological adaptation of grape growers and winemakers seeking particular wine styles (Iland and Gago, 2002).

The influence of temperature on wine attributes has been investigated primarily with indirect methods that compare cool and warm seasons within a region or thermally contrasting regions (Ashenfelter et al., 1995; Cozzolino et al., 2011; Duchêne and Schneider, 2005; Grifoni et al., 2006; Jones and Davis, 2000; Jones et al., 2005; Petrie and Sadras, 2008b; Soar et al., 2008; Webb, 2006). Although helpful, this indirect approach is inconclusive because temperature is correlated with other climatic factors and regional comparisons also involve confounded effects with soils and management practices (Sadras and Petrie, 2011a; Soar et al., 2008). Fruit from a hot environment, such as the Riverland, is likely to have lower acidity and higher pH than that of fruit from a cool environment such as Coonawarra, and these differences have been attributed primarily to temperature (Iland et al., 2011). The Riverland, however, is not only hotter but also has higher radiation, evaporative demand and vapour pressure deficit than Coonawarra (Sadras and Petrie, 2011a) and different soils, irrigation practices and production objectives (Dry et al., 2005; Maschmedt, 2004). Manipulation of temperature in the field showed that titratable acidity and pH of sun-exposed Merlot fruit did not respond to temperature whereas acidity declined and pH increased with increasing temperature in shaded fruit (Spayd et al., 2002). This illustrates the caution needed to draw conclusions using indirect methods.

Direct assessment of fruit traits in response to thermal regime under field conditions is scarce (Sadras and Moran, 2012; Spayd et al., 2002; Tarara et al., 2008a; Tarara et al., 2008b). Furthermore, there have been no direct assessments of thermal effects on wine sensory attributes. Despite strong interactions between complex wine attributes and the variability of human senses (Atanasova et al., 2005; Parr et al., 2004; van Beilen et al., 2011; Verdu Jover et al., 2004; Wood et al., 2008), trained panels and rigorous protocols consistently capture wine sensory responses to environmental factors and technology (Chapman et al., 2004; Chapman et al., 2005; García-Carpintero et al., 2011; Gomez-Miguez et al., 2007; Pineau et al., 2011; Rezaei and Reynolds, 2010; Ristic et al., 2010; Saenz-Navajas et al., 2011).

Against the background of warming trends, we are involved in a project where untreated control vines are directly compared with vines grown under elevated temperature using open-top chambers in realistic vineyard conditions (Sadras and Moran, 2012; Sadras and Soar, 2009). Here we report for the first time, the responses of wine attributes to elevated temperature in a factorial experiment where four cultivars (Semillon, Chardonnay, Shiraz, Cabernet Franc) were exposed to two temperature regimes over two seasons in the Barossa Valley, a premium wine region of Australia. We focused on juice titratable acidity and pH, and wine sensory traits. Anthocyanins and total soluble solids (TSS) have been discussed elsewhere (Sadras and Moran, 2012).

Methods

The experiments have been described in previous papers (Sadras and Moran, 2012; Sadras et al., 2012c). Briefly, a factorial experiment combining four cultivars and two temperature regimes was established on October 2009. Own-rooted cultivars were Cabernet Franc clone Penfold 58 planted in 1988, Chardonnay clone 277 planted in 1995, Semillon clone LRC147 planted in 1989 and Shiraz clone NSW 15 planted in 1997. The temperature regimes were a control with background ambient temperature, and a treatment in which daytime temperature was elevated using open-top chambers located below the canopy. Treatments were laid out in a split-plot design with three replicates. The main plot was allocated to cultivar and sub-plots to temperature treatments. Each replicate comprised nine vines, allowing for sampling in seven central vines and at least five buffer vines between replicates within a block.

Fruit harvest, winemaking and assessment

All fruit was hand harvested. We targeted fruit for harvest at ~ 22^{o}Brix for Chardonnay and Semillon and ~ 24^{o}Brix for Shiraz and Cabernet Franc (Iland et al., 2011). To achieve a similar concentration of total soluble solids in berries of heated and control treatments, we monitored TSS from weekly samples of 100 berries per replicate and staggered harvest date using projections of trajectories of when required.

In 2010, we harvested all the fruit from each of three replicates of Semillon, Shiraz and Cabernet Franc. Low yield precluded meaningful winemaking of Chardonnay and meant that two of the field replicates of the Semillon were combined for winemaking. In 2011, the wet season favoured bunch diseases, particularly rot caused by *Botrytis cinerea* (Smith, 2011). We therefore picked only visually healthy bunches from each of the three replicates. This reduced, but did not eliminate completely, the incidence of disease, which was particularly evident in one of the replicates where wines showed evident lacasse oxidation (see below).

Fruit from each replicate was separately crushed and pressed (white cultivars) or crushed (red cultivars) using hand-operated equipment. No attempt was made to split the field replicates into fermentation replicates. The must was treated with

potassium metabisulfite (0.1g kg/fruit). Immediately after crushing we assessed the juice using the guidelines of Iland et al. (2000) for total soluble solids (digital refractometer HANNA HI 96801 Woonsocket, RI, USA), titratable acidity and pH (autotitrator CRISON Barcelona, Spain).

The juice of white wine cultivars was settled at ~ 3^{o}C during 3–4 days after crushing, then it was racked and yeast (EC1118 at 0.5 g/L) and yeast nutrient (0.3 g/L) were added. Tartaric acid was added to each individual replicate as required to reach titratable acidity ~ 5.5 g/L. Approximately three weeks after crushing, the juice was racked off lees and treated with potassium metabisulfite (20 mg/L), tartaric acid as required to adjust titratable acidity of each sample, and copper sulfate (1 mg/L). Wines were racked after cold stabilisation.

The must of red wine cultivars was fermented on skins in 75 L plastic bins at ~ 22^{o}C, with the ferment plunged twice a day. After seven days, the must was pressed into 10 L demijohns and malolactic bacteria (*Oenococcus oeni* Lalvin VP 41, Lallemand Inc., Montreal, Quebec, Canada) were added. The wine was racked 24 days after crushing and treated with potassium metabisulfite (60 mg/L) and tartaric acid as required.

Wines were bottled (750 mL bottles in 2010, 375 mL bottles in 2011), sealed with screw-caps and stored at ~17^{o}C until the time of assessment on 15 June (2010) and 2 December (2011). The tasting panel comprised 12 (2010) or 15 (2011) professional winemakers from Treasury Wine Estates. Prior to the formal tasting, winemakers tasted the wines and selected flavour and aroma characteristics that were the most contrasting between the treatments; consensus on terms was easily reached as the winemakers regularly tasted together (Table 1). Tasting was completed in an open tasting room. Panellists were presented with paired samples corresponding to the wine derived from control vines and from vines exposed to elevated temperature (~25 mL per sample in 210 mL ISO standard tasting glasses). The pairs of wines were presented in random order and the glasses were covered by petri dishes prior to tasting. Panellists were asked to identify, for each pair and for each replicate, the wine for which each attribute was more intense in a two-alternative forced choice test (Perez-Magarino et al., 2011; Sun et al., 2011). Assessments included three replicates, except for Semillon in 2010 and Shiraz in 2011, where two replicates were available. In 2010 all wines were tested in a single session and in 2011 the tasting was split over two sessions.

Statistical analyses

Total soluble solids, titratable acidity and pH were assessed with analysis of variance accounting for the effect of temperature, cultivar, season and their interactions. This was complemented with analysis of variance testing the effect of temperature for each trait, cultivar and season. To disentangle high-order interactions, we derived norms of reaction for both pH and titratable acidity, as described by Sadras and colleagues (Sadras et al., 2009; Sadras and Trentacoste, 2011; Trentacoste et al., 2011). Briefly, four environments were defined that resulted from the combination of

two thermal regimes and two seasons. For each of these environments, we calculated the mean titratable acidity and pH across cultivars; these are the "environmental means". The actual value of the trait for each environment and cultivar and replicate was regressed against environmental mean, and the slope was taken as a measure of phenotypic plasticity (DeWitt and Scheiner, 2004).

Sensory traits were cultivar and season dependent; this precluded the statistical evaluation of interactions. The effect of temperature was therefore evaluated for each trait, cultivar and season individually. We assigned a score = 1 to higher intensity of the sensory attribute in controls and -1 to higher intensity in heated treatment. Hence, an average score not significantly different from zero indicated lack of temperature effect, significant positive scores indicated a more intense character in controls, and significant negative scores indicated a more intense character in wine from treated vines. The null hypothesis (score = 0) was evaluated with a two-tails non-parametric one sample sign test (Statview Reference 1999, SAS Institute Inc., Cary, NC, USA)

Results

Growing conditions

Relative to controls, the heated system elevated maximum temperature by 1.6^{o}C in 2009–10 and by 1.4^{o}C in 2010–11, with minor effect on minimum temperature (Sadras et al., 2012c). Maximum temperature was higher in 2009–10 than in 2010–11. Particularly important for wine attributes, maximum temperature during the ripening period of February and March was ~5^{o}C higher in the first season. Wet and relatively cool conditions favoured bunch rot caused by *Botrytis cinerea* and botrytis-derived lacasse oxidation in 2011 wines (Smith, 2011).

Total soluble solids, titratable acidity and pH in berry juice

All three traits were affected by cultivar, thermal regime and their interaction (Table 2). We achieved the aim of similar total soluble solids at harvest in 6 out of 8 cases. The exceptions were berries in the heated treatment that had higher total soluble solids than controls for Chardonnay in 2010 and for Cabernet Franc in 2011 (Table 2).

The response to temperature depended on the trait (pH, TA), the cultivar and the seasonal conditions (Table 2). Both pH and TA responded to the interaction between cultivar and temperature. To interpret these interactions, we developed the reaction norms of pH and titratable acidity (Figure 1). Cabernet Franc and Chardonnay had large plasticities for both traits, i.e. as the environment favoured higher acidity or higher pH (x-axis in Figure 1), both cultivars responded plastically by increasing the value of these traits (y-axis in Figure 1). Quantitatively, this was reflected in the magnitude and significance of the regression slopes (parameter *b* in Figure 1). The slope representing plasticity of titratable acidity was statistically indistinguishable from 0 for both Semillon had Shiraz (Figure 1b); this is a conservative conclusion as

the apparent non-linearity in the norms of reaction for these cultivars cannot be justified biologically (Keller, 2010b). Shiraz also showed lack of plasticity for pH (Figure 1d) whereas Semillon was highly plastic for this trait (Figure 1c).

Wine sensory attributes

Figure 2 summarises the effects of elevated daytime temperature on 2010 wines. Coefficients of variation were below 4% for all wine attributes except for tannins in Cabernet Franc (CV=18.2%). In Semillon, elevated temperature slightly reduced green leaf aromas and strongly increased ripe flavours. In Shiraz, elevated temperature reduced berry flavours and floral aromas and increased cooked fruit flavours. In Cabernet Franc, elevated temperature had no detectable effects on all four sensory attributes tested.

Figure 3 summarises the effects of elevated temperature on 2011 wines. Coefficients of variation were below 15% for all wine attributes except for tannins in Shiraz (CV=24.2%). In Semillon, elevated temperature reduced citrus and green leaf aromas and strongly increased 'rich mouth feel' and ripe (tropical fruit) flavours. A similar trend for reduced citrus and enhanced mouth feel with elevated temperature was recorded for Chardonnay, but these apparent effects were not statistically significant. In Shiraz, tannin structure, cooked fruit, floral and berry characters were indistinguishable in the wines from heated vines and controls. In Cabernet Franc, elevated temperature reduced the dark fruit character and enhanced the green leaf character.

Discussion

Context: experimental limitations and the background environment

The limitations of the heating system used in our trial have been discussed in detail (Sadras et al., 2012c; Sadras and Soar, 2009). Further limitations in this study include the lack of independent fermentation replicates and the incidence of disease in 2011. Logistic constraints impose winemaking tradeoffs that are solved according to the objectives and priorities of each particular study. For example, in the investigation of water regimes by Chapman et al. (2005), 'fruit from field replications of each of the irrigation treatments was combined' and divided into three fermentation replicates. Likewise, Ristic et al. (2010) combined all field replicates from each canopy management treatment to produce three winemaking replicates. In both these studies, fermentation replicates were obtained at the expense of field replicates leading to confounded field and fermentation effects. Instead, we chose to maintain the integrity of our field replicates, hence the composite of field and fermentation effects. The coefficients of variation in our sensory assessments (see section *Wine sensory attributes*) compare well with studies using duplicated fermentations (CV ≤ 19%, García-Carpintero et al., 2011; CV ≤ 23%, García-Carpintero et al., 2010).

By selecting visually healthy bunches in 2011, we reduced but did not fully eliminate the effect of bunch rot on grapes and wines; the more extreme case was a Shiraz replicate that had to be excluded from tastings. Wine attributes in 2011 therefore have a component of temperature and disease interaction that cannot be fully disentangled.

The biological effects of warming depend on plant type, trait and location-specific baseline temperature (Way and Oren, 2010). In hot regions currently producing quality grapes of well adapted cultivars, future warming could reduce the frequency of seasons conducive to balanced fruit and wine whereas cooler regions could benefit from warming leading to a higher frequency of favourable seasons (Jones et al., 2005). As an example of a rapid, region-dependent response to warming the European Union accepted Denmark as a commercial wine production nation in 2000 and the Association of Danish Winegrowers reached 1400 members in 2009 (Bentzen and Smith, 2009). It is therefore important to place the elevation of temperature in our trials in a regional context where the Barossa Valley is an intermediate region with long-term mean January temperature ~21.2^{o}C, compared to the hotter Riverland region with January mean temperature ~23.2^{o}C and the cooler Coonawarra region with mean January temperature ~19.5^{o}C (Dry et al., 2005). Extrapolation of our results to warmer or cooler regions is therefore not warranted.

Effects of temperature on titratable acidity and pH

Indirect evidence based on comparisons between seasons or regions supports the reduction of titratable acidity and increase in pH with high temperature (Iland et al., 2011; Keller, 2010b). This response,is, however, not universal. In experiments where manipulation of temperature in the field allowed for unequivocal results, titratable acidity declined and pH increased in shaded but not in sun-exposed Merlot fruit (Spayd et al., 2002). In our experiment, titratable acidity was affected by all three main sources of variation (temperature, cultivar, season) and all interactions except temperature by season (Table 2). pH responded to temperature, cultivar and the strong interactions between cultivar and temperature and cultivar and season (Table 2). These complex responses were clarified when data were analysed from the perspective of phenotypic plasticity (DeWitt and Scheiner, 2004; Sadras et al., 2009; Sadras et al., 2007; Sadras and Trentacoste, 2011; Trentacoste et al., 2011; Woltereck, 1909). Norms of reaction showed three types of responses for these traits (Figure 1). Chardonnay and Cabernet Franc conformed to the textbook pattern: titratable acidity declined and pH increased in response to elevated temperature in both seasons (Figure 1a,b). In Semillon, norms of reaction revealed a strong plasticity for pH in contrast to the lack of plasticity for titratable acidity (Figure 1b,c). This suggests a weak link between titratable acidity and pH that requires a closer look at their components, including dynamics of malate, tartrate, Na^{+} and K^{+} (Iland et al., 2011). The third type of response, represented by Shiraz, showed a remarkable lack of plasticity for both titratable acidity and pH (Figure 1b,d). Shiraz was indeed able to maintain a stable, high acidity and stable and relatively low pH in the range of environmental conditions that caused large responses in Chardonnay and Cabernet Franc. This is consistent with the proven adaptation of Shiraz to the Barossa Valley

(Fuller and Walsh, 1999). Indeed, Shiraz was traditionally cultivated in warmer regions because it was able to maintain a better acid level before the addition of acid became a standard production process.

Effects of temperature on wine sensory traits

We achieved the aim of similar TSS at harvest in 6 out of 8 cases. The exceptions were Chardonnay in 2010 and Cabernet Franc in 2011 (Table 2). No wine was made from Chardonnay in 2010, hence the only wine where differences in sugar ripeness may have been confounded with thermal effects was Cabernet Franc in 2011.

Semillon showed the more consistent pattern of thermal response in sensory traits in both seasons. Elevated temperature reduced green and citrus characters and enhanced rich mouth feel and ripe (tropical) flavours. This shift parallels the changes in sensory traits associated with delayed harvest (Iland and Gago, 2002). A similar, but slighter and non-significant trend was observed for Chardonnay in 2011. Practical experience suggests that white wine aromas develop more intensely in cooler seasons or regions (Duchêne and Schneider, 2005). Examples of classes of grape aroma compounds relevant for white wine aroma of cultivars, such as Semillon, Sauvignon Blanc or Riesling, are isoprenoids and pyrazines. Several studies indicated that after accounting for the effect of stage of maturation (total soluble solids), a higher temperature led to a lower level in white aromatic grape cultivars thus potentially reducing aromatic intensity (Belancic et al., 1997; Reynolds and Wardle, 1993). Likewise higher temperature and/or light exposure are correlated with a lower methoxypyrazine level in grapes (Lacey et al., 1991; Ryona et al., 2008). Significant thermal effects on berry sensory traits were detectable in Semillon that may have contributed to these wine responses (Sadras et al., 2012c). Berries grown at elevated temperature had enhanced skin fruity aromas and less developed pulp detachment and pulp juiciness in 2010, and less developed pulp sweetness and skin disintegration in 2011.

Sensory traits in Shiraz and Cabernet Franc were strongly season dependent. Shiraz showed a shift from berry to cooked fruit characters with increasing temperature in 2010, but no detectable thermal responses in 2011. Conversely, Cabernet Franc showed no detectable responses in 2010 and a marked reduction in dark fruit and enhancement of green leaf character with elevated temperature. The breakdown of the green aromas in Cabernet Franc could have been delayed relative to the sugar accumulation in 2011. This is consistent with observations in Sauvignon Blanc, where thinning advanced harvest and increased wine green characters (Naor et al., 2002). As 2010 was a significantly warmer season, it is likely that the green components were probably broken down by harvest. In relation to controls, elevated temperature consistently advanced seed ripening of Cabernet Franc in 2010 (Sadras et al., 2012c), but this had no detectable effect on wine sensory attributes (Figure 2). The more intense berry and floral characters in the Shiraz 2010 control wine (Figure 3) corresponded with detectable enhanced pulp aromas in berries (Sadras et al., 2012c).

Conclusion

The assumption that elevated temperature reduces titratable acidity and increases pH is an oversimplification that highlights the risk of generalisations based on indirect evidence where temperature is confounded with other factors. Our field experiments involving direct comparisons in a framework of plasticity revealed three out of the four possible thermal responses of titratable acidity and pH: (i) both traits were responsive (Cabernet Franc, Chardonnay), (ii) both traits were unresponsive (Shiraz) and (iii) pH but not acidity was responsive (Semillon). For our combination of cultivars and environments, no evidence was found for the fourth type, i.e. responsive titratable acidity and unresponsive pH. Expanding the range of cultivars and environments and a closer look at the dynamics of malate, tartrate, Na^+ and K^+ are required to improve our understanding of the genetic and environmental control of these important traits. Semillon showed the more consistent pattern of thermal response in sensory traits in both seasons, and the sensory attributes of Cabernet Franc and Shiraz were strongly influenced by the interaction between temperature regime and seasonal conditions.

Acknowledgements

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Table 1. Vintage and cultivar specific sensory attributes evaluated in wines from control and heated vines grown in the Barossa Valley of Australia.

Vintage	Cultivar	Attribute
2010	Semillon	green leaf aroma, rich mouth feel[(a)], ripe flavours (tropical fruit)
	Chardonnay	--
	Shiraz	floral aromas, berry flavours, cooked fruit flavours, ripe flavours (Shiraz)
	Cab Franc	green leaf aroma, light palate[(b)], tannin structure[(c)], varietal character[(d)]
2011	Semillon	citrus aroma, green leaf aroma, rich mouth feel, ripe flavours (tropical fruit)
	Chardonnay	apple aroma, citrus aroma, rich mouth feel, tropical fruit flavour
	Shiraz	tannin structure, cooked fruit flavours, floral aromas, berry flavours
	Cab Franc	dark fruit flavour, green leaf aroma, lighter palate, tannin structure

-- Insufficient fruit for wine production.

Descriptive terms similar to (a) 'flavour concentration', (b) 'thin',(c) a combination of 'drying' and 'dynamic' (Gawel et al., 2000). (d) 'the unique and distinctive character of a single grape cultivar as expressed in the wine it produces' (Stevenson, 2005).

Table 2. Total soluble solids (TSS), titratable acidity (TA) and pH of berry juice at harvest in four cultivars grown under two thermal regimes over two seasons in the Barossa Valley of Australia. The bottom of the table shows the *P* value for main factors and interactions from analysis of variance. As a complement, asterisks indicate significant temperature effect at P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***) for the pair-wise comparison of heated and control treatments for each cultivar.

Vintage	Variety	TSS (^{o}Brix)		TA (g L^{-1})		pH	
		control	heated	control	heated	control	heated
2010	Semillon	20.6 ± 0.22[a]	21.1 ± 0.42	6.4 ± 0.12	5.1 ± 0.39*	3.11 ± 0.0167	3.30 ± 0.0780
	Chardonnay	21.8 ± 0.38	23.6 ± 0.22*	4.9 ± 0.16	3.9 ± 0.12**	3.52 ± 0.0567	3.80 ± 0.0285*
	Shiraz	25.9 ± 0.51	24.7 ± 0.44	5.7 ± 0.35	7.5 ± 0.41*	3.44 ± 0.0458	3.40 ± 0.0318
	Cab Franc	25.4 ± 0.16	26.0 ± 0.16	5.3 ± 0.15	4.3 ± 0.10**	3.66 ± 0.0088	3.85 ± 0.0384**
2011	Semillon	22.6 ± 0.43	24.0 ± 0.79	4.9 ± 0.18	5.7 ± 0.69	3.37 ± 0.0318	3.54 ± 0.0361*
	Chardonnay	21.0 ± 0.32	21.9 ± 0.52	5.3 ± 0.20	4.5 ± 0.17*	3.57 ± 0.0265	3.82 ± 0.0713*
	Shiraz	23.5 ± 0.06	23.0 ± 0.35	7.2 ± 0.10	6.7 ± 0.18	3.37 ± 0.0231	3.43 ± 0.0463
	Cab Franc	23.8 ± 0.18	24.7 ± 0.20*	6.6 ± 0.06	6.0 ± 0.16*	3.50 ± 0.0120	3.65 ± 0.0208**

Source of variation			
variety (V)	0.0001	0.0001	0.0001
temperature (T)	0.0056	0.0185	0.0001
season (S)	0.0063	0.0011	0.3320
V x T	0.0014	0.0010	0.0008
V x S	0.0001	0.0002	0.0001
T x S	0.5038	0.7135	0.9675
V x T x S	0.3177	0.0001	0.5544

[a] standard error

Captions to figures

Figure 1. Norms of reaction of titratable acidity and pH of four grapevine cultivars in the Barossa Valley, Australia. Regression lines were fitted to the individual replicates, whereas the graph shows means and two standard errors; *b* is the slope of the regression, and *P* indicates its statistical divergence from 0. Error bars are two standard errors of the means from three replicates.

Figure 2. Sensory attributes of Semillon, Shiraz and Cabernet Franc wines as affected by elevated daytime temperature in 2010. Significant departure from score = 0 indicates treatment effect as assessed with a non-parametric one sample sign test (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$). Each value is the mean of 36 assessments (3 replicates x 12 panellists) for Shiraz and Cabernet Franc and 24 assessments for Semillon (2 replicates x 12 panellists); error bars are two standard errors of the means.

Figure 3. Sensory attributes of Semillon, Chardonnay, Shiraz and Cabernet Franc wines as affected by elevated daytime temperature in 2011. Significant departure from score = 0 indicates treatment effect as assessed with a non-parametric one sample sign test (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$). Each value is the mean of 45 assessments (3 replicates x 15 panellists) for Semillon, Chardonnay and Cabernet Franc and 30 assessments for Shiraz (2 replicates x 15 panellists); error bars are two standard errors of the means.

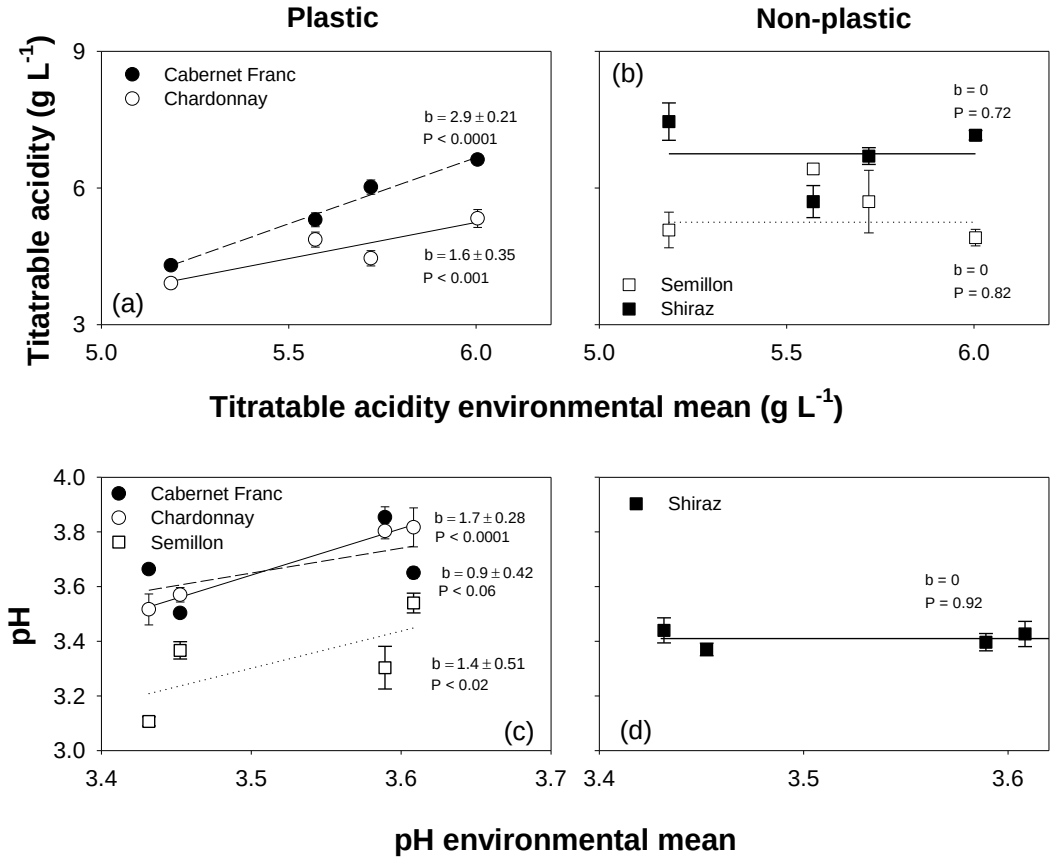

Plastic
Non-plastic
Titatrable acidity (g L-1)
Cabernet Franc
Chardonnay
b = 2.9 ± 0.21
P < 0.0001
b = 1.6 ± 0.35
P < 0.001
(a)
(b)
b = 0
P = 0.72
b = 0
P = 0.82
Semillon
Shiraz
Titratable acidity environmental mean (g L-1)
pH
Cabernet Franc
Chardonnay
Semillon
b = 1.7 ± 0.28
P < 0.0001
b = 0.9 ± 0.42
P < 0.06
b = 1.4 ± 0.51
P < 0.02
(c)
Shiraz
b = 0
P = 0.92
(d)
pH environmental mean

Fig. 8

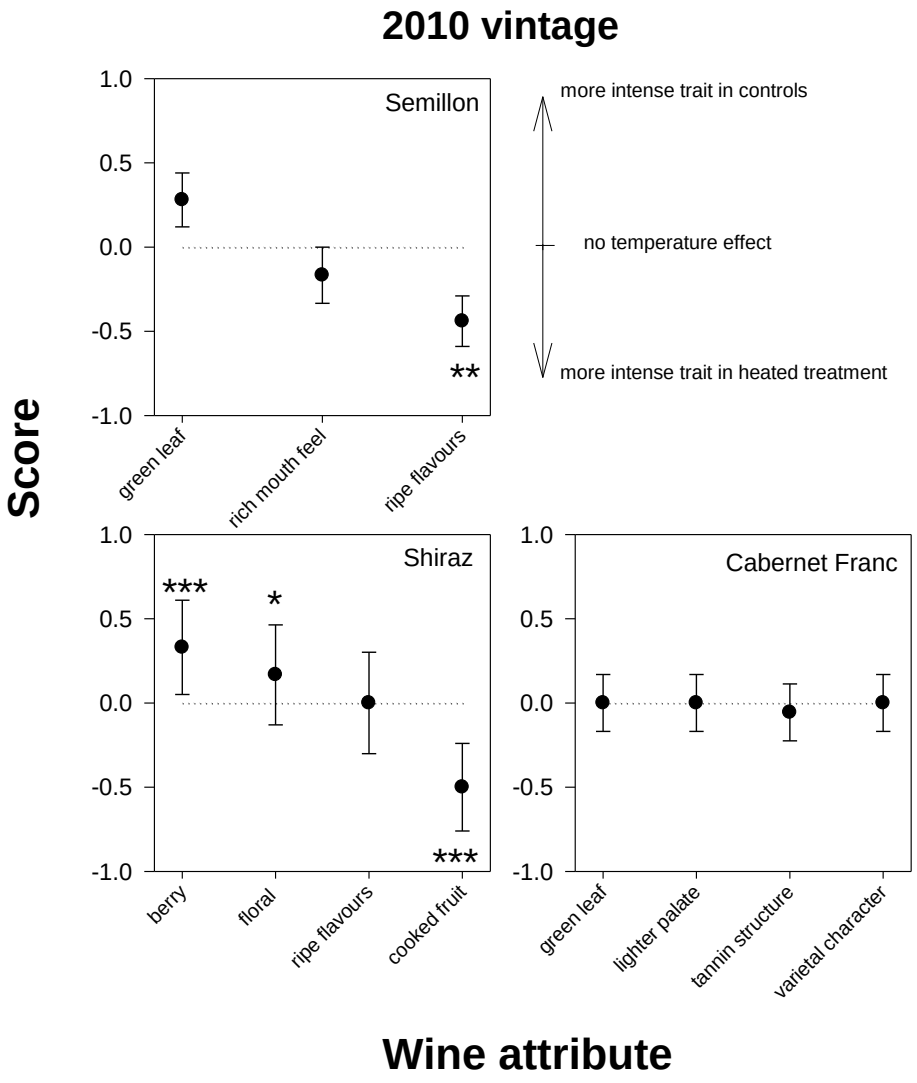

2010 vintage
Score
Semillon
Shiraz
Cabernet Franc
more intense trait in controls
no temperature effect
more intense trait in heated treatment
1.0
0.5
0.0
-0.5
-1.0
**
***
*
***
green leaf
rich mouth feel
ripe flavours
berry
floral
ripe flavours
cooked fruit
green leaf
lighter palate
tannin structure
varietal character
Wine attribute

Fig. 9

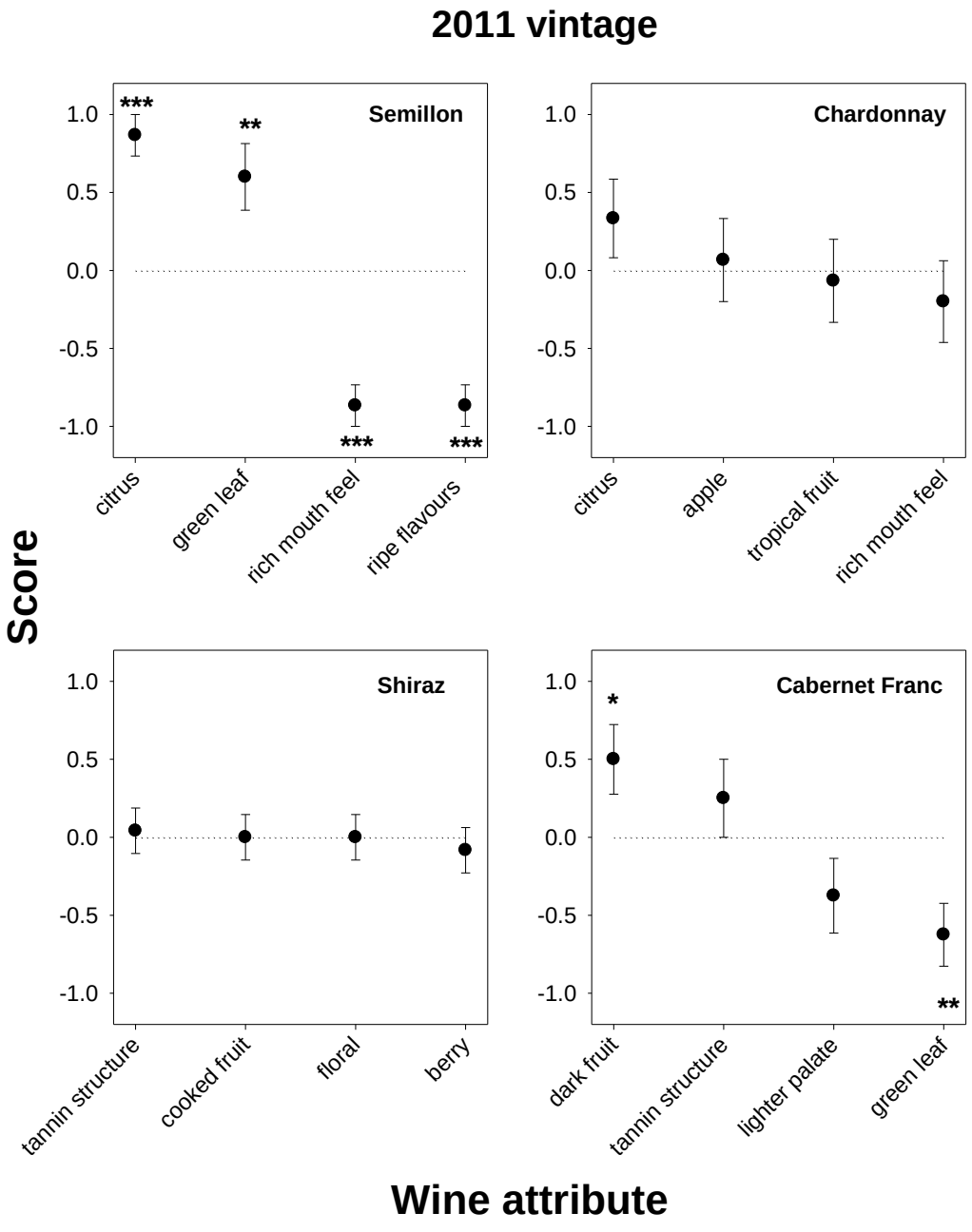

2011 vintage
Score
Semillon
Chardonnay
Shiraz
Cabernet Franc
1.0
0.5
0.0
-0.5
-1.0
***
**
***
***
*
**
citrus
green leaf
rich mouth feel
ripe flavours
citrus
apple
tropical fruit
rich mouth feel
tannin structure
cooked fruit
floral
berry
dark fruit
tannin structure
lighter palate
green leaf
Wine attribute

Fig. 10

Chapter 7. Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel

Marcos Bonada, Víctor Sadras, Sigfredo Fuentes

Abstract

Berry water loss at late stages of ripening is a variety dependent trait correlated with mesocarp cell death. We tested the hypotheses that elevated temperature anticipates the onset and increases the rate of mesocarp cell death. The implications of these putative effects on the time-course of berry shrivel were also investigated.

We assessed the progression of mesocarp cell vitality and the degree of shrivelling in berries from a factorial field experiment combining two thermal regimes (elevated temperature and control) and two varieties (Shiraz and Chardonnay). A bilinear model was used to objectively discriminate the onset of cell death and to quantify the rates of cell death before and after the inflection point in chronological and thermal scales. Elevated temperature advanced the onset of mesocarp cell death of berries and increased the rate of cell death in the period onset-harvest for both varieties. There was a close correlation between the percentage of living tissue and shrivel for Shiraz but not shrivel was observed in Chardonnay despite significant mesocarp cell death. Elevated temperature accelerated both berry shrivelling and mesocarp cell death in Shiraz and accelerated mesocarp cell death but had no impact on shrivel in Chardonnay. Mesocarp cell death seems necessary but not sufficient to explain berry shrivelling.

Awareness of functional links between berry shrivel and mesocarp cell death, and the environmental modulation of these traits would likely contribute to management practices that could reduce the severity of shrivel in a context of warmer conditions.

Introduction

Common in the plant kingdom, programmed cell death is a developmental process that takes place at specific tissues and times during plant ontogeny (Greenberg 1996, Danon et al. 2000, Thomas and Sadras 2001, Love et al. 2008, Thomas et al. 2009). This process involves genetic and epigenetic controls that integrate endogenous and environmental cues, and enzymatic reactions leading to the breakdown of membranes, mixing, disassembling and reallocation of cell compounds during senescence (Paliyath and Droillard 1992, Greenberg 1996, Huijser and Schmid 2011, Kuang et al. 2011). Recent studies in grapevines have shown that programmed death of cells in the mesocarp of berries is a likely process during late ripening stages, which may enhance berry sensory attributes (Krasnow et al. 2008, Tilbrook and Tyerman 2008, Coetzee and du Toit 2012). The lipoxygenase family of enzymes plays a crucial role in the membrane deterioration pathway and synthesis of derived metabolites related with berry flavour and aroma (Rogiers et al. 1998, Podolyan et al. 2010). This enzymatic pathway, and therefore programmed cell death, is modulated by biotic and abiotic stresses (Maccarrone et al. 2001). Thermal modulation of programmed cell death has been widely documented, particularly in monocarpic species (Zuppini et al. 2006, Chauhan et al. 2009). Cellular senescence shares common features in monocarpic and perennial plants (Munné-Bosch 2008) but the effects of temperature on the dynamics of cell death in berry mesocarp and its relation with berry shrivelling are largely unknown.

Fuentes et al. (2010) reported variety-dependent correlations between mesocarp cell death and berry shrivel. Some varieties such as Chardonnay show high level of cell death in the mesocarp of berries with minimal shrivel (Tilbrook and Tyerman 2009, Fuentes et al. 2010). In contrast, berry shrivel or late-season dehydration is more frequent in varieties like Shiraz (McCarthy 1999, Sadras and McCarthy 2007, Krasnow et al. 2010). Yield losses of up to 30% have been reported for Shiraz in Australia (Rogiers et al. 2006, Fuentes et al. 2010), affecting in some seasons more than half of the vineyard's fruit (Krasnow et al. 2010). Berry shrivel is a consequence of the combined effects of water loss by transpiration (McCarthy and Coombe 1999, Greer and Rogiers 2009), a reduction of phloem influx in the berry (Rogiers et al. 2006) and back flow of water from the berries to the plant through the xylem (Tyerman et al. 2004, Bondada et al. 2005, Keller et al. 2006, Tilbrook and Tyerman 2009). Berry shrivel occurs in the latest stage of ripening when berry has reached the maximum weight (Sadras and McCarthy 2007) and coincides with the onset of mesocarp cell death (Tilbrook and Tyerman 2008, Fuentes et al. 2010). From this point onwards, subsequent weight losses are a consequence of variety-dependent water relations in berries (Sadras and McCarthy 2007, Tilbrook and Tyerman 2008).

Phenological shifts associated with current and projected warming trends have been widely documented for grapevines (Duchene and Schneider 2005, Webb et al. 2007,

Petrie and Sadras 2008, Webb et al. 2011). Warmer conditions during berry ripening might exacerbate the incidence of berry shrivel due to: i) increased berry water loss under elevated evaporative conditions (Greenspan et al. 1994, McCarthy and Coombe 1999, Greer and Rogiers 2009), ii) a putative backflow of water to the parent plant (Keller et al. 2006, Tilbrook and Tyerman 2009), and/or iii) shift on the onset and development of mesocarp cell death, thus, leading an anticipated berry water loss.

Within the context of warming trends in viticulture worldwide (Jones et al. 2005, Webb et al. 2007, Petrie and Sadras 2008, Duchene et al. 2010, Galbreath 2011), the aim of this paper was to investigate the effects of elevated temperature on cell death in the mesocarp of berries and its relationship with berry shrivel in two contrasting varieties. The working hypotheses are outlined in Figure 1. We hypothesised that elevated temperature during berry growth and development will produce: 1) earlier onset of mesocarp cell death, and 2) increased rates of cell death in the phenological periods of anthesis-onset and onset-harvest. We measured the proportion of living tissue and the degrees of berry shrivel during berry development on plants submitted to two thermal regimes, i.e. elevated temperature and ambient temperature (control). We compared the varieties Chardonnay and Shiraz, due to their contrasting patterns of berry cell death and shrivel. Both varieties show similar pattern of mesocarp cell death after maximum weight, but, while Chardonnay maintains berry water content beyond this threshold, Shiraz berries often present berry shrivel symptoms (Tilbrook and Tyerman 2008, Fuentes et al. 2010).

Materials and methods

Vines and site

The trial consisted in own-rooted Chardonnay and Shiraz field-grown vines located at the SARDI's Nuriootpa Research Station vineyard in the Barossa Valley of South Australia (34°S, 139°E). Dry et al. (2004) described the climate and viticultural practices of the Barossa Valley. Summers are dry and warm and winter-dominant rainfall ranges between 500 to 700 mm. Vines are in the same block and were planted in 1995 (Chardonnay clone 277) and 1997 (Shiraz clone NSW) on a red brown earth soil (Northcote 1979). Row orientation is north-south and vines were planted at 2.25 m between vines and 3.0 m between rows. Vineyard management was the same for both varieties; vines were spur pruned to 40–50 buds per vine and trained to a single-wire trellis and were drip irrigated weekly from mid January at a flow rate of approximately 4 l h^{-1}, during 12 hours per irrigation event.

Heating system

An open-top system was used to increase the temperature and minimise any unrealistic effect on the canopies. The system consisted on modular rectangular units of polycarbonate located on both sides of the row. Each module stands on fold-out

legs that allow for height adjustment. This system increases day ambient temperature between 2 to 4°C in comparison to non-heated treatments (Sadras and Soar 2009). Some operational characteristics of this passive top open system reported by Sadras and Soar (2009), include: i) tracking of diurnal temperature dynamics, ii) maintenance of relative humidity, avoiding the interaction between temperature and vapour pressure deficit and iii) maintenance of normal canopy exposure to natural light intensity and quality. Detailed discussion of the system performance and limitations are in Sadras and Soar (2009).

Experimental design

A factorial trial of two varieties (Shiraz and Chardonnay) and two treatments (control and heated plants) was established on 2009. The passive heating system was permanently installed on October of this year. Panels were occasionally removed during sampling, harvesting, pruning and weed control. Data presented in this paper corresponds to the 2010-11 season. Treatments were laid out in a split-plot design with three replicates, assigning the variety to main plot and temperature to subplots. Each replicate included nine vines, and sampling was constrained to the seven central vines of each replicate, leaving the two external vines as buffers. The effect of panels on rain distribution was partially replicated in control treatments by covering the same ground area with high density polyethylene. To further check for the putative effect of chambers on rain distribution and vine water status, we measured pre-dawn water potential using the procedure of Hsiao (1990).

Our target temperature for heated vines was adjusted to warming predictions for grape growing regions of Australia by 2050 (insets figures 2ab). Increments in ambient temperature are expected to be between 0.4 to 2.6°C above average (Webb 2006). Treated plants were compared with control vines, which were maintained at air temperature (Fig.2). Temperature (T^{o}) and relative humidity (RH) from each replicate were continuously recorded at 15 minutes intervals using Tine tag Ultra2 temperature and humidity loggers (Hastings Dataloggers, Port Macquarie, Australia), which were located inside the canopy of the central vines.

Phenology and growing conditions

The inception of key phenological stages, e.g. budburst, anthesis, and veraison, was assessed using the modified E-L system proposed by Coombe (1995). Three target plants per replicate were weekly monitored during the growing season. Buds were considered burst when the first green tip was visible (E-L 4). The total of buds per plant left during winter pruning was assessed at each time (n ~ 40). The time of budburst of each replicate was defined when 50% or more of buds had burst (n ≥ 60). The course of anthesis was assessed monitoring 20 bunches per plant randomly chosen from primary shoots. The visual scoring system used considered three levels of capfall: 1 for < 30% caps off, 2 between 30 - 80% caps off and 3 for > 80% caps off. The day of anthesis of each replicate was estimated when half of bunches had

reached level 2. The beginning of veraison was defined when 50% of the assessed bunches per replicate (n=60) had at least one coloured berry (E-L 35).

Growing degree days (GDD) from anthesis was calculated for each replicate using actual temperature and a base temperature of 10°C (Amerine and Winkler 1944, Williams et al. 1985). Vapour pressure deficit (VPD) was calculated as a function of temperature and relative humidity in KPa (Jones 1992).

Berry sampling

Between January and March of 2011 we sampled Shiraz 12 times and Chardonnay 11 times. Around 70 berries were collected weekly from each replicate as follows: one berry per bunch from the top, middle and bottom part of 10 bunches per seven plants (n = 70), located on both sides of the row on the shaded part of the canopy. Berries were carefully cut with scissors preserving the pedicel and avoiding any possible alteration in the tissues by physical damage. Samples were collected into zip-lock plastic bags and placed immediately in ice-cooled containers to be transported to the laboratory for further analysis. From this sampling, sub-samples of 15 berries per replicate were randomly obtained for morphometric and fluorescent microscopy analysis. The remaining berries were kept as a backup and for extra chemical analyses (not presented in this paper).

Berry weight, total soluble sugar and osmolality

Twelve of the fifteen berries collected for each replicate were randomly selected and weighed after removing the pedicel by cutting transversally at the insertion point with a bistoury. The remaining three berries were used to measure osmolality using a Wescor 5500B vapour pressure osmometer (Wescor Inc., Utah, USA). The average osmolality of these berries was used to prepare the fluorescein diacetate (FDA) staining solution. Following the methodology used by Fuentes et al. (2010), berries were sectioned in halves longitudinally at the maximum diameter avoiding contact with the seeds. The juice from one half was extracted by manual crushing to measure total soluble solid (TSS) using a temperature compensated digital refractometer (Model PR101; ATAGO, Tokyo, Japan). The remaining halves were used for FDA staining and fluorescent microscopy imaging.

FDA staining and fluorescent microscopy imaging

Twelve half-berries per replicate were used to assess cell vitality using the FDA staining technique (Jones and Senft 1985, Krasnow et al. 2008, Tilbrook and Tyerman 2008, Fuentes et al. 2010). From a fresh 4.8 mM stock FDA solution in acetone, a sucrose solution of osmolality was prepared similar to that measured from berries (± 10%). This solution was applied on the cut section until a maximum concave meniscus was formed on top. The berries were then incubated in darkness

for 30 min. Dried sectioned berries were positioned under a Nikon SMZ 800 (Nikon Co., Tokyo, Japan) dissecting microscope at minimum magnification (0.5x objective lens) under ultraviolet light with a green fluorescent protein filter in place. Both, normal digital images (RGB in JPG digital format) and fluorescent field images, were obtained using a Nikon DS-5Mc (Tochigi Nikon Precision Co., Ltd, Otawara, Japan) colour cooled digital camera using a NIS-Elements F2.30 software maintaining the same gain and exposure settings for all images. RBG images were used to determine the location of the seeds during the image analysis to be excluded later as proposed by Fuentes et al. (2010).

Berry tissue vitality and morphometric analysis

A MATLAB® R2008a (Mathworks Inc., Natick, MA. USA) code was used to analyse the fluorescent field digital images (Fuentes et al. 2010). This analytical tool allows to semi-automatically measuring the following variables: i) berry perimeter (P, cm), ii) diameter (D, cm), iii) area (A, cm^2), and iv) tissue vitality as percentage of living tissue (LT %).

Berry shrivel (ShI) was calculated as a normalised index that ranges from 1 (maximum turgor) to 0 (maximum shrinkage) (Fuentes et al. 2010):

$$ShI = (R - Rmin) / (Rmax - Rmin) \quad \text{eq. 1}$$

where R is the ratio between the diameter and the perimeter for a single berry, Rmax is the maximum and Rmin is the minimum ratio; both Rmax and Rmin were derived from all the samples x dates for each variety. The values used in these calculations were 0.25 and 0.26 for Rmin, and 0.35 and 0.38 for Rmax, in Shiraz and Chardonnay respectively. To test for biased from aberrant berries, we plotted histograms of R, and verified that these parameters were well in the tails of the frequency distributions; for example, 2% of Shiraz berries had $0.25 \leq R \leq 0.27$ and 2% of berries had $0.33 \leq R \leq 0.35$.

Statistical analysis

The onset and rates of mesocarp cell death were quantified by fitting a bilinear model between the percentages of LT (y) and chronological or thermal scales (x) using a piece-wise routine of SigmaPlot (version 11.0, Systat Software Inc., San Jose, California):

$LT = a - b \cdot x$ if $x < x^*$ eq. 2a

$LT = a' - b'x$ if $x \geq x^*$ eq. 2b

This model defines three parameters (Fig 1): rate of change before the onset of rapid cell death (b), the onset of rapid cell death (x^*) and rate of change after the onset (b').

To set our chronological and thermal time scales, we used four reference points: date of budburst, date of anthesis, date of 8 Baume in berries, and day of year. All four references returned similar patterns; hence we used date of anthesis as a reference for comparison with published studies (Tilbrook and Tyerman 2008, Krasnow et al. 2008). Inaccuracy in the determination of date of anthesis therefore did not bias the estimates of model parameters.

Significance of differences in the onset and rate of mesocarp cell death between treatments was tested with the analysis of the variance (ANOVA). Relationships between variables where analysed with linear (e.g. ShI vs. LT) and quadratic (e.g. ShI vs. Time) regression analysis. Residual analysis was used to test the temperature-induced variation on the degree of shrivel in chronological and thermal time (Sadras and Moran 2012). The effect of temperature on residuals was tested with ANOVA.

Results

Growing conditions

Monthly mean minimum temperature ranged from 5.8 ± 0.04°C (September) to 14.5 ± 0.14°C (January), and was similar for both heated and control treatments and for both varieties (Fig 2a and b). Monthly mean maximum temperatures in controls ranged from 16.0 ± 0.17°C (September) to 35.2 ± 0.03°C (January). During the growing season, maximum temperature was elevated by 1.5 ± 0.06 for Shiraz and 2.3 ± 0.08°C for Chardonnay in the heated treatments compared to controls (Fig 2a and b). The more effective heating in Chardonnay was related to differences in canopy size (Sadras et al. 2012). Using pruning weight as indicator of canopy size, Chardonnay canopy in the heated treatment (0.76 ± 0.074 kg vine^{-1}) was less than half the size of its Shiraz counterpart (1.73 ± 0.108 kg vine^{-1}). Elevated temperature reduced pruning weight in Chardonnay by 20% (P = 0.04) but did not change pruning weight in Shiraz (P = 0.85).

The passive open-top system did not affect relative humidity; hence, the VPD tracked the regime of maximum temperature during the cycle (Fig 2c and d). Consistently, VPD calculated at maximum temperature was higher in the heated treatments compared to control treatments ($P < 0.002$) (insets fig 2c and d). In correspondence with the differences in maximum temperature between Chardonnay and Shiraz in the heated treatment, maximum monthly VPD ranged from 0.82 ± 0.086KPa (September) to 5.41 ± 0.383KPa for Chardonnay and from 0.65 ± 0.061KPa to 4.92 ± 0.342KPa (January) for Shiraz.

Comparison of long-term and actual rainfall for the period September-March, showed that the 2010/11 season was one of the wettest in the last sixteen years (Bureau of Meteorology 2011). The accumulated rainfall was 459mm, two-fold the historical record from the last 16 years (243mm). January was the driest month with 4mm, while 47% of the rainfall (~ 217mm) was concentrated in the period from berry softening to harvest. Six supplementary irrigations were applied from January 24th to March 7th, 2011 (6 irrigations @ 48 l vine^{-1} irrigation^{-1}). Pre-dawn leaf water potential was ~ -0.19 in early spring and peaked at ~ -1.0 at the end of the soil drying cycle immediately before the beginning of irrigation (Table 2). Pre-dawn leaf water potential was unaffected by the heating treatment, hence confirming that the chambers had no physiologically detectable effects on the water regime of the vines in comparison to controls (Table 2).

Onset and rate of cell death

Elevated temperature advanced the onset of rapid cell death for both varieties (Fig 3a and b, Table 1). For Shiraz, the onset was brought forward from 87 DAA in controls to 79 DAA in the heated treatments ($P = 0.04$). In Chardonnay, the onset was undetectable in controls (> 114 DAA) but a clear onset was found at 80 DAA under warmer conditions ($P < 0.01$). In Shiraz differences in onset were statistically undetectable on a thermal time scale ($P = 0.83$) (Fig 3c and d, Table 1).

On a chronological scale, elevated temperature increased the rate of cell death before the rapid onset for both varieties (Table 1). However the analysis of the variance indicated that these differences where only significantly different for Shiraz ($P = 0.02$). On a thermal scale, slopes where reduced and remained marginally different for Shiraz ($P = 0.02$).

The rates of cell death on the period onset of rapid cell death-harvest were 8 and 13 fold higher in relation to the period anthesis-onset for Shiraz, in chronological and thermal time scales respectively. Elevated temperature increased the rate of cell death after the onset on a chronological scale but differences were not significant on a thermal time scale (Table 1, Fig 3a and c).

In Chardonnay there was a distinctive pattern of cell death between treatments. While the progression of cell death in heated berries followed the two-phase pattern, defined by the parameters of the bilinear model (eq. 2a and b), control berries maintained the same rate of cell death until harvest (Fig 3b and d). The rate of cell death in heated berries after the onset was between 6 to 8 fold higher than in controls on chronological and thermal time scales, respectively (Table 1).

Berry shrivelling

Fresh weights for Shiraz berries ranged from 1.6 ± 0.12 to 1.5 ± 0.10 and from 1.3 ± 0.08 to 1.4 ± 0.08, for heated and control treatments respectively, in the period maximum fresh weight-harvest. In chardonnay, weights ranged from 1.1 ± 0.02 to 1.06 ± 0.06 in heated, while their counterpart ranged from 1.1 ± 0.13 to 0.9 ± 0.05 in the same period. More details of berry weights are reported in (Sadras and Moran, 2012).

Elevated temperature increased the rate of shriveling after the inception of rapid berry cell death in Shiraz but not in Chardonnay (Fig 4). In Chardonnay, the shrivel index was maintained constant and equal to zero ($P > 0.05$) independently of the scale (chronological or thermal). No comparison was done for this variety between treatments due to the absence of the second phase of cell death in control berries. In Shiraz, thermal effects on the rate of shrivelling was dramatically reduced when rates where compared on a thermal time scale basis (Fig 4b). Analysis of residuals showed highly significant differences between thermal regimes ($P < 0.01$) in chronological time which were undetectable on a thermal time scale ($P = 0.86$).

There was a strong and statistically significant correlation between ShI and LT for Shiraz ($R^2 = 0.83$; $P < 0.0001$), but no relationship was found for Chardonnay (Fig 5). In the case of Shiraz, the ShI declined from 0.8 to 0.6 when the percentage of living tissue decreased from 97% to 55%. In Chardonnay, the regression had a slope not different from zero and ShI was maintained ~ 0.9 beyond the inception of rapid mesocarp cell death, ranging from a minimum of 73% to a maximum of 97% (Fig 5).

Discussion

The hypothesis of temperatures-induced changes in berry growth and development (Sadras and Petrie 2011, Sadras and Petrie 2012) is a useful starting point for the analysis of effects of warmer conditions on the onset and rates of mesocarp cell

death. Here we found that a temperature-induced change resulted in earlier onset and faster rate of berry mesocarp cell death for both Shiraz and Chardonnay.

The selection pressure exerted by winemakers since the beginning of the wine industry has had a distinctive effect favouring some quality traits that make wine-grapes more suitable or desirable for winemaking. Some of these traits are: i) higher rates of must extractability during crushing, ii) lower water content and iii) development of desirable flavours and aromas, which might be favoured by mesocarp cell death (Tilbrook and Tyerman 2008, Coetzee and du Toit 2012). With a snap-shot focus at harvest, Fuentes et al. (2010) found that lower cell vitality in the mesocarp of berries correlated with higher shrivel. Here we provide new insight, based on the quantification of the dynamics of these traits.

The morphological method used in this work to quantify the level of shrivel assumes some changes on berry shape late in ripening associated to a progressive reduction in mesocarp cell viability (Fuentes et al. 2010). Changes in shape are mainly attributed to reduction in volume during the collapse of death cells in the mesocarp of shrivelling berries (Bondada and Keller 2012). As flesh cells collapse physic and morphological change in berry properties become more evident (Tilbrook and Tyerman 2008, Bondada and Keller 2012). In this sense, lower percentage of viable cells may result in a higher level of shrinkage and a reduction in the relation between berry diameter and perimeter. However, while this morphometrical-type approach has resulted to be a good estimator of berry shrivelling, additional studies need to be done on berry water content during late ripening for better interpretation of cell death and shrivelling.

While Shiraz showed a significant linear relationship between tissue vitality and shrivel, these traits were unrelated in Chardonnay (Fig 5). Two hypotheses can be put forward to account for the lack of correlations in Chardonnay. The first parsimonious interpretation is that both cell death and shrivel progress in parallel during berry ripening, but that the processes are not functionally linked. The second interpretation is that cell death is necessary but not sufficient for shrinkage to occur. Water relations in Chardonnay seem to be unrelated to the loss in membrane integrity and the hydraulic isolation of the berry during late ripening does not allow for putative backflow from the berry to the plant (Tilbrook and Tyerman 2009). If the link between cell death and shrivelling is functional in Shiraz, management practices to delay cell death might reduce shrivelling at the expense of flavour and aroma development.

Our study showed an environmental modulation of the berry shrinkage in Shiraz. These observations are in agreement with previous work that suggested a plastic behaviour of berry shrivelling in Shiraz (Sadras and McCarthy 2007). Therefore, the

simultaneous development of ripening in warming conditions is expected to interfere with the time course and severity of berry shrivelling by, i) increasing water losses by a putative backflow from the berry to the vine, drive by an accelerated disruption in membrane integrity; and/or ii) increasing the rates of transpiration, due to a higher vapour pressure deficit.

The baseline for our analysis were differences in VPD at level canopy, however, temperature induced change in VPD at berry level could be increased with managements practices that results in more exposed fruit, e.g. leaf thinning, or that result in less vigorous canopies, e.g. water stress. Also, factors inherent to each variety, e.g. berry colour, shape and bunch compactness, may result in differences on the energy balance of the berry (Smart and Sinclair 1976).

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Table 1. Rates of mesocarp cell death before (b) and after (b') the onset of rapid cell death in chronological (DAA) and thermal (GDD) scales for Shiraz and Chardonnay. Slopes b and b' represent rates of change on the percentage of living tissue per day after anthesis (% day^{-1}) and per increase in growing degree day (%°Cd^{-1}). The thresholds and slopes were estimated from curve fitting of bilinear segments model (Fig 1, eq. 2). Data are means ± s.e. and P values are from the analysis of the variance (ANOVA).

Cultivar	Treatment	DAA			GDD		
		b	Onset	b'	b	Onset	b'
Shiraz	**Control**	-0.076 ± 0.0026	87 ± 1.62	-0.622 ± 0.034	-0.005 ± 0.0002	1080 ± 23.52	-0.067 ± 0.0047
	Heated	-0.101 ± 0.0062	79 ± 2.02	-0.863 ± 0.0609	-0.006 ± 0.0003	1071 ± 31.61	-0.079 ± 0.0075
	P value	0.0208	0.03830	0.0242	0.0254	0.8383	0.2427
Chardonnay	**Control**	-0.084 ± 0.0091	> 114 ± 0.00	-	-0.006 ± 0.0007	> 1350 ± 9.77	-
	Heated	-0.139 ± 0.0223	80 ± 6.63	-0.533 ± 0.0560	-0.009 ± 0.0014	1137 ± 47.15	-0.048 ± 0.0060
	P value	0.0855	0.0066	-	0.2045	0.0114	-

Table 2: Pre-dawn leaf water potential (MPa) in Shiraz and Chardonnay vines grown under two temperature regimes. Values are means ± standard errors. P values account for differences between treatments at the same chronological time from the analysis of variance.

Date	Shiraz							Chardonnay						
	DAA	Control			DAA	Heated		P value	DAA	Control			DAA	Heated		P value
26/11/10	-2	-0.17	±	0.014	2	-0.18	± 0.035	0.7956	5	-0.17	±	0.022	9	-0.20	± 0.025	0.3752
21/1/11	54	-0.90	±	0.018	58	-0.95	± 0.029	0.1808	61	-1.08	±	0.090	65	-1.17	± 0.094	0.4881
25/2/11	89	-0.56	±	0.016	93	-0.57	± 0.015	0.8810	96	-0.47	±	0.024	100	-0.53	± 0.032	0.2128

FIGURES

1. Time-trajectory of mesocarp cell death for Shiraz berries in the study of Tilbrook and Tyerman (2008). The bilateral model fitted to the data (eq. 2) has three biologically relevant parameters: *x** (the onset of rapid cell death), b (slope before the onset) and b' (slope after the onset).

2. Comparison of temperature and vapour pressure deficit dynamics between heated (red dashed lines) and control treatments (solid line), during the growing period 2010-11. (a,b) Differences in daily maximum and minimum temperatures in Shiraz and Chardonnay replicates. Arrowheads indicate the timing of key phenological stages for control (full) and heated (empty) treatments: budburst (B), flowering (F), veraison (V) and harvest (H). Insets represent differences in maximum temperatures between treatments. (c,d) Difference in vapour pressure deficit at maximum temperature. Insets compare control and heated vapour pressure deficit; the line is y=x.

3. Dynamics of mesocarp living tissue (LT %) in chronological (DAA) and thermal (GDD) scales for Shiraz (a and c) and Chardonnay (b and d) berries. Each point corresponds to the mean of n=12 berries from control (full dots) and treated vines (empty dots). The model adjusted corresponds to eq. 2. Table 1 shows the parameters of the fitted models.

4. Progression of shrivelling in chronological (DAA) and thermal time (GDD) scales for Shiraz and Chardonnay berries during the stage of rapid cell death. Note that there were no data for Chardonnay control, as this treatment showed no evidence of rapid cell death (Fig. 3). Lines represent the best fit regression for each treatment (quadratic or linear). Each point corresponds the mean ± s.e., for control (full) and treated (empty) treatments across varieties: Chardonnay (diamond) and Shiraz (square). Insets compare the regression residuals in Shiraz for control and heated treatments (mean and one standard error).

5. Relation between shrivel and percentage of living tissue from the onset of rapid mesocarp berry cell death (refer to table 1), for Shiraz (full dots) and Chardonnay (empty dots). Lines represent the slopes of the regression for each variety. Each point is a replicate.

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Fig. 1

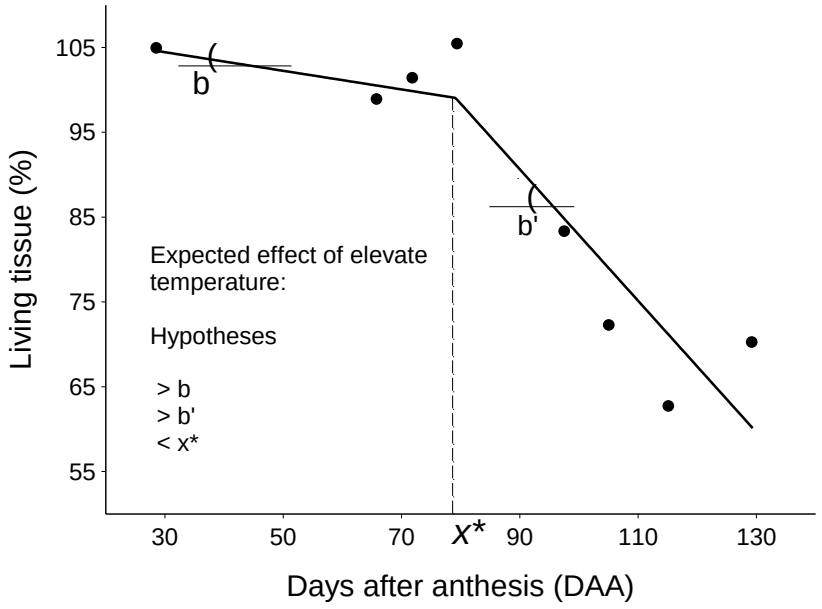

105
95
85
75
65
55
Living tissue (%)
b
b'
Expected effect of elevate temperature:
Hypotheses
> b
> b'
< x*
30
50
70
X*
90
110
130
Days after anthesis (DAA)

Fig. 2

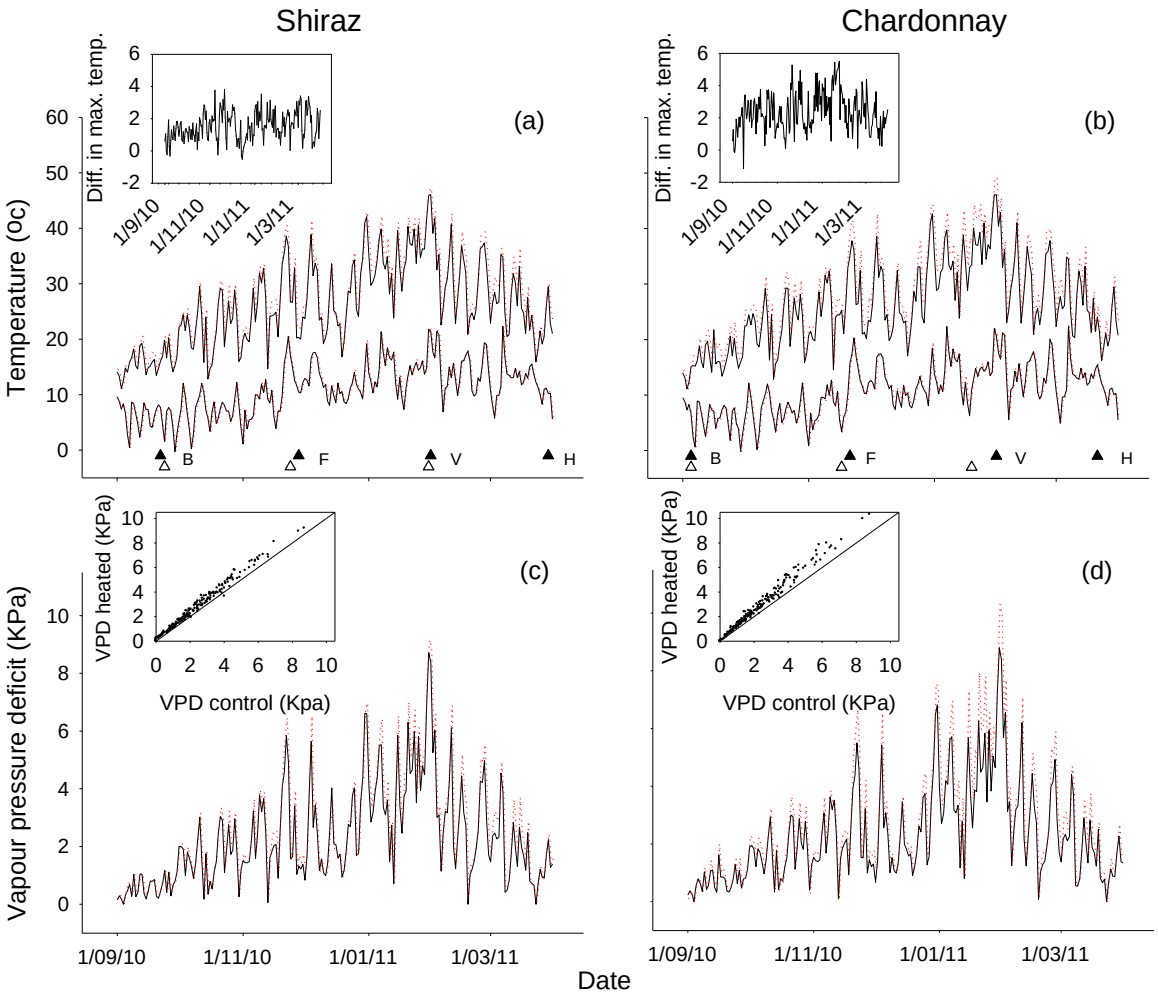

Shiraz
Chardonnay
(a)
(b)
(c)
(d)
Temperature (0c)
Diff. in max. temp.
Vapour pressure deficit (KPa)
VPD heated (KPa)
VPD control (Kpa)
VPD control (KPa)
B
F
V
H
1/9/10
1/11/10
1/1/11
1/3/11
1/01/11
1/03/11
Date

Fig. 3

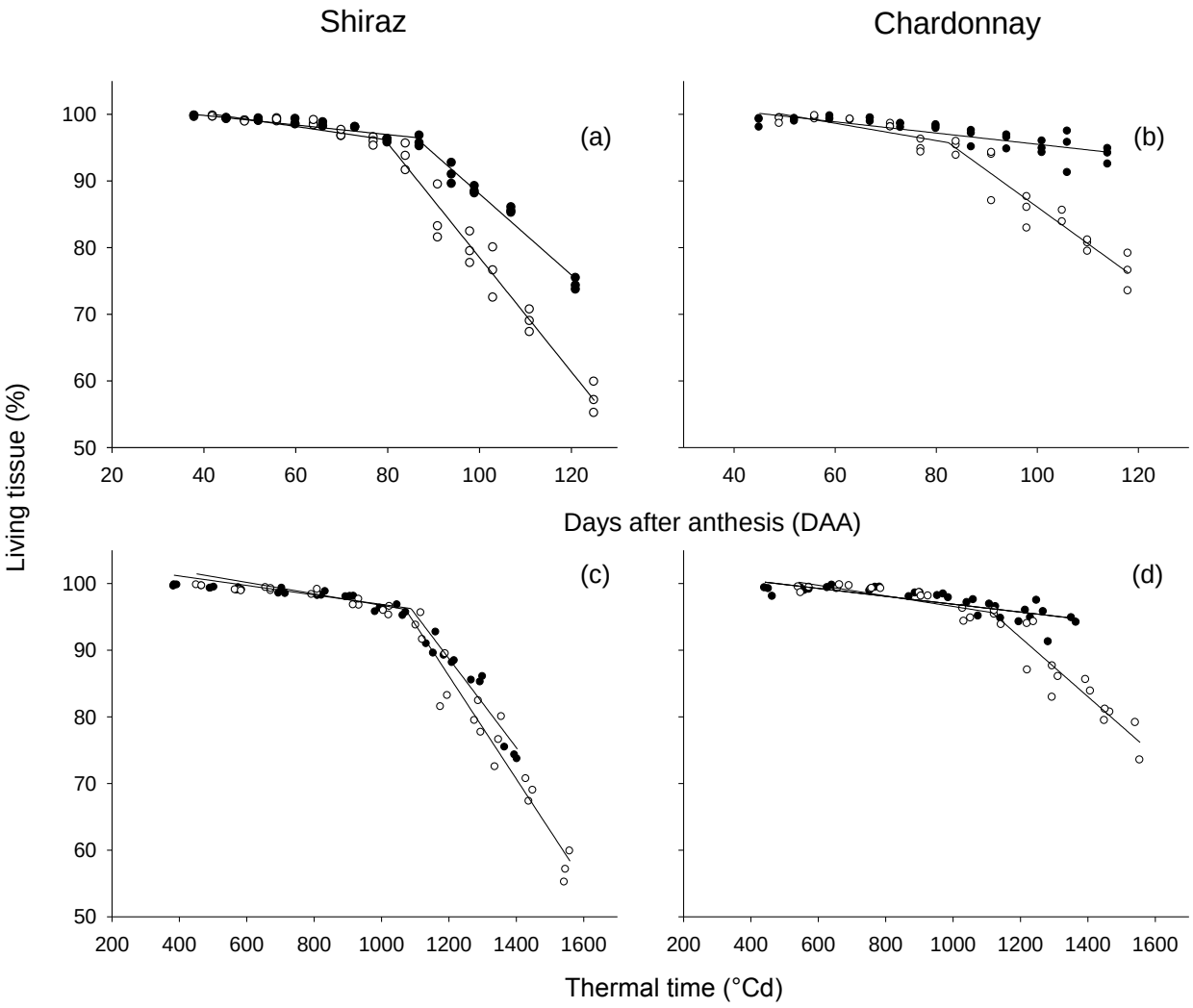

Shiraz
Chardonnay
(a)
(b)
(c)
(d)
Living tissue (%)
Days after anthesis (DAA)
Thermal time (°Cd)

Fig. 4

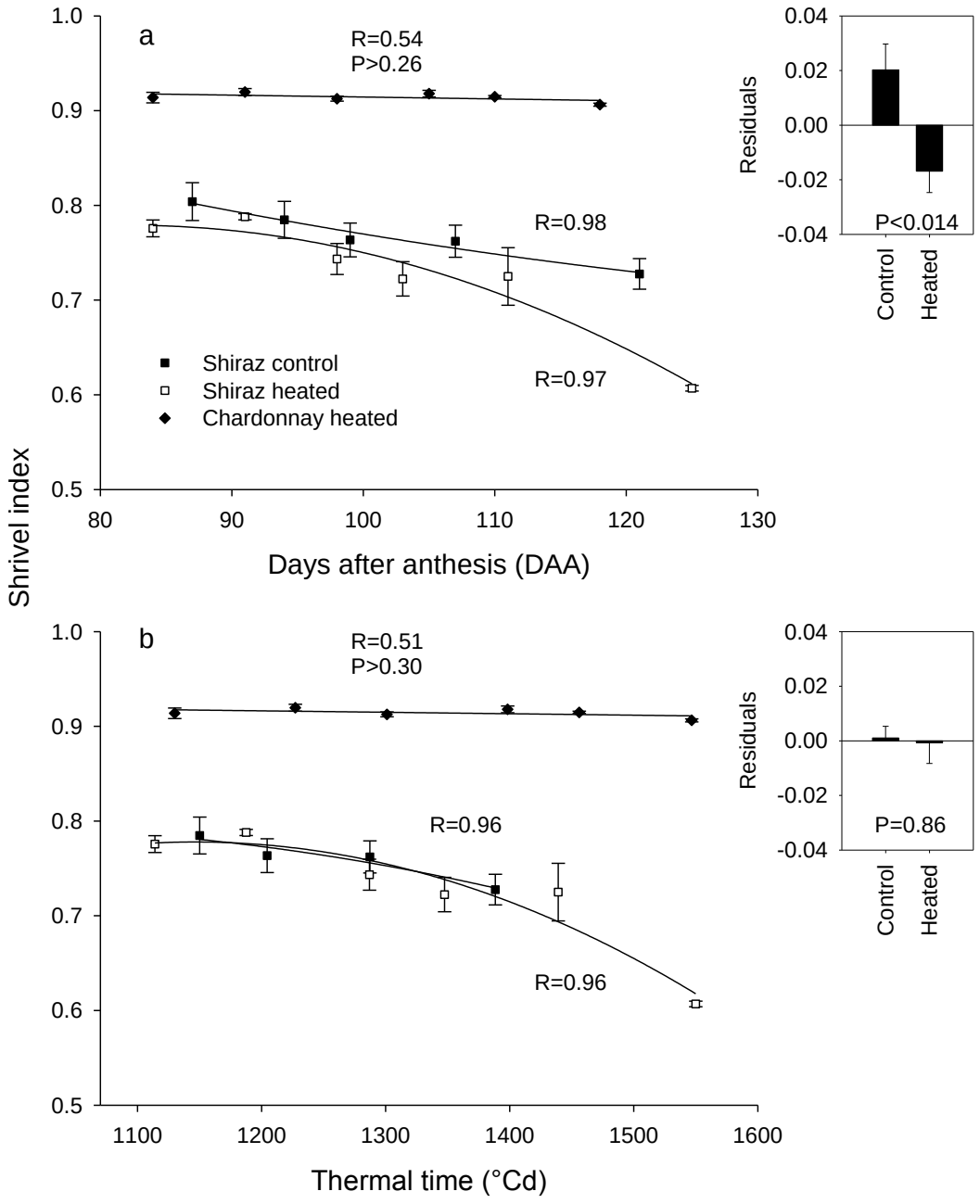
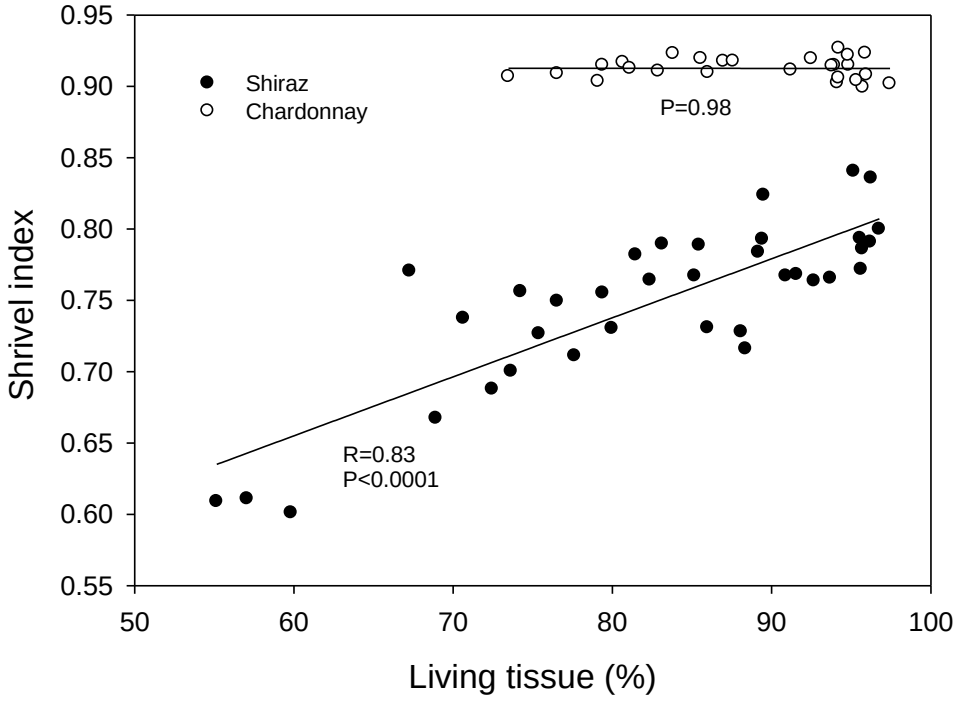

a
R=0.54
P>0.26
R=0.98
R=0.97
Shiraz control
Shiraz heated
Chardonnay heated
Shrivel index
Days after anthesis (DAA)
Residuals
P<0.014
Control
Heated
b
R=0.51
P>0.30
R=0.96
R=0.96
Thermal time (°Cd)
P=0.86

Fig. 5


0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
Shrivel index
50
60
70
80
90
100
Living tissue (%)
Shiraz
Chardonnay
P=0.98
R=0.83
P<0.0001

Chapter 8. Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine

VO Sadras, A Montoro, MA Moran, PJ Aphalo

Abstract

We measured the effect of elevated temperature, and its interaction with fruit load (exp. 1) and water supply (exp. 2), on the stomatal conductance (g_s) of *Vitis vinifera*, cv. Shiraz. Thermal regimes (control vs. elevated temperature using open-top chambers) were initiated in spring, thus affecting leaf development, and were maintained during the whole growing season. We used a top-down approach where reaction norms were derived that relate g_s for each treatment and the mean g_s across treatments; the slopes of reaction norms quantify phenotypic plasticity.

Stomatal conductance responded to neither the interaction between temperature and fruit load ($P = 0.37$) nor the interaction between temperature and water regime ($P = 0.33$). We therefore dealt with each factor separately. Reaction norms of g_s under elevated temperature and control treatments diverged, i.e. elevated temperature had no effect on g_s under conditions conducive to low conductance (below ~ 100 mmol m^{-2} s^{-1}) but increased g_s in relation to controls under conditions favouring high conductance. Stomata density was 159 ± 6.7 mm^{-2}, and was unaffected by temperature. Stomata length increased from 20.6 µm in controls to 23.2 µm in the heated treatment ($P < 0.0001$) and width increased from 14.4 µm in controls to 16.8 µm in the heated treatment ($P < 0.0001$). Thus, longer and wider stomata contributed to the enhanced plasticity of stomatal conductance under elevated temperature. Consistently, reaction norms of light-saturated photosynthesis were divergent, i.e. elevated temperature did not affect photosynthesis under conditions conducive to low g_s and photosynthesis, but increased photosynthesis in relation to controls under more favourable conditions. A high source:sink ratio reduced the plasticity of stomatal conductance. Water regime had a minor effect on the plasticity of stomatal conductance, but the reaction norms for irrigated and water deficit treatments were offset by ~ 60 mmol m^{-2} s^{-1} thus indicating a consistent effect of water deficit across environmental conditions.

We conclude that the responses of stomata derived from transient temperature treatments in fully expanded leaves are unsuitable to model the dynamics of gas exchange in response to projected warming. Our experiments support a conceptual model where current stomatal conductance is a function of (i) the prevailing environmental conditions during early stages of leaf development that set the upper limit of conductance through the modulation of stomatal density and size, (ii) the current environmental conditions and (iii) the source:sink ratio.

1. Introduction

Two aspects of projected warming are relevant to viticulture: the increasing incidence of heat waves and the gradual increase in average temperature (Beniston et al., 2007; Duchêne et al., 2010; Moriondo et al., 2011). In this paper, we are interested in the increase in average temperature, predicted to be between 0.4 and 2.6°C in grape-growing regions of Australia by 2050 (Webb, 2006). Warming projections have also been reported for grape-growing regions of Europe and North America, and their viticultural implications have been investigated with a range of approaches, from empirical correlations to physiological models (Bindi et al., 1996; Jones et al., 2005; Lobell et al., 2006; Moriondo et al., 2011). Experimental work necessary to improve these models is scarce (Bindi et al., 2001a; Bindi et al., 2001b; Salazar Parra et al., 2010); Schultz and Stoll (2010) summarised the gaps in environmental physiology of grapevines, including the environmental modulation of stomatal conductance, the focus of this study.

Stomata play at least three roles: regulation of gas exchange and water use efficiency (Cowan, 1977; Cowan and Farquhar, 1977; McAdam and Brodribb, 2011; Schultz and Stoll, 2010), thermal modulation of canopies (Burke and Upchurch, 1989; Nautiyal et al., 2012; Radin, 1994; Sadras and Soar, 2009; Soar et al., 2009; Upchurch, 1988) and prevention of embolism (Sperry et al., 2002; Tyree and Sperry, 1989). Recognising the multiple functions of stomata is important because multiple functions cannot be optimised simultaneously (Dudley and Gans, 1991; Farnsworth and Niklas, 1995; Piersma and van Gils, 2011). In response to short spells of high temperature in the field, well-watered Shiraz increased stomatal conductance and evaporative cooling at the expense of water use efficiency (Sadras and Soar, 2009; Soar et al., 2009). Studies in annual crops further support the notion that regulation of canopy temperature under elevated ambient temperature could override the putative role of stomata in maximising water use efficiency for some combinations of genotypes and environments (Fischer et al., 1998; Nautiyal et al., 2012; Radin, 1994; Rizza et al., 2012).

The environmental modulation of stomatal conductance involves both the density and size of stomata, and the degree of opening of the pore (Weyers and Meidner, 1990). Stomatal density and size are responsive to environmental conditions during leaf development and are invariant after the leaf is fully expanded (Düring, 1980; Rogiers et al., 2011). In contrast, the size of the pore opening is adjusted reversibly in response to the environment internal and external to the plant (Aphalo and Jarvis, 1991; Cowan, 1977; Damour et al., 2010; Gollan et al., 1986; Harris et al., 2010; Kramer and Boyer, 1995; Rahnama et al., 2010; Zeiger et al., 1987). The responsiveness of stomata to the environment hence results from the combination of both invariant and reversible responses operating at different time scales (Bresta et al., 2011).

In contrast to the bottom-up perspective where stomatal regulation is correlated to specific environmental factors (Damour et al., 2010; Marsal et al., 2008; Schultz and Stoll, 2010), in this paper we use a top-down perspective with a focus on reaction norms and plasticity. Reaction norms are mathematical functions relating phenotype and environment (DeWitt and Scheiner, 2004; Woltereck, 1909). For example, Reymond et al. (2003) derived linear reaction norms for maize hybrids that relate leaf

expansion rate and vapour pressure deficit, where the slope quantifies the phenotypic plasticity of the trait (DeWitt and Scheiner, 2004; Dudley, 2004). We measured stomatal conductance of Shiraz in two field experiments where control vines were compared with vines continuously exposed to elevated temperature during the growing season. We ask two questions: how does elevated temperature affect the reaction norms and plasticity of stomatal conductance? How does fruit load and water supply interact with temperature in affecting the reaction norms and plasticity of stomatal conductance? Manipulation of source:sink ratio and water supply are widespread viticultural practices with deep impact on vine physiology (Keller, 2010b).

2. **Methods**

2.1. Vines and site

We established two field experiments on a red brown earth at Nuriootpa (34 ^{o}S, 139 ^{o}E) in the Barossa Valley of South Australia (Dry et al., 2005; Gladstones, 2005). Experiment 1 used Shiraz (clone 1654) on Paulsen 1103 rootstock and experiment 2 used own-rooted Shiraz (clone 1654). The vines are arranged with a row by vine spacing of 3.0 x 2.25 m, cordon-trained to a single-wire trellis, have been spur pruned and managed with supplementary irrigation since planting in 2004 (double 2.2 l h^{-1} drippers per vine).

2.2. Experiment 1

We combined two thermal regimes and two fruit loads in a factorial experiment laid out in a split-plot design with three replicates. Thermal regime was assigned to main plot and fruit load to sub-plot. Each subplot comprised nine vines and measurements were taken in the three central vines of each replicate, yielding a buffer of six vines (~ 13 m) between fruit load treatments within a replicate. The temperature regimes, which were established on 18 November 2010, were: control with background ambient temperature (Fig. 1c), and elevated daytime temperature using a passive, open-top chamber (Fig. 1a). Daytime temperature was elevated up to ~ 3 ^{o}C, with smaller effects on minimum temperature (Fig. 1e). The system has the desirable property of minor effects on relative humidity during the growing season (Hall and Sadras, 2009), hence the increased vapour pressure deficit in parallel to the increase in temperature (Figure 1g). Sadras and Soar (2009) present details of the construction, performance and limitations of the heating system. Fruit loads were: thinned vines, where 2/3 of bunches were removed before veraison (6 January 2011) and untreated controls.

2.3. Experiment 2

We combined two thermal and two water regimes in a factorial experiment laid out in a split-plot design with three replicates. Temperature regime was assigned to main plot and water regime to sub-plot. Each subplot comprised nine vines; three buffer

vines separated the water treatments within a plot and measurements were constrained to the three central vines in each replicate, yielding an effective buffer of nine vines (~ 20 m) between water regimes. Temperature regimes, initiated on 7 October 2010, were: control with background ambient temperature (Fig. 1d), and elevated day/night temperature. We used an open-top heating system described by Sadras et al. (2012a) (Fig. 1b). It aims at a warming range (Figure 1f) consistent with projections for south-eastern Australia (Webb, 2006). The system has small effects on relative humidity, hence the enhancement in vapour pressure deficit associated with increased temperature (Fig. 1h). Water regimes were: controls, which were watered weekly between January 24th and March 7th, 2011 (7 weeks @ 35 l vine^{-1} week^{-1}), and water deficit which was irrigated twice on 21st and 28th February (2 weeks @ 35 l vine^{-1} week^{-1}).

2.4. Measurements in experiments 1 and 2

Temperature and relative humidity were measured continuously and recorded at 15 minute intervals using shielded sensors that were located in the centre of each replicate at mid-canopy level (TinyTag Ultra2 loggers, Hastings Dataloggers, Port Macquarie, Australia). Vapour pressure deficit was calculated as a function of temperature and relative humidity (Jones, 1992). Phenological stage in the E-L system (Coombe, 1995) was monitored weekly on three tagged vines per replicate.

Stomatal conductance was measured periodically during the growing season; six times in experiment 1 and eight times in experiment 2. Three tagged, fully expanded and well lit leaves per replicate were measured on clear days between 12.00 and 15.00 (local time) using a steady-state leaf porometer (Decagon SC1, Pullman, WA, USA).

2.5. Additional measurements in experiment 2

Experiment 2 was used to probe for causes and consequences of the putative changes in the plasticity of stomatal conductance. Thus, all the measurements described in this section were preformed on leaves of similar age and exposition as those used in the measurement of stomatal conductance.

To evaluate the magnitude and timing of stress associated with the water treatments, we monitored pre-dawn leaf water potential regularly; four fully expanded leaves per replicate were measured with a pressure bomb using the procedure of Hsiao (1990) (Table 1).

To test wether the plasticity of stomatal conductance could be related to the density and size of stomata, we sampled four leaves per treatment on March 1st, 2011. To account for the hypostomatous character of *Vitis vinifera* (Keller, 2010b), sticky-tape imprints were made from the abaxial epidermis, and stomatal density was derived from counts at six-0.125 mm^2 sectors of each leaf at a magnification of x 200. Stomatal length and width were measured from the same samples.

To evaluate the consequences of putative changes in the plasticity of stomatal conductance on leaf photosynthesis, we measured light-saturated photosynthesis periodically during the growing season using a Li-Cor 6400 system (Li-Cor

Environmental Sciences, Lincoln, Nebraska, USA). Measurements were taken at temperature and vapour pressure deficit conditions corresponding to each treatment, and saturating light (photosynthetically active radiation ≥ 2000 □m m^{-2} s^{-1}) on two leaves in two replicates per treatment. Leaf photosynthesis is proportional to the specific leaf mass, i.e. leaf mass:area ratio (Loomis and Connor, 1996). Specific leaf mass was derived from measurements of leaf area and dry weight in four leaves per replicate sampled on March 1st, 2011.

2.6. Reaction norms and plasticity

For stomatal conductance and photosynthetic rate, we: (i) calculated the environmental mean for each trait, (ii) derived reaction norms by fitting linear regressions between the value of the trait for each treatment and the environmental mean, and (iii) used the slopes of the regressions as a measure of plasticity. This approach has been widely applied to phenological development, yield, stem water potential and quality traits in annual and perennial crops (Calò et al., 1975; Finlay and Wilkinson, 1963; Lacaze et al., 2009; Peltonen-Sainio et al., 2011; Sadras and Lawson, 2011; Sadras et al., 2009; Sadras and Trentacoste, 2011; Trentacoste et al., 2011). Our null hypothesis is that the intercepts of the regressions were not different from 0 and the slopes were not different from 1, hence indicating lack of treatment effect on trait plasticity. To account for error in both *x* and *y*, we used Model II regression (Niklas, 1994).

3. Results

3.1. Effects of temperature and fruit load on stomatal conductance

Across treatments, stomatal conductance declined from 366 to 43 mmol m^{-2} s^{-1} as the vines depleted soil moisture, canopies expanded and evaporative demand increased from spring to summer (Fig. 2a). The initiation of a weekly cycle of irrigation lifted stomatal conductance to 246 mmol m^{-2} s^{-1} (Fig. 2a).

Analysis of variance indicated that stomatal conductance did not respond to the interaction between temperature and fruit load ($P = 0.37$), thus we dealt with the reaction norms for temperature and fruit load separately. The reaction norms of stomatal conductance for control and heated treatments were divergent (Fig. 2b). The intercepts were not different from zero ($P > 0.60$), and the slope was greater than 1 for heated treatment ($P < 0.020$) and smaller than 1 for the controls ($P < 0.022$). Likewise, the reaction norms for thinned and unthinned treatments diverged significantly (Fig. 2c). The intercepts were not different from zero ($P > 0.23$), and the slope was greater than 1 for unthinned controls ($P < 0.002$) and smaller than 1 for the thinned treatment ($P < 0.001$).

3.2. Effects of temperature and water regime on stomatal conductance

Across treatments, stomatal conductance declined from 630 to 56 mmol m^{-2} s^{-1} in the initial part of the growing season (Fig. 3a). After reaching its minimum, the establishment of differential irrigation treatments increased average stomatal conductance to 200-260 mmol m^{-2} s^{-1} (Fig. 3a).

Analysis of variance indicated that stomatal conductance did not respond to the interaction between temperature and water treatments ($P = 0.33$), thus we dealt with the reaction norms for temperature and water regime separately. The reaction norms of stomatal conductance for control and heated treatments were divergent (Fig. 3b). Intercepts were no different from zero ($P > 0.32$) and the slope was greater than 1 for heated treatment ($P < 0.002$) and smaller than 1 for the controls ($P < 0.0025$).

In comparison to irrigated controls, the water deficit treatment had lower pre-dawn leaf water potential during approximately four weeks (Table 1). The reaction norms of stomatal conductance for irrigated and water deficit treatments were parallel, i.e. the slopes were not different from 1 in both treatments ($P > 0.15$) and the intercepts were offset by ~ 60 mmol m^{-2} s^{-1} (Fig. 3c).

3.3. Effect of temperature on stomata density and size

Across treatments, stomata density was 159 ± 6.7 mm^{-2}, and was unaffected by temperature. Mean stomata length increased from 20.6 µm in controls to 23.2 µm in the heated treatment ($P < 0.0001$) and mean width increased from 14.4 µm in controls to 16.8 µm in the heated treatment ($P < 0.0001$) (Fig. 4). Both stomata density and dimensions of stomata were within the ranges reported for grapevine (pp. 39-40 , Keller, 2010b).

3.4. *Effects of temperature and water regime on photosynthesis and specific leaf mass*

Across treatments, light-saturated photosynthesis increased from 7.7 to 11.0 µmol $m^{-2}s^{-1}$ in response to significant rainfall and declined to 5.4 µmol m^{-2} s^{-1} with leaf aging (Fig. 5a). Independent measurements with the gas exchange system showed divergent reaction norms for light-saturated photosynthesis with temperature (Fig. 5b) that were consistent with the reaction norms for stomatal conductance measured with a steady-state porometer (Fig. 3b). Owing to significant rain towards the end of the season, reaction norms for the irrigated and water deficit treatments were statistically similar and not different from the $y = x$ line (Fig. 5c).

In mature leaves of experiment 2, the leaf mass : area ratio was reduced from 68.2 g m^{-2} in controls to 60.3 g m^{-2} under elevated temperature ($P < 0.01$) and was unaffected by water regime ($P > 0.10$) and water by temperature interaction ($P > 0.91$).

4. Discussion

4.1. Effects of elevated temperature on the plasticity of stomatal conductance

In both experiments, elevated temperature increased the phenotypic plasticity of stomatal conductance of Shiraz vines under realistic field conditions (Figures 3b and 4b). Owing to intra-specific variation in stomatal physiology, caution is needed in extrapolating this finding to other varieties (Schultz, 2003; Soar et al., 2006).

In Experiment 2, the increased plasticity of stomatal conductance was partially related to longer and wider stomata in leaves grown at high temperature (Fig. 4). Counteracting the reduction in mass per unit area typical of leaves under elevated temperature (Craufurd et al., 1999; Zhou et al., 2011), larger stomata likely contributed to the increased plasticity of light-saturated photosynthesis (Fig. 5). Likewise, previous studies in the same environment showed that both stomatal conductance and photosynthetic rate increased with transient exposition of whole vines to high temperature (Soar et al., 2009). Although the response is similar, there is a fundamental difference between our current study, where leaves were continuously exposed to elevated temperature during development, and the transient exposition to elevated temperature of mature leaves. In the conceptual model of Bresta et al. (2011), the long-term treatment elicits both structural responses which are irreversible (e.g. changes in stomata size), and transient functional responses (e.g. stomata closure). In contrast, a short-term treatment would primarily trigger transient functional responses of stomatal conductance on fully developed leaves. An important corollary from these observations is that the thermal responses of both stomata and photosynthesis derived from transient temperature treatments in fully expanded leaves (e.g.Gamon and Pearcy, 1990; Kriedemann, 1968; Soar et al., 2009) are unsuitable to model the dynamics of gas exchange in response to projected warming.

4.2. Implications for modelling

Many models of stomatal conductance in response to environmental factors, reviewed by Damour et al. (2010), have the form:

$$g_s = g_s^{\max} \cdot f(E) \qquad (1)$$

where g_s is actual stomatal conductance as a function of $g_s^{\max}$, an upper limit of conductance, which is scaled as a function of environmental factors *f (E)*. Depending on the objectives of the model, the environmental functions range from the simple assumption of a linear decline in conductance with increasing vapour pressure deficit to more complex functions accounting for vapour pressure deficit, temperature, radiation and soil or plant water status (Macfarlane et al., 2004; Monteith, 1995; Stewart, 1988). Maximum stomatal conductance is a function of stomatal density (D) and average stomatal size (S) (Weyers and Meidner, 1990). These traits are

responsive to environmental conditions during an early, critical time of leaf ontogeny, t_c (Düring, 1980; Rogiers et al., 2011):

$$g_s^{max} = f_D[E(t_c)], f_S[E(t_c)] \quad (2)$$

where $f_D[E(t_c)]$ and $f_S[E(t_c)]$ are functions relating stomatal density and stomata size to environmental conditions during leaf development. From eqs. (1) and (2):

$$g_s[t] = f_D[E(t_c)], f_S[E(t_c)], f[E(t)] \quad (3)$$

Our experiments support the conceptual model in eq. (3), which makes explicit that $g_s[t]$, the conductance at time *t*, is a function of both the prevailing environmental conditions during early stages of leaf development that set the upper limit of stomatal conductance through the modulation of stomatal density and size, and the current conditions which are most commonly emphasised in models of stomatal conductance (Damour et al., 2010). Equation (3) thus partially allows for the combined effects of previous history and current conditions. From a complementary perspective, Bresta et al. (2011) emphasised the coordination between structural (e.g. stomata density, leaf vein density) and functional regulation of gas exchange in response to long-term environmental conditions.

4.3. Interactions

Under our experimental conditions, we found no interaction between water and thermal regime on stomata conductance. The actual direction and magnitude of interactions between water and temperature is, however, likely to change with the relative intensity of both stresses, and would involve temperature-vapour pressure deficit interactions. Monteith (1995) defined three regimes for the short-term response of stomatal conductance to vapour pressure deficit, depending on how transpiration rate is affected. Furthermore the bulk air vapour pressure deficit is not directly sensed by stomata, either bulk leaf transpiration rate (Monteith, 1995), or possibly a component of this flux (Aphalo and Jarvis, 1991) are sensed instead. Owing to the non-linear relationship between saturated vapour pressure and temperature, an increase in leaf temperature at constant relative humidity sharply increases vapour pressure deficit. Such an effect would in most cases lead to stomatal closure, while the response to an increase in leaf temperature per se (under constant vapour pressure deficit at the leaf surface) is more difficult to predict (Aphalo and Jarvis, 1991; Monteith, 1995). All these short-term responses are superimposed on the longer-term effect of temperature through stomatal density and size.

In their comprehensive review with a focus on grapevine, Chaves et al. (2010) stated that "…dramatic reductions in plant carbon assimilation may occur due to severe decline in photosynthesis under supra-optimal leaf temperatures combined with water deficits…" This proposition was primarily supported by research in Mediterranean environments where temperature and water deficit increase in parallel from spring to summer, hence the interaction between thermal and water stress. None of the original sources supporting this statement, however, used factorial experiments required to quantify the interaction between water deficit and elevated temperature (Chaves et al., 2007; de Souza et al., 2003; de Souza et al., 2005; dos Santos et al., 2007; Flexas et al., 2002; Flexas et al., 1998; Maroco et al., 2002). This highlights the need for further factorial field experiments combining water and thermal regimes of varying intensities.

In addition to environmental factors, our study showed an additive effect of temperature and fruit load on stomatal conductance that needs to be accounted for in models of conductance and photosynthesis. The reduced plasticity of stomatal conductance after bunch removal (Fig. 2c) is consistent with the documented feedback inhibition of photosynthesis in vines with increased source : sink ratio (p. 139, Keller, 2010a). The reduction in stomatal conductance in response to increased source : sink ratio is expected to contribute to the maintenance of plant water status particularly under dry conditions. Consistent with this expectation, the decline in stem water potential with increasing water deficit was sharper in trees with lower source:sink ratio (Sadras and Trentacoste, 2011; Trentacoste et al., 2011).

5. Conclusion

The thermal responses of both stomata and photosynthesis derived from transient temperature treatments in fully expanded leaves are unsuitable to model the dynamics of gas exchange in response to projected warming. Our experiments support a conceptual model where current stomatal conductance is a function of the prevailing environmental conditions during early stages of leaf development that set the upper limit of conductance through the modulation of stomatal density and size, the current environmental conditions and the source:sink ratio. Our findings reinforce the key role of stomata regulation in the adaptation of Shiraz vines to elevated temperature mediated by evaporative cooling (Sadras and Soar, 2009; Soar et al., 2009).

Acknowledgements

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Table 1. Pre-dawn leaf water potential (MPa) in Shiraz grown under two water regimes at the Barossa Valley; experiment 2. Values are means ± s.e.

	Water regime		
Date	Irrigated	Water deficit	*P*[1]
26/11/10 (-56)[2]	-0.17 ± 0.022	-0.14 ± 0.026	
21/1/11 (0)	-0.79 ± 0.033	-0.80 ± 0.028	
28/1/11 (7)	-0.58 ± 0.043	-0.74 ± 0.049	*
4/2/11 (14)	-0.75 ± 0.041	-0.88 ± 0.053	
8/2/11 (18)	-0.29 ± 0.033	-0.87 ± 0.033	***
13/2/11 (23)	-0.60 ± 0.056	-0.85 ± 0.098	*
15/2/11 (25)[3]	-0.50 ± 0.027	-0.92 ± 0.058	***
18/2/11 (28)	-0.38 ± 0.016	-0.48 ± 0.037	*
21/2/11 (31)[4]	-0.24 ± 0.026	-0.30 ± 0.018	
25/2/11 (35)	-0.41 ± 0.047	-0.46 ± 0.042	

[1] *P* from analysis of variance for the effect of water treatment; * < 0.05, *** < 0.0001.

[2] Days from beginning of differential irrigation treatment.

[3] 64 mm of rainfall were recorded on 16/2/11 and 19/2/11.

[4] End of differential irrigation treatment.

Captions to figures

1. Heating systems used in (a) experiment 1 and (b) experiment 2. Both are open-top systems where the canopies are fully exposed to natural radiation conditions. In (a) heating is passive and the panels are fixed. This means there is no fine regulation of temperature, the system does not heat in overcast days or during the night, and there is no capacity to release excess heat typical of clear, still spring days (Sadras and Soar, 2009). In (b) there is a combination of passive heating and active heating with pipes circulating hot water; the mobile panels allow for release of excess heat as required (Sadras et al., 2012a). (c, d) Daily maximum (red) and minimum (blue) temperature in controls. Arrow-heads indicate time of full bloom (E-L stage 23), veraison (E-L stage 35) and harvest (E-L stage 38); closed symbols are for control and open symbols for heated treatments. (e, f) Difference in daily maximum (red) and minimum (blue) temperature between heated and control treatments. Owing to a wet spring, vigorous canopies develop that required trimming at the dates indicated by the arrows. Note the improvement in heating capacity after trimming, particularly in (e), where the system is fully dependent on passive heating. (g, h) Vapour pressure deficit in controls at 15 minute intervals. Insets compare vapour pressure deficit in control and heated treatments; red lines are y = x.

2. Stomatal conductance of Shiraz in a factorial experiment combining two temperatures and two fruit loads. (a) Time course of stomatal conductance averaged across treatments. Arrow heads show: T, implementation of fruit load treatments; I, beginning of the weekly cycle of irrigation. (b) Reaction norms of stomatal conductance for heated and control treatments. (c) Reaction norms for thinned and unthinned treatments after the implementation of fruit load treatments. In (b,c) error bars are two standard errors of the means; fitted lines are reduced major axis regressions (Model II) for each treatment, with parameters *a* (intercept) and *b* (slope). All slopes, which are a measure of plasticity, were statistically significantly different from 1 ($P < 0.05$).

3. Stomatal conductance of Shiraz in a factorial experiment combining two temperatures and two water regimes. (a) Time course of stomatal conductance averaged across treatments. Arrow head indicates initiation of differential irrigation between control and water deficit treatments. (b) Reaction norms of stomatal conductance for heated and control treatments. (c) Reaction norms for irrigated and water deficit treatments after the establishment of the water treatments. In (b,c) error bars are two standard errors of the means; fitted lines are reduced major axis regressions (Model II) for each treatment with parameters *a* (intercept) and *b* (slope). The slope, a measure of plasticity, departed from 1 in (b) but in not in (c). A sample of these data was published in Fig. 7, Agricultural and Forestry Meteorology 154– 155 (2012) 187– 194.

4. Frequency distribution of stomata length and width as affected by temperature in field-grown Shiraz grapevines.

5. Light-saturated photosynthesis of Shiraz leaves in a factorial experiment combining two temperatures and two water regimes. (a) Time course of photosynthesis. Horizontal lines indicate significant rainfall during the period of measurements (b) Reaction norms of stomatal conductance for heated and control treatments. (c) Reaction norms for irrigated and water deficit treatments. In (b,c) error bars are two standard errors of the means; fitted lines are reduced major axis regressions (Model II) with parameters *a* (intercept) and *b* (slope).

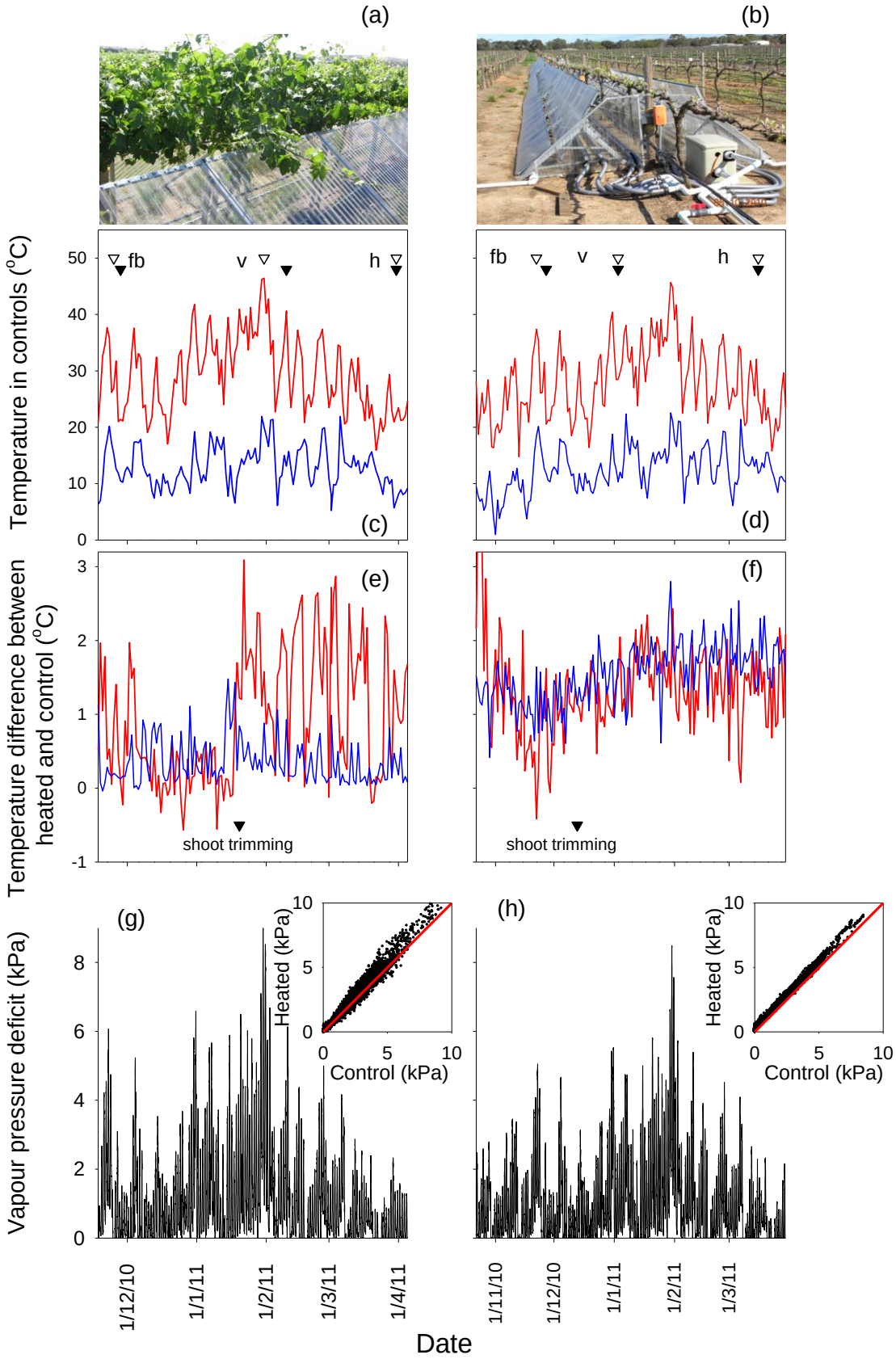

(a)
(b)
fb
v
h
(c)
(d)
Temperature in controls (°C)
(e)
(f)
shoot trimming
Temperature difference between heated and control (°C)
(g)
(h)
Heated (kPa)
Control (kPa)
Vapour pressure deficit (kPa)
1/12/10
1/1/11
1/2/11
1/3/11
1/4/11
1/11/10
1/12/10
1/1/11
1/2/11
1/3/11
Date

Fig. 11

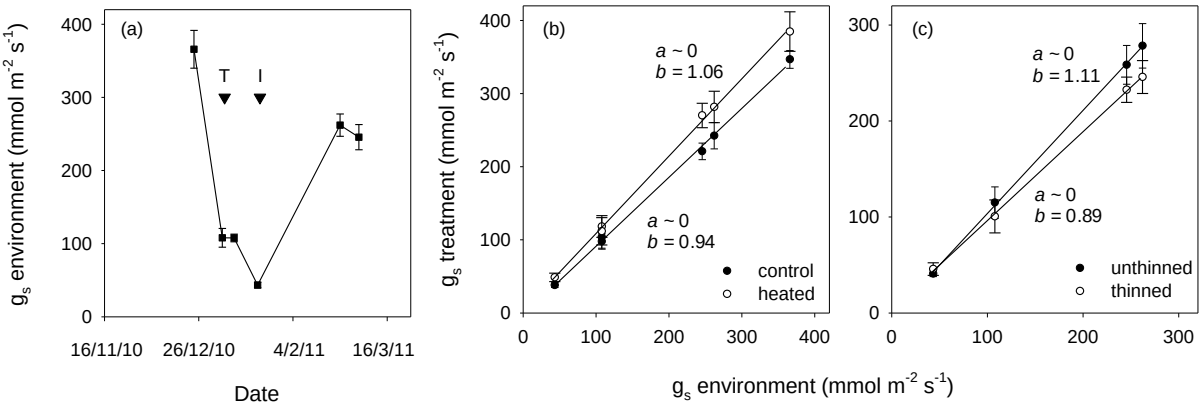

(a)
T
I
g_s environment (mmol m^{-2} s^{-1})
400
300
200
100
0
16/11/10
26/12/10
4/2/11
16/3/11
Date
(b)
g_s treatment (mmol m^{-2} s^{-1})
a ~ 0
b = 1.06
a ~ 0
b = 0.94
control
heated
0
100
200
300
400
g_s environment (mmol m^{-2} s^{-1})
(c)
a ~ 0
b = 1.11
a ~ 0
b = 0.89
unthinned
thinned
300
200
100
0

Fig. 12

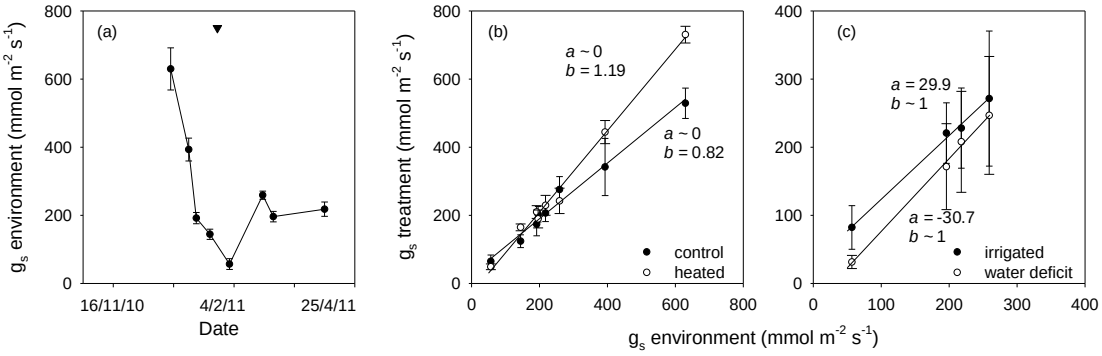

(a)
(b)
(c)
g_s environment (mmol m^{-2} s^{-1})
g_s treatment (mmol m^{-2} s^{-1})
Date
16/11/10
4/2/11
25/4/11
a ~ 0
b = 1.19
a ~ 0
b = 0.82
control
heated
a = 29.9
b ~ 1
a = -30.7
b ~ 1
irrigated
water deficit
g_s environment (mmol m^{-2} s^{-1})

Fig. 13

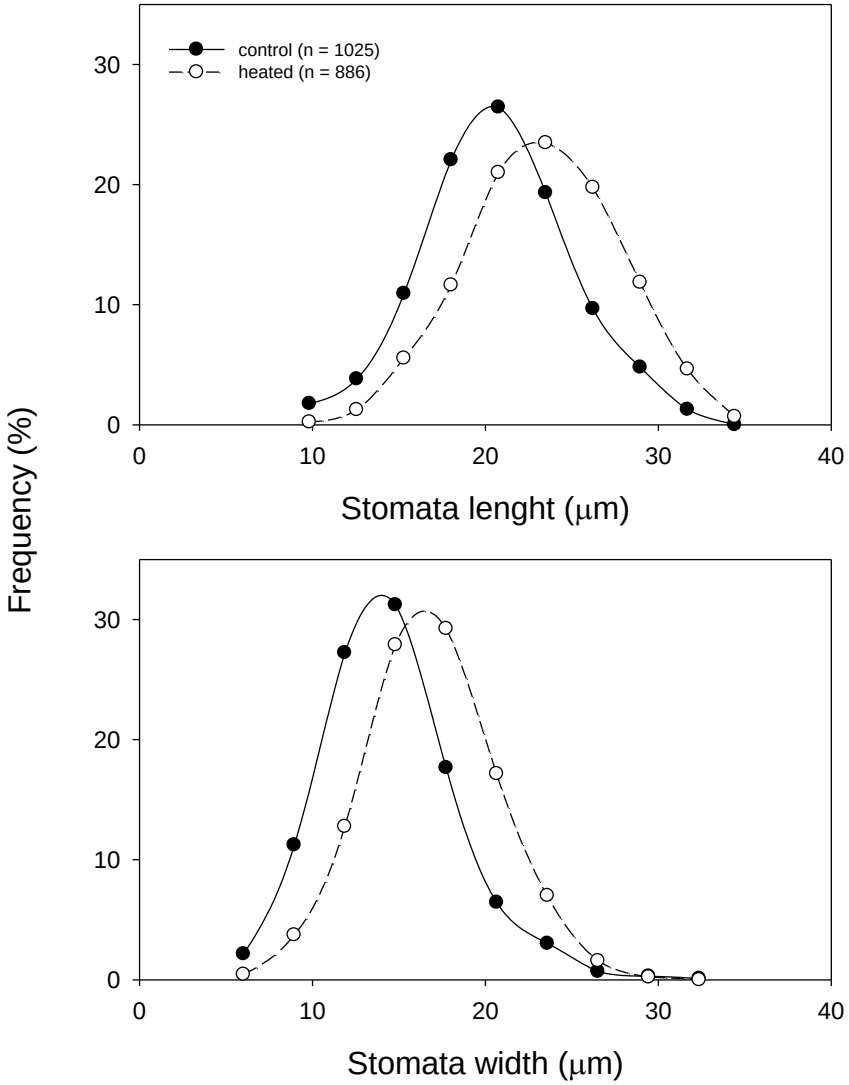

control (n = 1025)
heated (n = 886)
Frequency (%)
Stomata lenght (μm)
Stomata width (μm)
0
10
20
30
40

Fig. 14

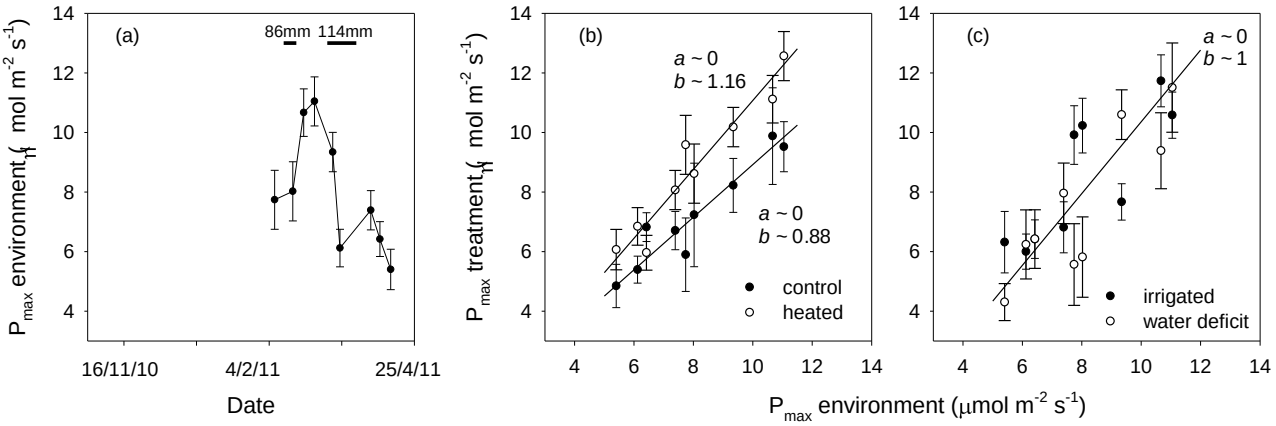

(a)
86mm 114mm
P_{max} environment (μ mol m^{-2} s^{-1})
16/11/10
4/2/11
25/4/11
Date
(b)
a ~ 0
b ~ 1.16
a ~ 0
b ~ 0.88
control
heated
P_{max} treatment (μ mol m^{-2} s^{-1})
(c)
a ~ 0
b ~ 1
irrigated
water deficit
P_{max} environment (μmol m^{-2} s^{-1})

Fig. 15

Chapter 9. Quantifying the onset, rate and duration of sugar accumulation in berries from commercial vineyards in contrasting climates of Australia

VO Sadras, PR Petrie

Abstract

To predict the trajectory of sugar accumulation in berries for planning of harvest and post-harvest operations, we derived a simple model that is consistent with the known biological and viticultural drivers of berry ripening and requires inputs that are readily available to industry.

We used data from three vintages in climatically contrasting regions of Australia to derive a relationship between total soluble solids (TSS) and thermal time. A linear-plateau function was fitted to the 90th percentile data, hence providing a boundary function representing the potential for each variety-environment combination. Biologically relevant parameters were derived including the rate of change in TSS, maximum TSS (TSS_{max}), the threshold thermal time when berries reached $TSS_{max,}$ the onset and the duration of the period of linear increase in TSS. Gaps, calculated as the difference between actual TSS and the boundary function, correlated positively with vapour pressure. A model with the observed onset of ripening as an input and a variety and site specific rate as parameter accounted for 81-92% of the variation in TSS for independent data sets.

In conclusion, the trajectory of TSS in berries can be modelled “as if” the main environmental and management sources of variation affect the onset rather than the rate of sugar accumulation. Our onset-rate model can be coupled with (i) short-term temperature forecasts to predict the trajectory of TSS for management purposes, and (ii) long-term records of temperature to produce probabilistic profiles of maturity date.

Introduction

Growth of plants or plant parts is defined as the irreversible increase in size whereas development is the continuous change in plant form and function with characteristic transition phases (Amzallag, 1999; Loomis and Connor, 1996). Growth is primarily associated with capture and allocation of resources whereas development is typically related to non-resource environmental clues such as temperature, photoperiod and light quality (Ballaré et al., 1995; Körner, 1991; Loomis and Connor, 1996; Monteith, 1994).

In grapevine, temperature-driven models capture a large part of the phenotypic variation in the timing of budburst, flowering and veraison (Baldwin, 1966; Caffarra and Eccel, 2009; Duchêne et al., 2010; Due et al., 1993; Godwin et al., 2002; Williams et al., 1985). In contrast, the morphological and biochemical transformations of the berry between veraison and maturity involve both resource- and temperature-driven processes where the limits between growth and development are blurred (Bindi et al., 2001b; Coombe, 1980; Coombe, 1992; Dry et al., 2005; Godwin et al., 2002; Sadras et al., 2008; Williams et al., 1985). Quantitative models of berry growth and development and predictive models of grapevine maturity span a wide range of detail and approaches, from empirical correlations between timing of maturity and weather variables to models with a narrower and deeper physiological focus (De Cortazar-Atauri et al., 2009; Due et al., 1993; Godwin et al., 2002; Sadras et al., 2008; Williams et al., 1985). Godwin et al. (2002) and Williams et al. (1985), for example, combined thermal and source-sink drivers to predict maturity. Dai et al. (2010) recently reviewed modelling approaches for water and carbon fluxes in grape berries.

In addition to the biological interest in berry growth and development, predicting the time-course of sugar accumulation is important for the logistics of harvest and post-harvest operations. A common approach to predict maturity for management purposes is to combine frequent measurements of berry sugar concentration and locally developed rules of thumb, e.g. berries accumulate 1 ^{o}Bé per week (Petrie unpublished).

In this paper, we outline and test a practical model of the time-course of sugar concentration in grapevine berries for management applications. We advance a novel approach to predict maturity based on boundary functions (Cade and Noon, 2003; Milne et al., 2006a; Milne et al., 2006b) and gap analysis (Casanova et al., 1999; French and Schultz, 1984; Grassini et al., 2009; Sadras and Angus, 2006). The aim is to derive a model with three attributes: it is parsimonious, i.e. has the minimum number of parameters and maximum predictive power (Landau et al., 2000), requires inputs that are readily available to industry, and is consistent with the known biological and viticultural drivers of berry growth and development.

Method

Environments and varieties

We analysed date from three vintages (2007 to 2009) in three regions: Coonawarra, a cool region with mean January temperature between 19.0 and 20.9 ^{o}C, Riverland, a hot region with mean January temperature above 23 ^{o}C, and Barossa Valley, an intermediate region with mean January temperature between 21.0 and 22.9 ^{o}C (Abbate et al., 2004). Boundary functions (see below) were developed for Shiraz, Cabernet Sauvignon, Chardonnay and Merlot in all three regions, and for Grenache, Mataro (Mourvedre), and Semillon in Barossa, and Colombard, Mataro and Muscat Gordo Blanco in Riverland. Gap analysis was performed for all varieties and regions, except for Merlot in the Barossa Valley where there were insufficient observations for this analysis.

Data source for boundary functions

Company representatives or vineyard staff collected maturity samples at approximately weekly intervals in the period leading up to harvest. Sampling was normally coordinated so that a minimum of four samples were collected prior to the block being picked. Samples were taken from both exposed and shaded fruit from both sides of the canopy across the block (Anon., 2009). Approximately 30 bunches per block were sampled to account for the typical variation of a vineyard block (Bramley, 2005; Krstic et al., 2003). Vineyard staff had an incentive to avoid sample bias because divergence between the sample maturity reading and the actual maturity of the fruit delivered to the winery may result in a financial penalty (Anon., 2009). In the Riverland, total soluble solids of fruit was measured on site by the vineyard staff. The fruit was usually macerated in the plastic bag used for collection, and a sample decanted for sugar analysis using an analogue refractometer or hydrometer; these measurements were normally not compensated for temperature with the consequent bias as quantified by Bayindirli (1993). Juice from the samples from the Barossa and Coonawarra was extracted using a roller crusher and analysed using a FOSS WineScan FT120 (FOSS, Hillerød, Denmark). The calibration of the WineScan was checked daily against a hydrometer with temperature correction.

Boundary functions

The method is described graphically in Fig. 1. For each combination of variety and region, the relationship between total soluble solids (TSS, ^{o}Bé) and thermal time (TT, ^{o}Cd) was described with the linear-plateau model:

$$TSS = a + b\,TT \quad \text{if } TT \le TTx \qquad \text{eq. 1a}$$

$TSS = a + b\ TTx$ if $TT > TTx$ eq. 1b

where *a* is the intercept and *b* is the rate of increase in TSS during the linear phase, TTx is the threshold thermal time when berries reach the maximum concentration of soluble solids, TSS_{max}. Considering that sampling for management purposes generally starts around 8 ^{o}Bé, we derived two additional parameters: the onset calculated as (8-*a*)/*b,* and the duration calculated as the difference between TTx and onset. We used a base temperature of 10 ^{o}C (Williams et al., 1985) and a starting point of December 1st for the calculation of thermal time. This base temperature was used as a convenient default, despite evidence of its variation with both variety and stage of development (Duchêne et al., 2010; Moncur et al., 1989). The starting point was selected to capture the fastest developing varieties in the hottest Riverland environment; it is arbitrary but does not affect the relevant parameters of the model (Sadras et al., 2008). Daily temperature to calculate thermal time was obtained from the Australian Bureau of Meteorology for three locations central to the target regions, i.e. Loxton (34^{o} 25' S, 140^{o} 35' E) for Riverland, Nuriootpa (34º 29'S, 139º 00'E) for Barossa Valley and Coonawarra (37º 18'S, 140º 49' E) for the Coonawarra region. To derive boundary functions using quantile regression (Fig. 1a), we segmented the data in 50 ^{o}Cd intervals, calculated the 90th percentile for TSS for each thermal time interval, and fitted equation 1 between the mid point of the thermal time interval (x) and the 90th percentile of TSS (y) (Cade and Noon, 2003; Casanova et al., 1999; Sadras, 2004).

Gap analysis

Gap analysis included two complementary approaches with a focus on meteorological drivers or the parameters of the linear-plateau model.

First, gaps were calculated as the difference between actual total soluble solids and the upper limit derived from the boundary function (vertical arrow, Fig. 1b). Gaps were segmented in 0.5 ^{o}Bé intervals and associations between the mean gap for an interval and meteorological variables were explored using regression analysis. Daily weather data were obtained from the stations described above and included maximum, minimum and average temperature, reference evapotranspiration, solar radiation, rainfall, vapour pressure, and relative humidity at maximum and minimum temperature. To account for the time interval between measurements, meteorological variables were averaged for the 5-day period previous to each measurement of soluble solids.

Second, linear-plateau or linear models when plateaus were statistically undetectable were fitted to the 75th, 50th, 25th and 10th percentiles to assess whether the gap

resulted from either a slower rate (blue line, Fig. 1b) or a delayed onset (black line, Fig. 1b).

Model evaluation

An onset-rate model to estimate the trajectory of total soluble solids in berries was tested using independent data sets from the Riverland, Sunraysia and Barossa Valley. These data sets were derived from (i) Cabernet Sauvignon during three seasons in vines exposed to five treatments combining irrigation and fruit load in the Riverland (Sadras et al., 2007), (ii) Cabernet Sauvignon in a single season at one location in each of the Riverland and Sunraysia from vines where source-sink ratios were manipulated with hand or mechanical bunch removal (Petrie and Clingeleffer, 2006), (iii) Shiraz in the Riverland during two seasons and eight irrigation regimes (McCarthy, 1997), and (iv) studies in the Barossa comparing five varieties in a single season (Sadras et al., 2008) and three varieties under two treatments: elevated daytime temperature using the method of Sadras and Soar (2009) and untreated controls (unpublished data). Estimated trajectories of total soluble solids were derived using (a) an initial observed value as input, (b) the linear rate of increase in total soluble solids for each variety and location, and (c) actual daily average temperature. Root mean square error and parameters of Model II regression were used for the statistical comparison of measured and estimated total soluble solids (Fila et al., 2003).

Results

Boundary functions

A linear-plateau boundary model provided a statistically strong fit ($0.86 \leq R^2 \leq 0.99$, $P < 0.005$) for all the varieties in the Barossa Valley (Fig. 2) and Coonawarra (Fig. 3), and for four out of seven varieties in the Riverland (Fig. 4). The break point (TTx) and plateau were statistically undetectable for Mataro, Chardonnay and Muscat Gordo Blanco in the Riverland, where linear functions fit the data with R^2 of 0.93, 0.97 and 0.93 respectively (all $P < 0.0001$). The plateau in the model is likely to have been driven by the target harvest TSS of the fruit as after the block is harvested no more samples are collected. Where no plateau was detected it is likely that harvest decision for these varieties was made to facilitate winery operations. In the case of Chardonnay this would have allowed processing to focus on red varieties and for Muscat Gordo Blanco and Mataro the end of processing for the season.

The onset, rate and duration of the increase in the concentration of soluble solids derived from the boundary function can be considered a measure of the potential for each combination of variety and region (Table 1). The average onset for Shiraz, Cabernet Sauvignon, Chardonnay and Merlot, the varieties common to all three regions, was 528 ^{o}Cd for Coonawarra, 410 ^{o}Cd for the Barossa and 312 ^{o}Cd for the

Riverland. This gradient reflects differences in background temperatures among the regions, as outlined in the section *Environments and varieties*. The rate during the linear phase was, however, very similar in all three regions, i.e. the average for the four common varieties was 0.014 ^{o}Bé ^{o}Cd^{-1} for both Barossa and Coonawarra and 0.012 ^{o}Bé ^{o}Cd^{-1} for the Riverland. In Barossa and Coonawarra, there was a strong varietal influence on the maximum concentration of soluble solids (Figures 2 and 3, Table 1). In the Barossa, maximum TTS ranged from 12.1 ^{o}Bé in Semillon to 15.5 ^{o}Bé in Shiraz. In Coonawarra, it ranged from 13.0 ^{o}Bé in Chardonnay to 15.4 ^{o}Bé in Shiraz. In the Riverland, Colombard peaked at 11.3 ^{o}Bé whereas Merlot, Shiraz, and Cabernet Sauvignon all peaked at a similar value, close to 14 ^{o}Bé. Differences in maximum concentration of soluble solids were related to the duration rather than the rate (Fig. 5).

Gap analysis

Owing to the use of the 90th percentile to derive the boundary function, about 10% of gaps were negative, as illustrated in Fig. 6. The gap was concentrated between -1 and 3 ^{o}Bé. For Cabernet Sauvignon, Shiraz and Chardonnay, for example, this interval contained 98% of the gaps in Coonawarra, and at least 85% of the gaps in Barossa Valley and Riverland (Fig. 6).

Gap analysis emphasised meteorological drivers and further consideration of the parameters of the linear-plateau model. Out of the 17 cases resulting from the combination of varieties and regions, we detected significant ($P < 0.05$) associations of the gap with relative humidity at maximum temperature in nine cases and with vapour pressure in seven cases. These were the most widespread and biologically consistent associations between gap and meteorological variables, as illustrated for Coonawarra where a common relationship between gap and vapour pressure was found for all four varieties (Fig. 7). Statistical associations were less frequent for maximum temperature, minimum temperature, rainfall, radiation, reference evapotranspiration, and relative humidity at minimum temperature. Owing to the lack of independence between meteorological variables, however, the links between the gap and meteorological variables need to be interpreted cautiously. For example, relative humidity at maximum temperature and vapour pressure were positively correlated with reference evapotranspiration, radiation and rainfall, and negatively correlated with maximum temperature. Significant correlations between gap and rainfall ($0.71 \leq r \leq 0.80$; $P < 0.05$) suggest a dilution effect associated with pulses of water but part of the association is attributable to the link between rainfall and relative humidity and vapour pressure. Likewise, negative correlations between the gap and radiation ($-0.87 \leq r \leq -0.73$, $P < 0.05$) can be interpreted in terms of the sequence high radiation → greater availability of reduced carbon mediated by current photosynthesis→ smaller gap, but part of this association is attributable to the negative association between radiation and vapour pressure.

At a given thermal time, concentration of soluble solids in berries could be below the potential outlined by the boundary function if there was a delay in the onset (black line, Fig. 1b), a reduced rate (blue line, Fig. 1b) or a combination of delayed onset and reduced rate. To assess the gaps from this perspective, we fitted boundary functions to data for the 75^{th}, 50^{th}, 25^{th} and 10^{th} percentile and derived the rate and onset for each case. Rates were largely unrelated to percentiles but there was a clear association between onset and percentile (Fig. 8).

A simple onset-rate approach to estimate the trajectory of total soluble solids

The gaps between the actual concentration of soluble solids and the upper limit established with the boundary function were closely related to shifts in onset, rather than to changes in rates (Fig. 8). We therefore propose that, for practical applications, the trajectory of sugars in berries can be predicted as a function of an observed initial value close to 8 ^{o}Bé, a site and variety specific rate, and actual temperature.

Comparison of measured and calculated concentration of soluble solids for three independent data sets from Cabernet Sauvignon and Shiraz in the hot Riverland and Sunraysia regions (Fig. 9a) returned a root mean square error = 0.96 ^{o}Bé, and a model II regression line with intercept not different from zero ($P > 0.75$) and a slope close to (1.09) but different from 1 ($P < 0.001$). The model with observed onset and fixed rate accounted for 93% of the observed variation in total soluble solids which derived from a combination of seasons, sites, varieties, source:sink ratios and irrigation regimes (McCarthy, 1997; Petrie and Clingeleffer, 2006; Sadras et al., 2007).

Comparison of measured and calculated concentration of soluble solids for an independent data set including six varieties in the Barossa Valley in 2007 and four varieties by two temperature regimes in 2010 (Fig. 9b) returned a root mean square error = 1.22 ^{o}Bé, and a model II regression line with intercept not different from zero ($P > 0.96$) and slope not different from 1 ($P > 0.30$). The model with observed onset and fixed rate accounted for 81% of the observed variation in total soluble solids which derived from a combination of seasons, varieties, and temperature regimes (Sadras et al., 2008; Sadras and Soar, 2009).

Discussion

Our current understanding of the closely linked carbon and water economies of berries (Dai et al., 2010) provides the framework for the analysis of our findings. In particular, the association between the onset of sugar accumulation in berries and the drop in turgor pressure of mesocarp cells below a threshold (Matthews et al., 2009; Thomas et al., 2008) allows for two complementary analyses.

First, relative humidity at maximum temperature and vapour pressure were the meteorological variables more closely associated with the gap between actual and

potential sugar accumulation in our study. This is consistent with the sequence: lower evaporative demand → lower transpiration rate → lower sugar accumulation (Rebucci et al., 1997). Furthermore, the functional link between berry sugar accumulation and fruit transpiration justifies the assumption that water efflux is the driving force of berry sugar import and accumulation in the model of Dreier et al. (2000). The mechanistic model of Dreier et al. (2000) and other models summarised in Dai et al. (2010) are increasingly important to summarise current knowledge and further our understanding of berry physiology. Owing to input requirements these models are unsuitable for management purposes, hence the need for a parsimonious approach (Landau et al., 2000). However the use of a simple empirical model driven by relative humidity, if this would prove robust, would also be limited as reliable forecasts of this variable are rarely available.

Second, Matthews and colleagues (Matthews et al., 2009; Thomas et al., 2008) provide a solid physiological insight on the two-fold importance of the onset of sugar accumulation: as a major driver of the actual dynamics of sugar accumulation in berries, and as a link with environmental drivers of this process. In physically constrained berries, the threshold cell turgor pressure ≈ 0.1 MPa under the experimental conditions of Matthews et al. (2009) was delayed by two weeks in relation to controls, and a similar delay was recorded for the onset of sugar accumulation. Our study supported a simple empirical approach to estimate the trajectory of sugar accumulation in berries for viticultural applications using an observed onset as input, and a variety and site specific rate calculated on thermal units. The correspondence between the findings of Matthews et al. (2009) and our onset-rate model is, however, only superficial. In the case of Matthews et al. (2009), the importance of the onset of sugar accumulation and its dependence on the achievement of a threshold turgor pressure is demonstrated directly, and has a physiological explanation. In our case, the use of an observed onset that captures the myriad of genetic, environmental and management factors that affect the dynamics of sugars in berries is only a consequence of the statistical structure of our data sets. Nonetheless, our data sets are a comprehensive sample of the main varieties in three climatically contrasting environments that ensure the robustness of our onset-rate approach.

Many studies support the notion that variation in the time to reach a target sugar concentration is primarily related to the onset of sugar accumulation, although some factors can also alter the rate. Petrie and Clingeleffer (2006) reduced bunch number and yield by 24 to 45% in thinning experiments with Cabernet Sauvignon in the hot Riverland and Sunraysia regions of Australia. Consistently with the increase in source:sink ratio in thinned vines, soluble solids at harvest increased by 1.6% in relation to controls. This difference was fully accounted for by changes in the onset, i.e. the trajectories of soluble solids in thinned and control crops were parallel (Petrie and Clingeleffer, 2006). Other studies where close-to-parallel trajectories of sugar concentration indicate a dominant effect of onset over rate includes a trial with Shiraz involving a five-fold variation in bunch:canopy ratio (Just et al., 2003) and experiments with Cabernet Sauvignon with four treatments ranging from full fruit load to 75% bunch removal (Nuzzo and Matthews, 2006). Furthermore, Fig. 7 in the experiment of Nuzzo and Matthews (2006) shows parallel lines relating date of target

sugar concentration and yield that strongly support changes in onset. Keller et al. (1998) established a factorial experiment with Cabernet Sauvignon where they dramatically varied both the supply of nitrogen and radiation intensity during ripening. Nitrogen supply, in a ten-fold range, did not alter the rate of increase in sugar concentration but high nitrogen supply delayed the onset of rapid sugar accumulation. The rate of sugar accumulation was unchanged by a reduction in radiation intensity from 100% in controls to 20%; almost complete darkness (2% radiation) was required to reduce the rate. Mobilisation of carbohydrate reserves and increased specific leaf area might contribute to the stability of rates under low irradiance (Keller et al., 1998). Other sources of variation that affect the rate of change in sugar concentration include variety (Sadras et al., 2008) and evaporative demand (Rebucci et al., 1997).

Modelling involves a trade-off between the improved predictive capacity associated with greater model complexity and increased error associated with more parameters (Passioura, 1996). Our aim in this study was to derive a simple model for practical applications. The onset-rate model developed here requires (a) an observed starting point for sugar concentration, (b) a site and cultivar specific rate on a thermal time basis, and (c) mean daily temperature. The Australian Bureau of Meteorology forecasts mean daily temperature with a lead time of five days that partially suits the predictions required for scheduling of harvest. Our method can also be used with long-term records of temperature to produce profiles of maturity date for seasons corresponding to, say, the 90th percentile warmer or cooler for a region. A limited test indicated this simple approach could account for about 80% of the observed variation in the trajectory of sugar accumulation of independent data sets. Improvement over this performance is unlikely without a significant cost in terms of complexity and additional parameters. To illustrate this trade-off, consider our approach in comparison to the model of De Cortazar-Atauri et al. (2009). They modelled the dynamics of water concentration in berries, and its complement the dynamics of sugar concentration, using a thermal (or chronological) time scale of development assuming a potential rate, and a correction for water deficit derived from additional modelling to derive canopy temperature. This approach is justified by our understanding of the links between the carbon and water economies of berries (Dai et al., 2010). The predictive capacity of their model, however, was similar to that of our simpler approach; compare Fig. 10 in De Cortazar-Atauri et al. (2009) and Fig. 9 in this paper, with the additional consideration that we used independent data for our test.

In summary, we produced boundary functions that quantify the onset, rate and duration of sugar accumulation of major grapevine varieties in three contrasting climatic regions of Australia. It is proposed that, for practical purposes, the trajectory of sugar accumulation can be modelled “as if” the main sources of variation in sugar dynamics affect the onset rather than the rate of accumulation. This assumption is consistent with some experimental observations, but in our study it is rather a reflection of the statistical structure of our data sets. Further improvements, at the expense of complexity and input requirements, could be achieved by consideration of direct influences of environmental and management sources of variation on the rate of sugar accumulation. Potential rates of increase in concentration of soluble solids

calculated in this study can also be used as a diagnostic tool – for example, where the actual sugar concentration measured in a given vineyard falls below this benchmark, growers could explore for factors such as water deficit or diseases causing faster than expected decline in canopy function.

Acknowledgements

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Table 1. Parameters of the linear + plateau model relating concentration of total soluble solids (TSS) and thermal time fitted to the 90th percentile data. Rate is the rate of increase in soluble solids up to a threshold thermal time (TTx) when the maximum (TSSmax) is reached. Two derived parameters are also shown: onset, calculated as the thermal time when TSS = 8 ^{o}Bé, and duration, calculated as TTx – onset. Threshold and plateau were undetectable for Mataro, Chardonnay and Muscat Gordo Blanco in the Riverland for which linear models provided the best fit.

Region	Variety	Rate (^{o}Bé ^{o}Cd^{-1})	TTx (^{o}Cd^{-1} from Dec 1st)	TSSmax (^{o}Bé)	Onset (^{o}Cd^{-1} from Dec 1st)	Duration (^{o}Cd^{-1})
Barossa	Semillon	0.013 ± 0.0028	769 ± 54.1	12.1 ± 0.21	453	316
	Chardonnay	0.012 ± 0.0023	735 ± 43.9	12.7 ± 0.18	334	401
	Merlot	0.015 ± 0.0030	862 ± 67.6	14.5 ± 0.34	449	412
	Cab. Sauvignon	0.013 ± 0.0021	932 ± 27.4	14.6 ± 0.13	431	501
	Mataro	0.011 ± 0.0018	1111 ± 41.1	15.0 ± 0.09	440	671
	Shiraz	0.017 ± 0.0013	883 ± 29.7	15.5 ± 0.17	424	459
	Grenache	0.015 ± 0.0037	945 ± 65.3	15.3 ± 0.23	475	470
Coonawarra	Chardonnay	0.017 ± 0.0017	857 ± 27.5	13.0 ± 0.38	564	293
	Merlot	0.013 ± 0.0012	1053 ± 34.4	15.0 ± 0.17	504	548
	Cab. Sauvignon	0.014 ± 0.0017	1014 ± 17.3	15.0 ± 0.24	548	465
	Shiraz	0.013 ± 0.0004	1066 ± 10.4	15.4 ± 0.20	495	571
Riverland	Colombard	0.015 ± 0.0010	640 ± 16.2	11.3 ± 0.17	432	207
	Merlot	0.014 ± 0.0011	775 ± 21.2	13.7 ± 0.12	342	433
	Shiraz	0.014 ± 0.0010	770 ± 19.6	14.1 ± 0.10	343	428
	Cab. Sauvignon	0.010 ± 0.0008	955 ± 19.6	14.1 ± 0.07	313	643
	Mataro	0.009 ± 0.0007			339	
	Chardonnay	0.011 ± 0.0004			248	
	Musc. Gordo Blanco	0.010 ± 0.0011			467	

Captions to figures

1. (a) Method to derive boundary functions and (b) key parameters. In (a), actual data (open circles) are split in 50 ^{o}Cd intervals (vertical lines). For each of these intervals, the 90th percentile is determined (closed circles) and the boundary function is fitted (red line). In (b) increase in concentration of total soluble solids proceeds at a steady rate (slope of thick red line) during a period between the onset and threshold thermal time (TTx) when maximum total soluble solids (TSS_{max}) is reached. The open circle represents grapes with either a slower rate (blue line) or a delayed onset (black line) and associated gap (vertical arrow). Slower rate and delayed onset are not mutually exclusive.
2. Total soluble solids as a function of thermal time for seven grapevine varieties in commercial vineyards in the Barossa Valley in 2007, 2008 and 2009. Linear + plateau boundary models (red lines, eq. 1) were fitted to the 90th percentile of the data.
3. Total soluble solids as a function of thermal time for seven grapevine varieties in commercial vineyards in the Coonawarra in 2007, 2008 and 2009. Linear + plateau boundary models (red lines, eq. 1) were fitted to the 90th percentile of the data. Parameters of the model are in Table 1.
4. Total soluble solids as a function of thermal time for seven grapevine varieties in commercial vineyards in the Riverland in 2007, 2008 and 2009. Linear + plateau boundary models (red lines, eq. 1) were fitted to the 90th percentile of the data except for Mataro, Chardonnay and Muscat Gordo Blanco for which the break point and plateau were statistically undetectable. Parameters of the model are in Table 1.
5. Relationship between maximum total soluble solids and rate and duration of the linear phase of increase. All three variables were derived from boundary functions fitted to the 90th percentile of the data, hence represent a measure of their potential.
6. Frequency distribution of gaps in total soluble solids for Cabernet Sauvignon, Shiraz and Chardonnay in the Coonawarra, Barossa Valley and Riverland. Gaps are the difference between actual total soluble solids and the potential estimated with boundary functions.
7. Relationship between the gap in total soluble solids and vapour pressure at Coonawarra. The gap is the difference between actual total soluble solids and the upper limit established with the boundary function for the 90th percentile.
8. Rate and onset of accumulation of soluble solids in grapevine berries for the 90th, 75th, 50th, 25th and 10th percentile of the data. Cultivars are Cabernet sauvignon (closed circle), Chardonnay (open circle), Grenache (closed triangle), Mataro (square), Semillon (open triangle), Shiraz (inverted triange).
9. Comparison of measured and calculated total soluble solids for independent data sets from the Riverland and Barossa Valley.

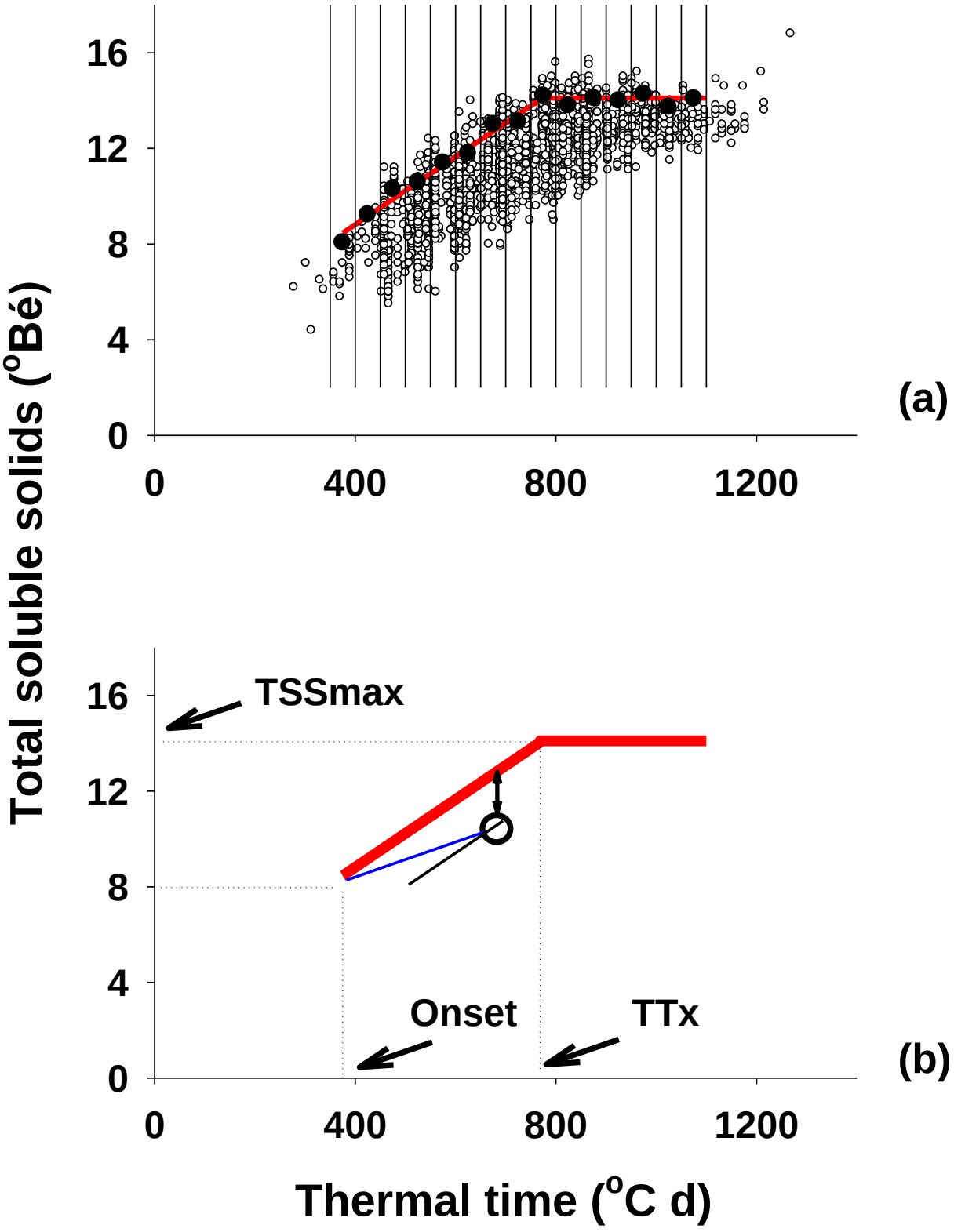

Total soluble solids (°Bé)
16
12
8
4
0
0
400
800
1200
(a)
TSSmax
Onset
TTx
(b)
Thermal time (°C d)

Fig. 1

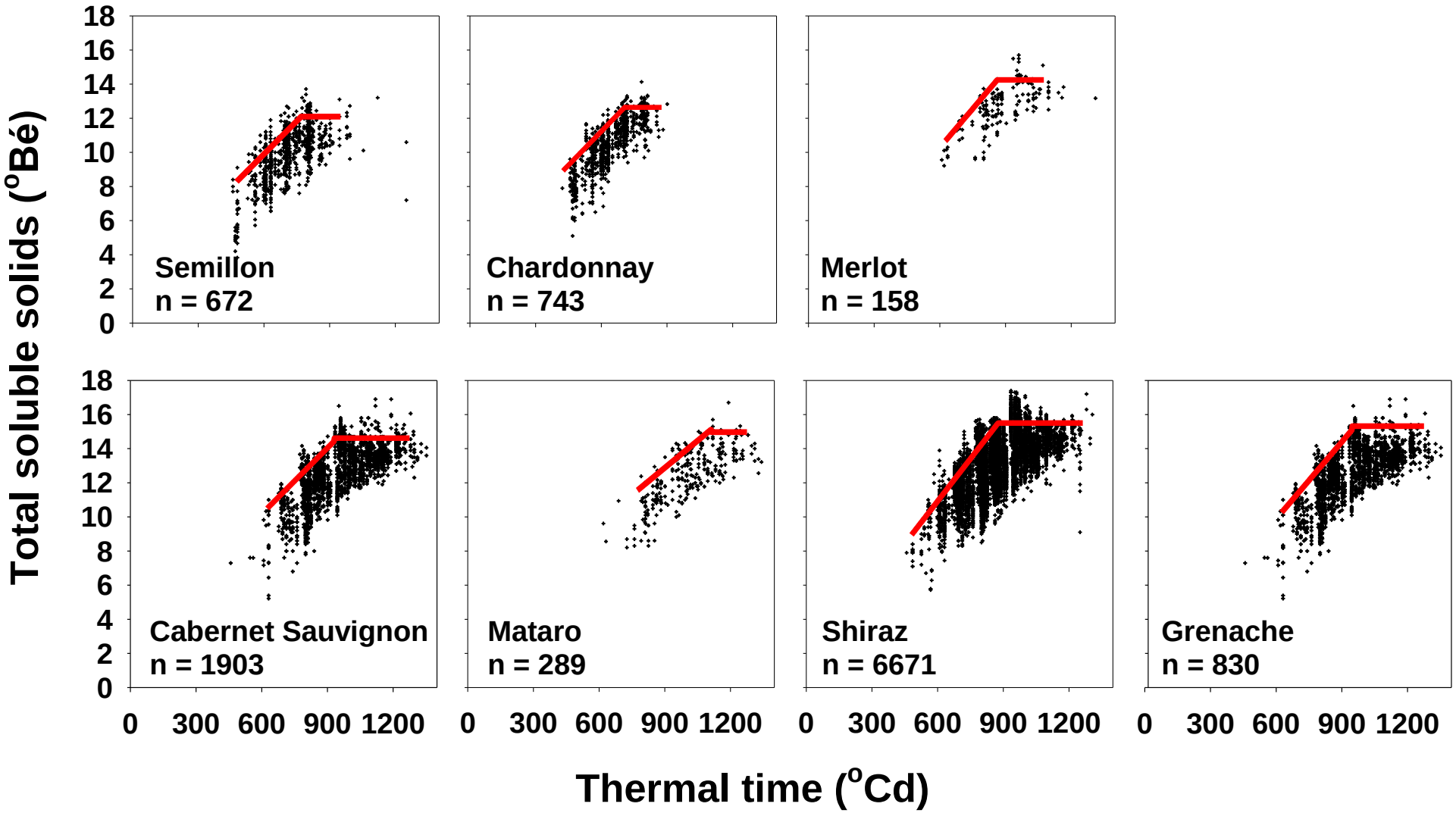

Total soluble solids (°Bé)
Thermal time (°Cd)
Semillon
n = 672
Chardonnay
n = 743
Merlot
n = 158
Cabernet Sauvignon
n = 1903
Mataro
n = 289
Shiraz
n = 6671
Grenache
n = 830
0
2
4
6
8
10
12
14
16
18
0
300
600
900
1200

Fig. 2

Fig. 3

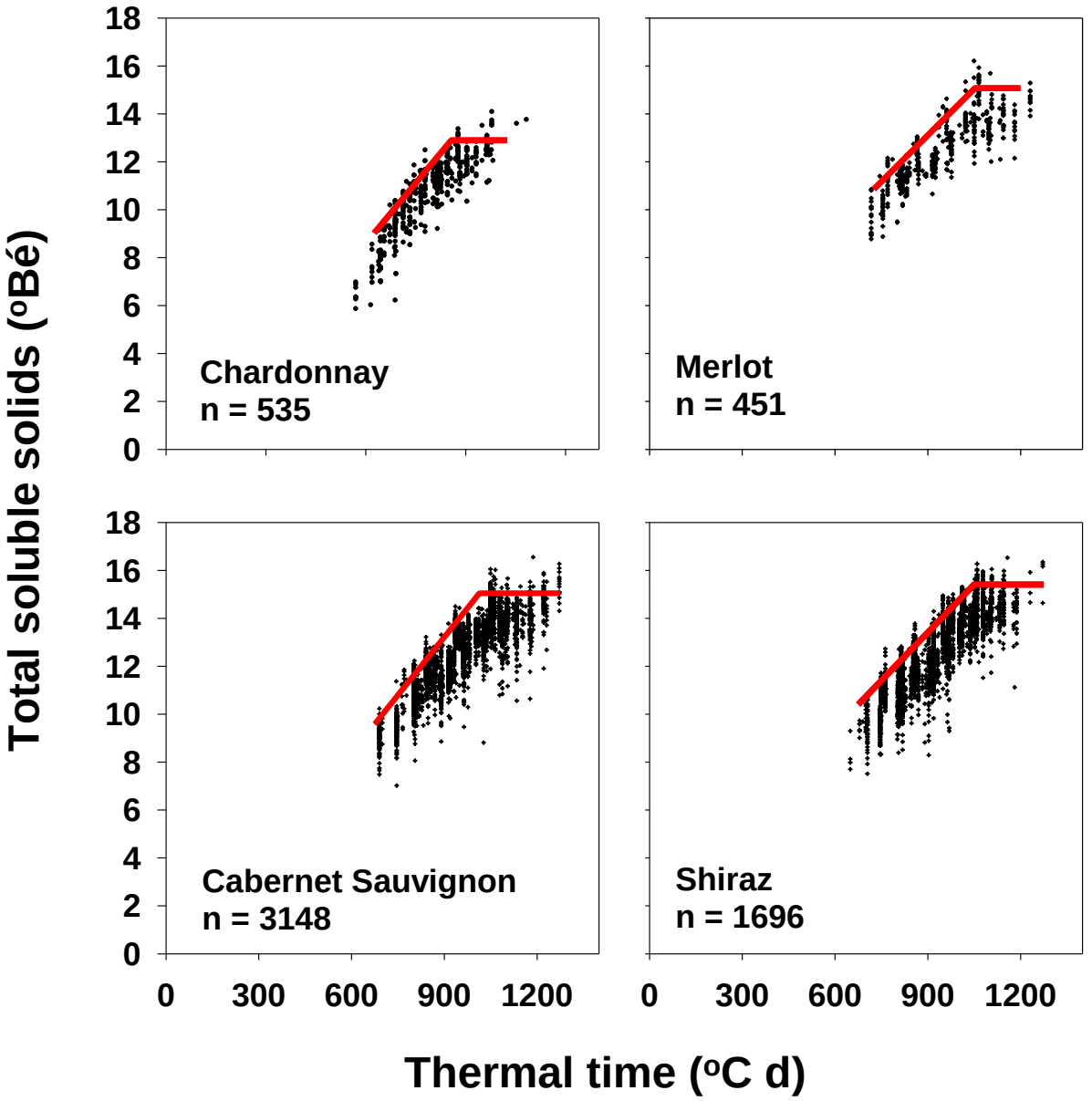

Total soluble solids (ºBé)
Thermal time (ºC d)
Chardonnay
n = 535
Merlot
n = 451
Cabernet Sauvignon
n = 3148
Shiraz
n = 1696
0
2
4
6
8
10
12
14
16
18
0
300
600
900
1200

Fig. 4

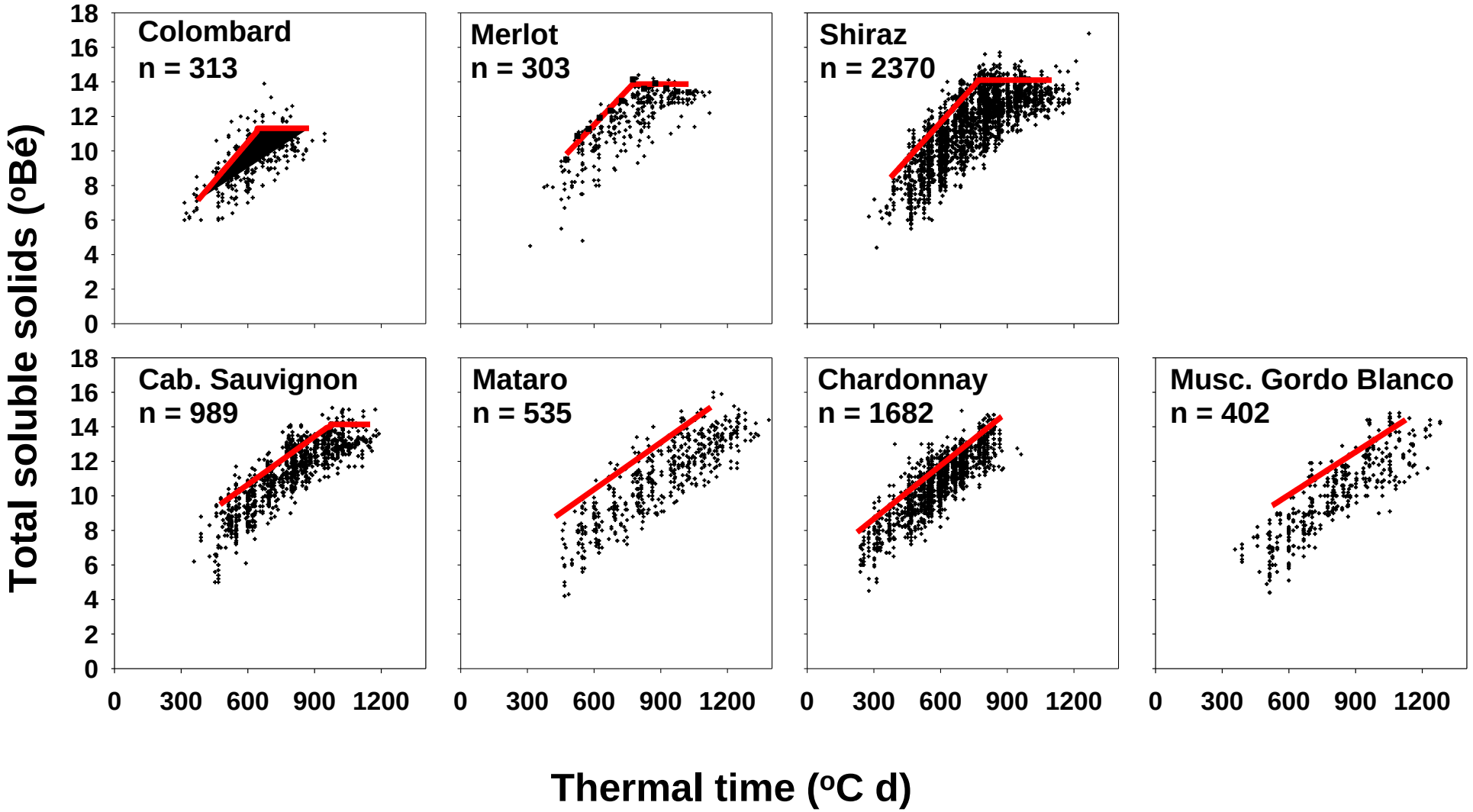
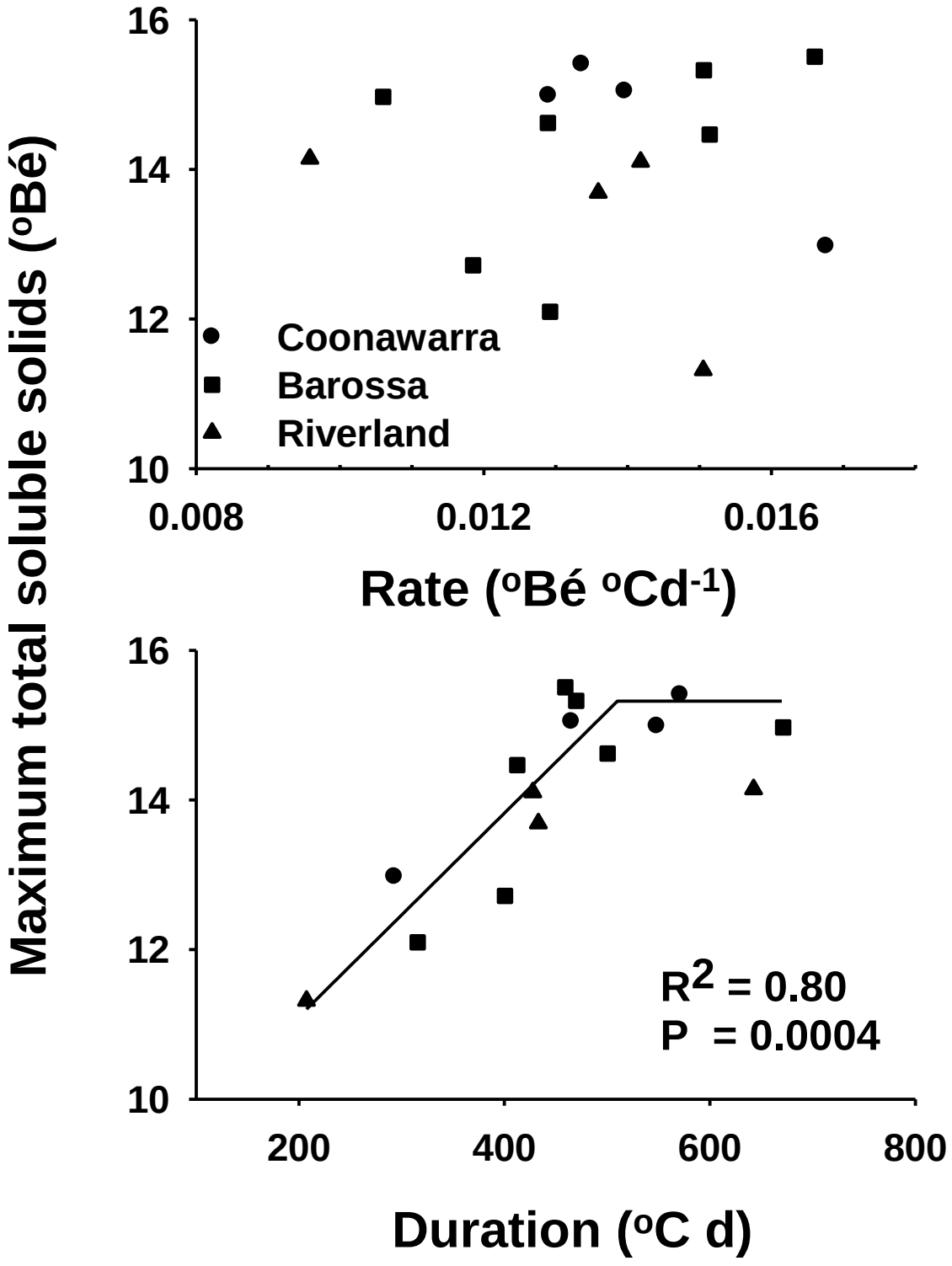

Colombard
n = 313
Merlot
n = 303
Shiraz
n = 2370
Cab. Sauvignon
n = 989
Mataro
n = 535
Chardonnay
n = 1682
Musc. Gordo Blanco
n = 402
Total soluble solids (ºBé)
Thermal time (ºC d)
0
2
4
6
8
10
12
14
16
18
0
300
600
900
1200

Fig. 5


Maximum total soluble solids (°Bé)
16
14
12
10
Coonawarra
Barossa
Riverland
0.008
0.012
0.016
Rate (°Bé °Cd-1)
16
14
12
10
R2 = 0.80
P = 0.0004
200
400
600
800
Duration (°C d)

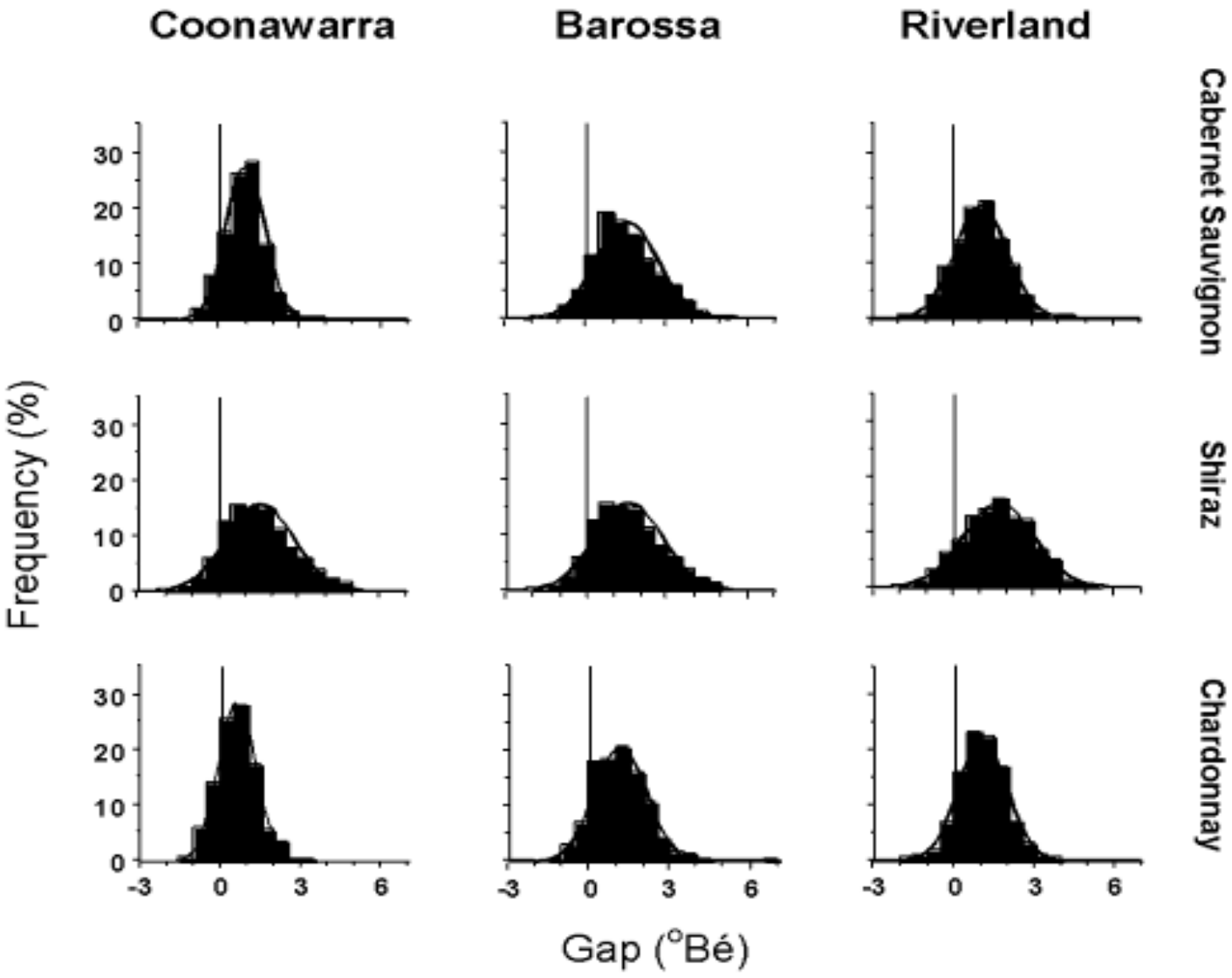

Coonawarra
Barossa
Riverland
Cabernet Sauvignon
Shiraz
Chardonnay
Frequency (%)
30
20
10
0
-3
0
3
6
Gap (°Bé)

Fig. 6

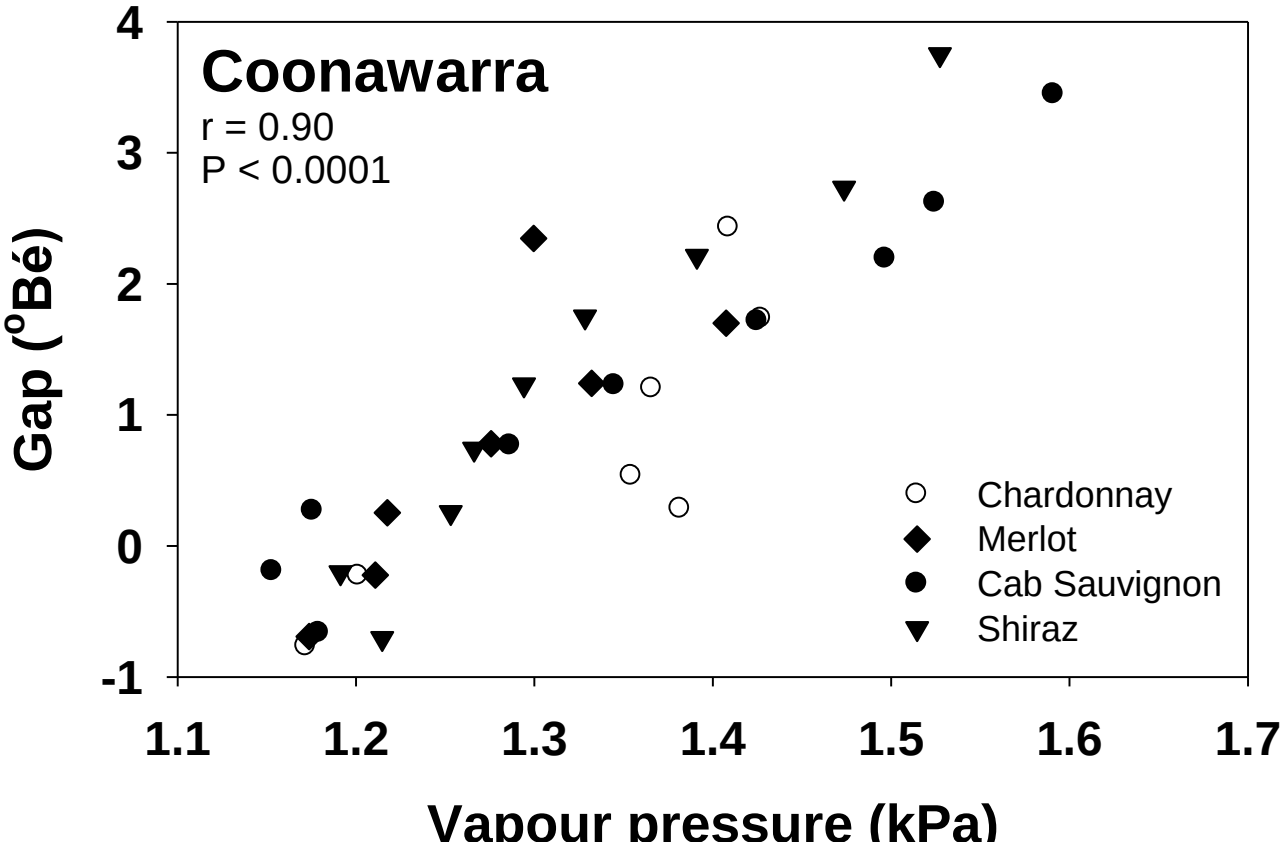

Coonawarra
r = 0.90
P < 0.0001
Gap (°Bé)
4
3
2
1
0
-1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Vapour pressure (kPa)
Chardonnay
Merlot
Cab Sauvignon
Shiraz

Fig. 7

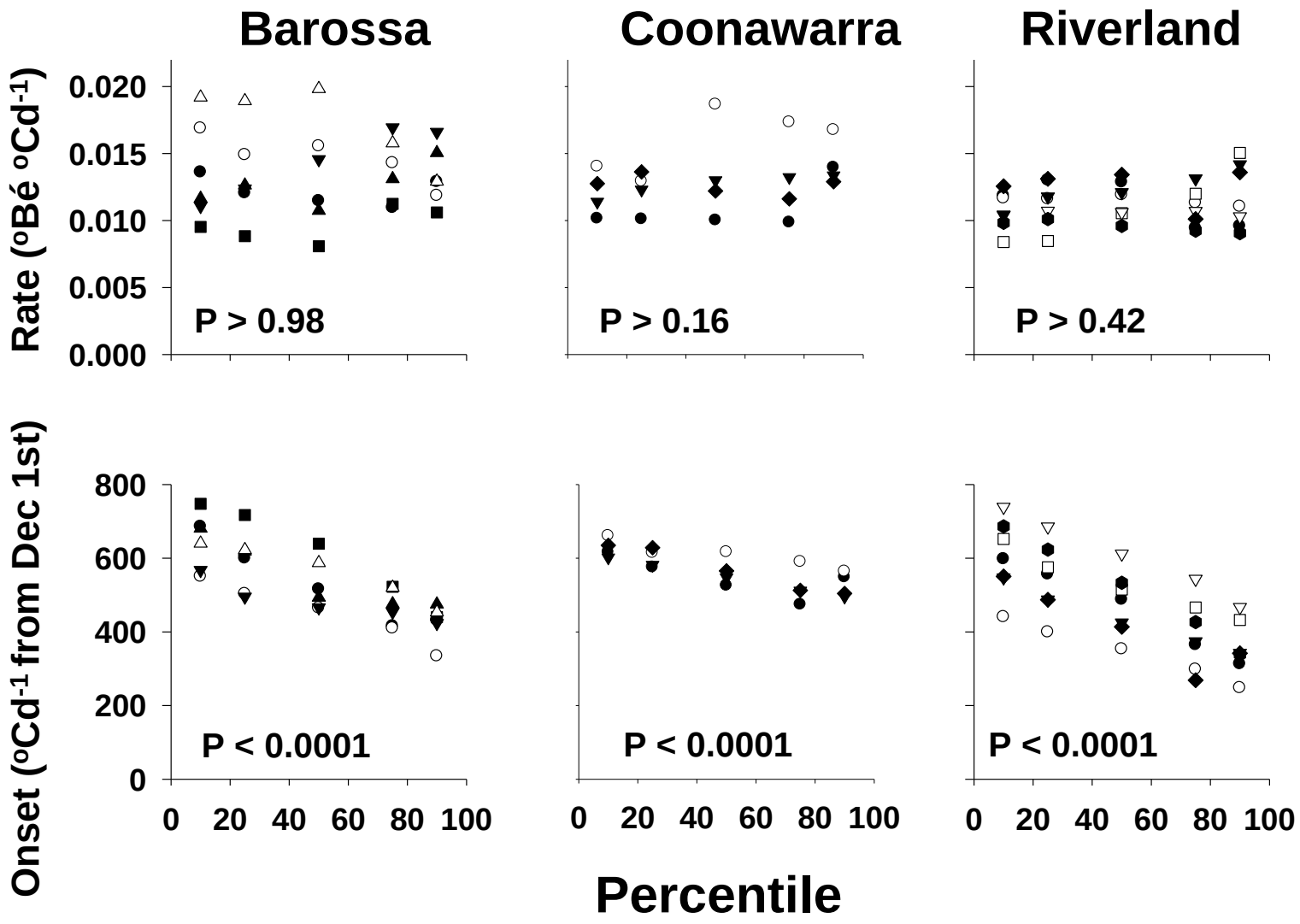
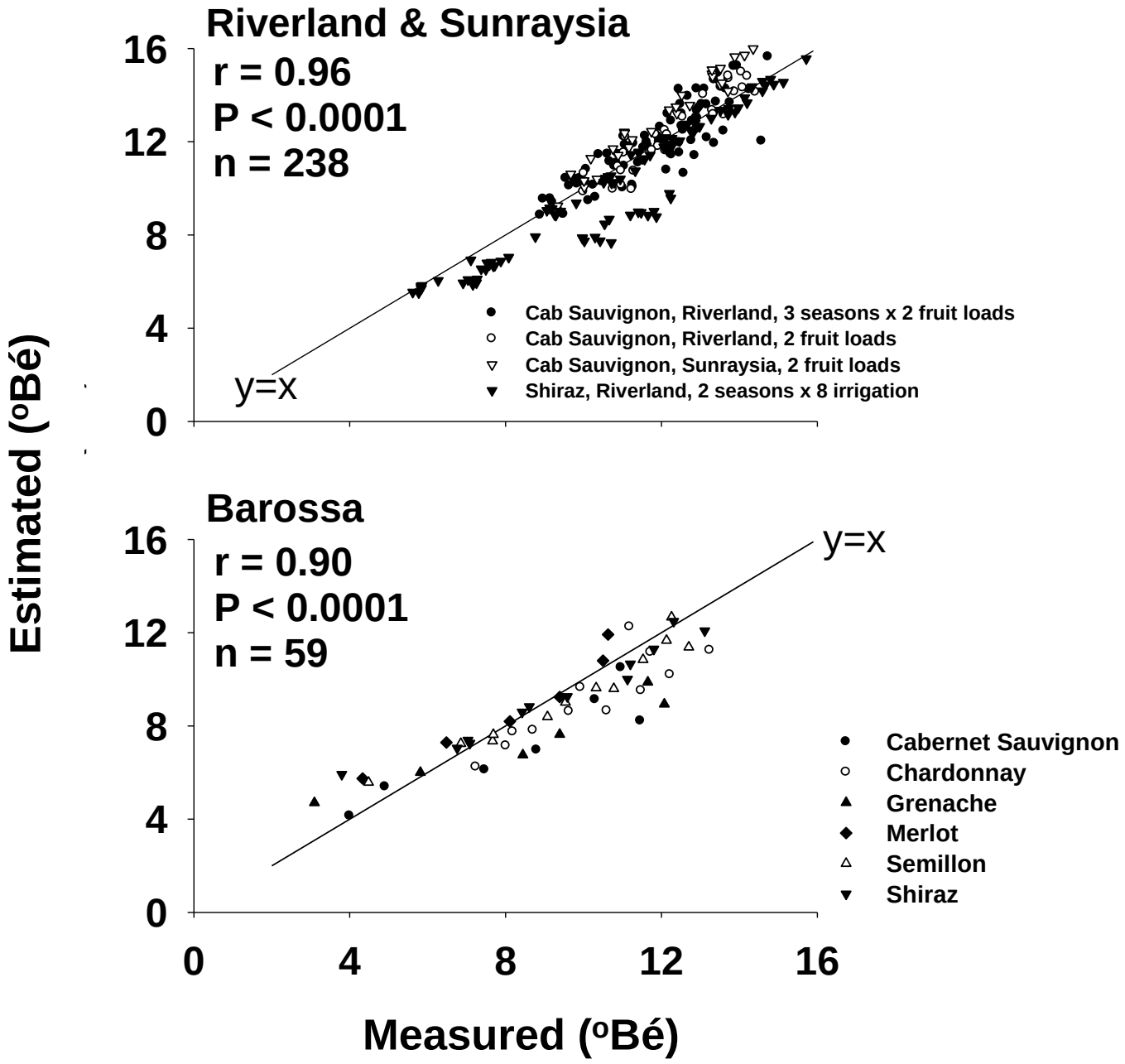

Barossa
Coonawarra
Riverland
Rate (°Bé °Cd-1)
0.020
0.015
0.010
0.005
0.000
P > 0.98
P > 0.16
P > 0.42
Onset (°Cd-1 from Dec 1st)
800
600
400
200
0
P < 0.0001
P < 0.0001
P < 0.0001
0 20 40 60 80 100
0 20 40 60 80 100
0 20 40 60 80 100
Percentile

Fig. 8

Fig. 9


Riverland & Sunraysia
r = 0.96
P < 0.0001
n = 238
y=x
Cab Sauvignon, Riverland, 3 seasons x 2 fruit loads
Cab Sauvignon, Riverland, 2 fruit loads
Cab Sauvignon, Sunraysia, 2 fruit loads
Shiraz, Riverland, 2 seasons x 8 irrigation
Barossa
r = 0.90
P < 0.0001
n = 59
y=x
Cabernet Sauvignon
Chardonnay
Grenache
Merlot
Semillon
Shiraz
0
4
8
12
16
Estimated (°Bé)
Measured (°Bé)

Chapter 10. Climate shifts in south-eastern Australia: early maturity of Chardonnay, Shiraz, and Cabernet Sauvignon is associated with early onset rather than faster ripening

VO Sadras, PR Petrie

Abstract

Early maturity of grapevine associated with recent warming is well documented but poorly understood. Here we ask the question: is early maturity associated with early onset of ripening or with increased rate of sugar accumulation?

We used records of total soluble solids (TSS) for Chardonnay, Shiraz and Cabernet Sauvignon collected in commercial vineyards between 1995 and 2009. The combination of 15 vintages and three climatic regions yielded a range of average seasonal temperature between 15.5 and 20.5 ^{o}C. Using boundary functions of TTS vs time, we derived the onset and rate of increase in TSS, and the time of maturity set at 12 ^{o}Bé for comparative purposes. Pooled across varieties, the rate of change in maturity was -9.8 ± 0.94 $^{o}Cd^{-1}$. Shifts in onset accounted for 86% of the variation in time of maturity, and rate of ripening for the remaining variation.

In conclusion, early maturity associated with higher temperature (and related factors including higher radiation and higher vapour pressure deficit) is primarily driven by early onset of ripening under a wide range of production systems in south-eastern Australia. Viticultural practices aiming at delaying maturity to counteract the effect of high temperature, high radiation and high vapour pressure deficit are more likely to be successful if they target the onset of ripening.

Introduction

The shift in phenological development of plants and poikilotherm animals is the most conspicuous biological effect of recent warming (Dell et al., 2005; Doi and Katano, 2008; Edwards and Richardson, 2004; Hays et al., 2005; Menzel et al., 2006; Moza and Bhatnagar, 2005; Sparks et al., 2005). Associated with warming trends over the last few decades, early maturity of grapevines has been reported for Europe, North America and Australia (Duchêne et al., 2010; Duchêne and Schneider, 2005; Etien et al., 2009; Jones and Davis, 2000; Petrie and Sadras, 2008b; Wolfe et al., 2005).

From viticultural and oenological perspectives, temperature-driven phenological shifts in grapevine may lead to increased potential alcohol and decoupling of berry traits with consequences for wine balance (Duchêne and Schneider, 2005; Jones et al., 2005; Petrie and Sadras, 2008b). From a biological perspective, the understanding, prediction and management of berry ripening requires consideration of both temperature-driven development and resource-driven growth (Dai et al., 2010; Sadras and Petrie, 2011b).

The trajectory of sugar accumulation in berries after veraison conforms to a sigmoidal pattern with characteristic lag-phase, maximum rate, duration and peak concentration (Sadras et al., 2008). For other biological processes with similar dynamics, chiefly grain growth in annual crops, the sigmoidal pattern is often simplified with a linear + plateau model which allows for estimates of onset, rate and duration of grain fill, and peak weight (Borrás and Otegui, 2001; Calderini et al., 1999; Rondanini et al., 2007; Sadras and Egli, 2008). This framework has proven useful to model the seasonal dynamics of sugar in grapevine berries grown under contrasting climates of south-eastern Australia (Sadras and Petrie, 2011b). Against this framework, in this paper we ask whether the observed shift in maturity of grapevine associated with increasing ambient temperature is related to an early onset, a higher rate of increase in sugar concentration, or a combination of early onset and higher rate. This question was not asked before (Duchêne et al., 2010; Duchêne and Schneider, 2005; Etien et al., 2009; Jones and Davis, 2000; Petrie and Sadras, 2008b; Wolfe et al., 2005). However, identifying the dominant component of this shift is important for management practices to counteract early maturity and some of its undesirable consequences for fruit composition. For example in Europe, defoliation to reduce canopy-to-fruit ratio is being considered as a means to slow ripening in response to warming (Stoll et al., 2010). Within viticultural ranges, source:sink ratio and resource availability seem to alter the onset more than the rate of sugar accumulation in berries (Keller et al., 1998; Nuzzo and Matthews, 2006; Petrie and Clingeleffer, 2006).

The aim of this study is to analyse the shifts in maturity of Chardonnay, Shiraz, and Cabernet Sauvignon grown under a combination of spatial and temporal sources of variation yielding a 5 ^{o}C range in mean temperature during the growing season. Our focus is on the underlying components of early maturity, i.e. early onset and higher rate of increase in sugar concentration.

Method

Varieties and climates

Our focus is on Chardonnay, Shiraz, and Cabernet Sauvignon which collectively accounted for 60% of the 2010 Australian vintage (www.wineaustralia.com).

To probe a wide range of climates, we combined 15 successive vintages since 1995 and three climatically contrasting regions including the Coonawarra as representative of cool regions, Riverland as representative of hot regions, and Barossa Valley as an intermediate region (Dry et al., 2005). Daily climate records were obtained from the Australian Bureau of Meteorology for three locations central to these regions, i.e. Coonawarra (37º 18'S, 140º 49' E), Loxton (34^{o} 25' S, 140^{o} 35' E) and Nuriootpa (34º 29'S, 139º 00'E), respectively. Data included maximum, minimum and average temperature, reference evapotranspiration, solar radiation, rainfall, and vapour pressure deficit at maximum and minimum temperature. Climate data were averaged for a vintage season from September to April (Coonawarra) or September to March (Barossa, Riverland). These averages were analysed in two complementary ways to provide a background climatic description for our assessment of grapevine ripening. First, we calculated time trends using linear regression. This analysis provides a coarse indication of trends in an otherwise short time series (15 years), rather than a critical assessment of climate shifts that would require both longer time series and more refined statistics (Viola et al., 2010). Second, we calculated the averages for each climatic variable for the 5 coolest and 5 hottest vintages in the 15-year series.

Total soluble solids

We described the approaches used in sampling and measurement of total soluble solids elsewhere (Petrie and Sadras, 2008b; Sadras and Petrie, 2011b). Briefly, company representatives or vineyard staff collected maturity samples at approximately weekly intervals yielding a minimum of four samples per block prior to harvest. Approximately 30 bunches per block from both exposed and shaded crop sections were sampled to account for the typical variation of a vineyard. Vineyard staff had an incentive to avoid sample bias because divergence between the sample maturity reading and the actual maturity of the fruit delivered to the winery may result in a financial penalty (Anon., 2009). In the Riverland, total soluble solids of fruit was measured on site. The fruit was usually macerated in the plastic bag used for collection, and a sample decanted for sugar analysis using an analogue refractometer or hydrometer; these measurements were normally not compensated for temperature with the consequent bias as quantified by Bayindirli (1993). Juice from the samples from the Barossa and Coonawarra was extracted using a roller crusher and analysed using a FOSS WineScan FT120 (FOSS, Hillerød, Denmark). The calibration of the WineScan was checked daily against a hydrometer with temperature correction. The number of observations ranged from 2395 for Chardonnay in Coonawarra to 24,196 for Shiraz in Barossa.

Onset and rate of increase in total soluble solids

We estimated onset and rate of increase in total soluble solids and other biologically and viticulturally relevant parameters using the approach of Sadras and Petrie (2011b) with small modifications. Briefly, we (i) plotted total soluble solids (TSS, ^{o}Bé) as a function of time *t* (DOY, day of year), (ii) segmented the data in 7 d intervals, (iii) calculated the 90th percentile of TSS for each time interval, and (iv) fitted equation 1 between the mid point of the time interval and the 90th percentile of TSS:

$TSS = a + b\,t$ if $t \leq tx$ eq. 1a

$TSS = a + b\,tx$ if $t > tx$ eq. 1b

This boundary function provides an upper envelope for the data set (Casanova et al., 1999; Sadras, 2004) with parameters *a* = intercept, *b* = rate of increase in TSS during the linear phase, and *tx* = threshold time when berries reach the maximum recorded concentration of soluble solids, TSS_{max}. We emphasise "recorded" in the definition of TSS_{max} because winemaking criteria determine sugar concentrations for harvest that are not necessarily biological maxima. Considering that sampling for management purposes generally starts around 8 ^{o}Bé, we derived two additional parameters: the onset calculated as (8-*a*)/*b,* and the duration calculated as the difference between *tx* and onset. Using eq. 1, we also derived the date of nominal maturity which was set at 12 ^{o}Bé for comparative purposes, as in Petrie and Sadras (2008b).

Results

Climate shifts and comparison of cool and hot vintages within regions

Fig. 1 shows the time trends of climatic variables between 1995 and 2009. This time series analysis is necessary as background for our assessment of grapevine ripening, but obviously insufficient to draw any conclusions on climate change. In all three regions maximum temperature increased at a rate ≈ 0.1 ^{o}C y^{-1}, minimum temperature had no detectable trend, and average temperature increased at 0.06 to 0.08 ^{o}C y^{-1}. Solar radiation increased in Coonawarra and Riverland, and reference evapotranspiration increased in Riverland. Vapour pressure deficit at maximum temperature increased in all three regions, and vapour pressure deficit at minimum temperature increased at Coonawarra and Riverland.

The difference in maximum temperature between the five coolest and five hottest vintages was ≈ 1.7 ^{o}C and the difference in minimum temperature ranged from 1 ^{o}C in Coonawarra to 1.5 ^{o}C in Riverland (Table 1). Hotter vintages had slightly greater evaporative demand, and markedly higher vapour pressure deficit (Table 1). The range of climatic factors explored in this study is primarily given by the difference between the coolest vintages in Coonawarra and the hottest vintages in Riverland, e.g. 6.5 ^{o}C maximum temperature, 3.6 ^{o}C minimum temperature, 5 ^{o}C average

temperature, 2.9 mm d^{-1} potential evapotranspiration, 4.0 MJ m^{-2} d^{-1} solar radiation, and 1.4 kP vapour pressure deficit at maximum temperature (Table 1). The range in vapour pressure deficit corresponds with a ~ 15% range of relative humidity. The 95 mm range in rainfall is large but is likely buffered by the intensive use of irrigation in the Riverland (Dry et al., 2005).

Ripening traits and their associations with climate

The boundary functions fitted to the 90th percentile data were statistically strong ($0.84 \leq r^2 \leq 0.98$) and represent the dynamics of total soluble solids under conditions conducive to fast ripening for each combination of variety and environment (Figures 2-4). Scatter plots and fitted boundary functions highlight the marked shift in the onset and the relative stability of rates in comparisons between cool and hot seasons for each variety (Figures 2-4).

The onset of the linear increase in total soluble solids varied 5-fold, from 19 December (DOY -12) for Chardonnay in hot vintages at the Riverland to 11 February (DOY 42) for Cabernet Sauvignon in cool vintages in the Coonawarra. The onset was closely associated with temperature. Pooling the data across varieties, vintage types and regions, the onset changed at a rate of -8.7 d ^{o}C (Fig. 5a). The association between onset and average temperature was significant for each individual variety and for the pooled data (Table 2).

The rate of increase in total soluble solids varied 2-fold, from 0.10 ^{o}Bé d^{-1} for Chardonnay in the Riverland to 0.19 ^{o}Bé d^{-1} for Shiraz in hot vintages in the Barossa. For the pooled data, the rate was unrelated to average temperature (Fig. 5b). There were some cases of increased rate with increasing temperature. For example, the rate for Shiraz increased from 0.12 ± 0.010 ^{o}Bé d^{-1} in cool vintages to 0.19 ± 0.039 ^{o}Bé d^{-1} in hot vintages in the Barossa Valley, and from 0.11 ± 0.008 ^{o}Bé d^{-1} to 0.17 ± 0.018 ^{o}Bé d^{-1} in Riverland. However, the response of the rate to temperature was inconsistent (Fig. 5b), and a significant association was only found for Cabernet Sauvignon (Table 2). For the pooled data, the rate was directly related to vapour pressure deficit (inset Fig 5b).

The threshold time when fruit reached the maximum recorded concentration of soluble solids advanced at an average rate of 10.7 d $^{o}C^{-1}$ (Fig. 5c, Table 2). The maximum recorded concentration of soluble solids ranged between 12.7 and 15.6 ^{o}Bé and was unrelated to temperature (Fig. 5d, Table 2).

The duration of the linear increase in total soluble solids varied 2-fold, from 30 days for Cabernet Sauvignon in hot vintages in the Riverland to 60 days for Shiraz in cool vintages in the Coonawarra. The duration was unrelated to temperature for the pooled data (Fig. 5e) and was weakly shortened with increasing temperature for Shiraz and Cabernet Sauvignon (Table 2).

The date of nominal maturity set at 12 ^{o}Bé for comparative purposes ranged from 11 January (DOY 24) to 9 March (DOY 81) and was the variable more closely related to temperature (Fig. 5f, Table 2). Averaged across varieties, vintage type and region, maturity was advanced at a rate of 9.8 d $^{o}C^{-1}$.

Maturity as a function of onset and rate of ripening

For the pooled data, the onset and rate of ripening were unrelated ($P > 0.67$). The lack of association between these variables was also verified with data split by variety ($P > 0.11$), region ($P > 0.23$) and vintage type, i.e. cool vs hot ($P > 0.41$). The independence of onset and rate for our data set allowed for the assessment of their individual contribution to changes in maturity in the climates under study. Onset accounted for by 86% of the variation in maturity, and rate accounted for 21% (Fig. 6). The rate accounted for 95% of the residual variation in time of maturity unaccounted for by onset (inset Fig. 6).

Discussion

Using boundary functions, we derived a rate of change in grapevine maturity between - 8.6 and -10.7 d $^{o}C^{-1}$ (Table 2, Fig. 5f). This range brackets the rate of -9.3 ± 2.67 d $^{o}C^{-1}$ derived with a direct regression approach using a partially common data set (Petrie and Sadras, 2008b). The agreement between the two approaches supports the conclusion that boundary functions returned realistic traits associated with the ripening of grapevine in response to climate drivers. Indeed, boundary functions were useful to extract meaningful information from highly scattered data sets resulting from the many sources of variation operating under vineyard conditions (Figures 2-4). Previous studies of grapevine phenological shifts associated with warming in Europe and North America report rates in days per year (Duchêne and Schneider, 2005; Jones and Davis, 2000; Wolfe et al., 2005), rather than days per ^{o}C required for comparisons when the rate of change in temperature is different between regions.

We showed that the time of maturity of grapevines can be analysed in terms of onset and rate of ripening, and that this approach can capture differences between thermal regimes. Two considerations are necessary in relation to the combination of regions and seasons to generate a range of thermal conditions. First, a single relationship described the association between average temperature and a number of important traits including onset of ripening, time when berries reach their maximum recorded concentration of soluble solids and time of maturity (Fig. 5). This means a consistent climatic effect irrespective of whether it changes with space or time. Second, when we consider vintage-to-vintage variation and medium term climate shifts, “warming” is an oversimplification of a syndrome that typically involves variation in other climatic variables (Fig. 1, Table 1). For example, onset of ripening was negatively associated with average temperature (Fig. 5a), vapour pressure ($P < 0.02$) and positively associated with reference evapotranspiration, vapour pressure deficit and radiation (all $P < 0.0001$). The associations between ripening traits and temperature are

therefore a convenient way to capture climate influences, but we need caution in establishing cause-effect relationships. Nonetheless, our understanding of temperature-driven development of grapevines (Moncur et al., 1989) justifies the conclusion that the differences in ripening traits were partially associated with differences in temperature in space and time. Likewise, faster development associated with high radiation and high vapour pressure deficit were consistent with known physiological processes of production and transport of assimilates to berries (Matthews et al., 2009; Rebucci et al., 1997; Thomas et al., 2008). Separating the individual effects of temperature and related variables such as radiation requires direct manipulation of these variables as illustrated in the studies of Tarara et al. (2000; 2008b).

The early maturity of grapevines associated with recent warming is well documented but the underlying mechanisms are poorly understood. We showed that onset of ripening accounted for 86% of the variation in maturity, and rate for the remaining variation (Fig. 6). This represents an important advancement in our understanding of climate effects on grapevine maturity with important viticultural and biological corollaries. First, however, we need to frame this finding in the context of our work – the dominant influence of the onset of ripening on grapevine maturity is valid for Chardonnay, Cabernet Sauvignon and Shiraz in a range of mean seasonal temperature from 15.5 to 20.5 ^{o}C, and for soils and management practices associated with fully irrigated, high yield production systems in the Riverland to the complementary irrigation systems seeking high quality fruit in Barossa and Coonawarra (Dry et al., 2005; Maschmedt, 2004). Departures in one or more of these environmental and management conditions might shift the relative importance of onset and rate in driving the time of maturity. From a viticultural viewpoint, manipulation of time of maturity is more likely to be effective by implementation of practices that act upon the onset of ripening. From a biological viewpoint, our finding is consistent with many physiological studies showing that manipulation of microclimate, source:sink relationships and resource availability all tend to alter the onset more strongly than the rate of increase in sugar concentration. Matthews et al. (2009) provide strong evidence for the high responsiveness of the onset of berry ripening to environmental factors. In physically constrained berries, the threshold cell turgor pressure ≈ 0.1 MPa associated with veraison under their experimental conditions was delayed by two weeks in relation to controls, and a similar delay was recorded for the onset of sugar accumulation (Matthews et al., 2009). Petrie and Clingeleffer (2006) reduced bunch number and yield by 24 to 45% in thinning experiments with Cabernet Sauvignon. Consistently with the increase in source:sink ratio in thinned vines, soluble solids at harvest increased by 1.6 ^{o}Brix in relation to controls. This difference was fully accounted for by changes in the onset, i.e. the trajectories of soluble solids in thinned and control crops were parallel (Petrie and Clingeleffer, 2006). Wolf et al. (2003) and Nuzzo and Matthews (2006) also reported large shifts in onset compared to changes in rates of Shiraz and Cabernet Sauvignon in response to manipulation of source:sink ratio. Keller et al. (1998) also found the onset of ripening of Cabernet Sauvignon was more responsive to the supply of nitrogen and radiation intensity than the rate of sugar accumulation, except in the more extreme treatments (2% radiation). Mobilisation of carbohydrate reserves and increased specific leaf area might contribute to the stability of rates under low

irradiance (Keller et al., 1998) and hydraulic isolation of berries post-veraison may contribute further to stable rates (Thomas et al., 2006). Sources of variation that affect the rate of change in sugar concentration include variety (Sadras et al., 2008) and evaporative demand (Rebucci et al., 1997).

In summary, we have shown that shifts in the onset of ripening, rather than faster accumulation of solutes, are the more likely driver of early maturity of grapevines in response to recent warming in south-eastern Australia. This finding is consistent with known physiological responses of ripening to climate, source:sink manipulation and resource availability. Viticultural practices aiming at delayed maturity to counteract the effect of increased temperature - and associated high radiation and high vapour pressure deficit – are more likely to be successful if they target the onset of ripening.

Acknowledgements

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Table 1 Climatic comparison of cool and hot vintages in Coonawarra, Barossa and Riverland. Values are averages for the five coolest and five hottest vintages between 1995 and 2009. Variables are maximum temperature (Tmax, ^{o}C), minimum temperature (Tmin, ^{o}C), average temperature (Tav, ^{o}C), solar radiation (Rad, MJ m^{-2}), reference evaporation (Evap, mm), vapour pressure deficit at maximum temperature (VPDmax, kPa), vapour pressure deficit at minimum temperature (VPDmin), rainfall (Rain, mm). All variables are daily, except for rainfall that is accumulated over the vintage window between September 1st and April 30th (Coonawarra) or September 1st and March 31st (Barossa, Riverland). Range is the difference (absolute value) between the coolest vintages in Coonawarra and the hottest vintages in Riverland.

Region	Vintage type	Tmax	Tmin	Tav	Peak Tmax	Evap	Rad	VPDmax	VPDmim	Rain
Coonawarra	Cool	22.2	8.6	15.4	40.5	5.0	18.7	1.66	0.10	252
	Hot	24.0	9.6	16.8	41.7	5.5	19.3	1.99	0.16	319
	P	0.001	0.001	0.001	0.300	0.058	0.110	0.005	0.001	0.094
Barossa	Cool	24.2	10.5	17.3	40.8	6.5	21.6	2.15	0.27	270
	Hot	26.0	11.8	18.9	41.8	7.4	22.0	2.55	0.42	214
	P	0.006	0.001	0.001	0.196	0.020	0.249	0.007	0.001	0.185
Riverland	Cool	27.0	10.7	18.8	42.0	7.1	22.2	2.64	0.24	158
	Hot	28.7	12.2	20.5	43.6	7.9	22.7	3.04	0.36	157
	P	0.001	0.001	0.001	0.168	0.008	0.167	0.003	0.006	0.959
Range		6.5	3.6	5.0	3.1	2.9	4.0	1.38	0.25	95

Table 2. Slope of linear regressions between daily average temperature during the vintage period (x) and parameters derived from boundary functions (y) including: the onset of the linear period of increase in total soluble solids (TSS); the rate of increase in TSS; *tx*, the threshold time when berries reached the maximum recorded concentration of soluble solids; TSSmax, the maximum recorded concentration of soluble solids; the duration of the period of linear increase in TSS, and the time when berries reached 12 ^{o}Bé. * P<0.1, ** P<0.05, *** P<0.01, ns P > 0.1.

Variety	**Onset** (d $^{o}C^{-1}$)	**Rate** (^{o}Bé d^{-1} $^{o}C^{-1}$)	***Tx*** (d $^{o}C^{-1}$)	**TSSmax** (^{o}Bé $^{o}C^{-1}$)	**Duration** (d $^{o}C^{-1}$)	**Time of 12 ^{o}Bé** (d $^{o}C^{-1}$)
Chardonnay	-10.9 ± 1.46**	Ns	-7.6 ± 1.79*	ns	ns	-8.6 ± 1.09***
Shiraz	-7.6 ± 1.41**	Ns	-12.7 ± 2.16**	ns	-5.1 ± 1.95*	-10.7 ± 2.11***
Cab Sauvignon	-7.5 ± 1.54**	0.012 ± 0.0041**	-11.6 ± 1.55**	ns	-4.1 ± 1.56*	-10.1 ± 1.58***
All	-8.7 ± 1.54***	Ns	-10.7 ± 1.36***	ns	ns	-9.8 ± 0.94***

Captions to figures

1. Time trends in climate variables at Coonawarra, Barossa Valley and Riverland. Variables are maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tav), solar radiation (Rad), reference evaporation (Evap), vapour pressure deficit at maximum temperature (VPDmax) and vapour pressure deficit at minimum temperature (VPDmin). Each point is the average of daily values for a vintage window between September 1st and April 30th (Coonawarra) or September 1st and March 31st (Barossa, Riverland). Regression lines are shown when the slope is significant at $P < 0.05$ (solid) or $P < 0.10$ (dashed).
2. Seasonal dynamics of berry total soluble solids for Chardonnay, Shiraz and Cabernet Sauvignon in the five coolest (COOL) and five hottest (HOT) vintages between 1995 and 2009 in Coonawarra. The red line is the linear-plateau model fitted to the 90th percentile.
3. Seasonal dynamics of berry total soluble solids for Chardonnay, Shiraz and Cabernet Sauvignon in the five coolest (COOL) and five hottest (HOT) vintages between 1995 and 2009 in Barossa. The red line is the linear-plateau model fitted to the 90th percentile.
4. Seasonal dynamics of berry total soluble solids for Chardonnay, Shiraz and Cabernet Sauvignon in the five coolest (COOL) and five hottest (HOT) vintages between 1995 and 2009 in Riverland. The red line is the linear-plateau model fitted to the 90th percentile.
5. Relationships between average vintage temperature and (a) onset of linear increase in total soluble solids, (b) rate of increase in concentration of soluble solids, (c) *tx*, threshold time when total soluble solids reach its maximum, (d) maximum total soluble solids, (e) time to 12 ^{o}Bé, a nominal measure of maturity, (f) duration of linear stage of increase in total soluble solids. Inset in (b) is the relationship between rate of increase in concentration of soluble solids and vapour pressure deficit at minimum temperature. Data derived from samples collected from Chardonnay, Shiraz and Cabernet Sauvignon vines in a broad range of thermal environments resulting from the combination of three regions and thermally contrasting vintages between 1995 and 2009.
6. Relationship between time of nominal maturity (12 ^{o}Bé) and onset and rate of linear increase in total soluble solids in fruit of Chardonnay, Shiraz and Cabernet Sauvignon in a broad range of thermal environments resulting from the combination of three regions and thermally contrasting vintages between 1995 and 2009. Inset is the relationship between the residuals of the regression between time to 12 ^{o}Bé and onset vs rate.

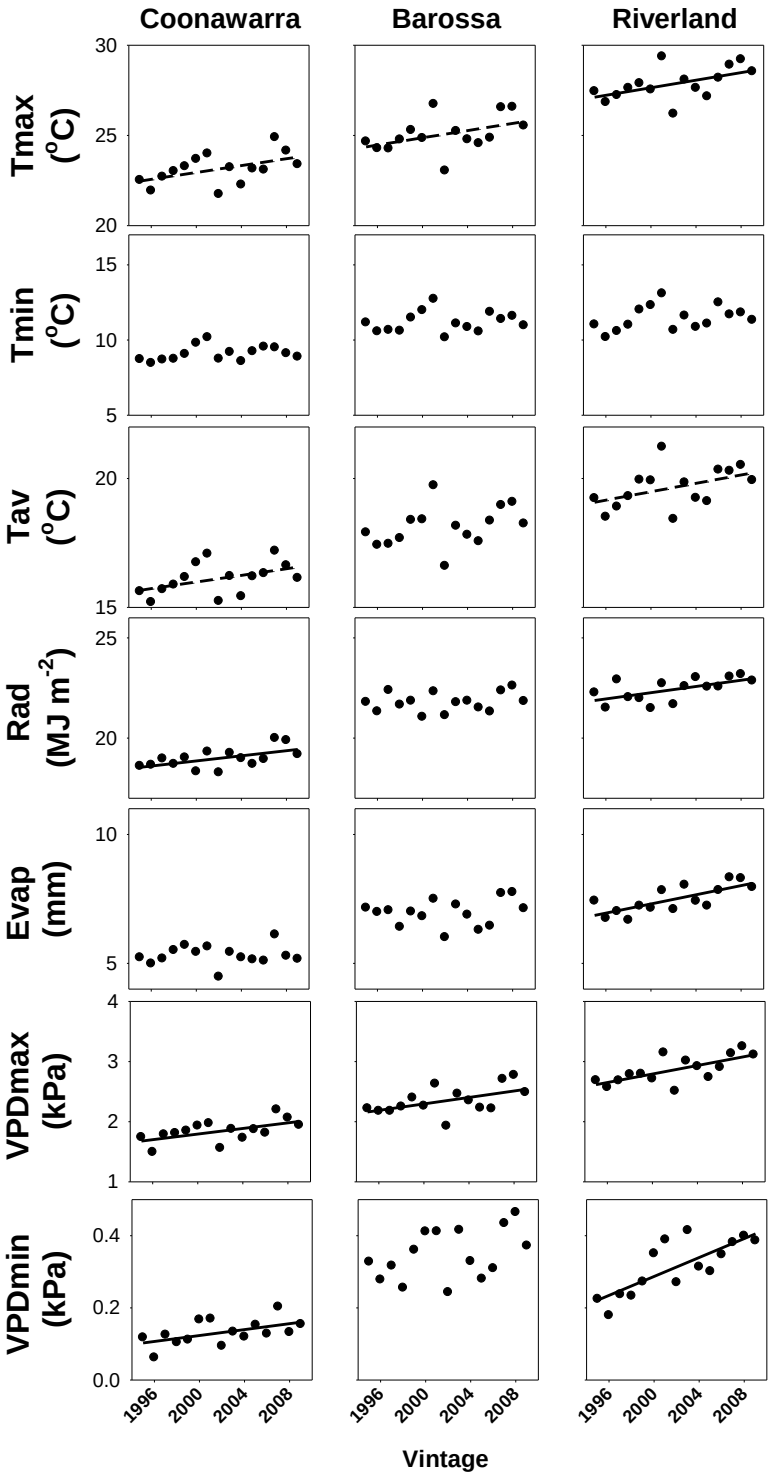

Coonawarra
Barossa
Riverland
Tmax (°C)
Tmin (°C)
Tav (°C)
Rad (MJ m-2)
Evap (mm)
VPDmax (kPa)
VPDmin (kPa)
30
25
20
15
10
5
20
15
25
20
10
5
4
3
2
1
0.4
0.2
0.0
1996
2000
2004
2008
Vintage

Fig. 1

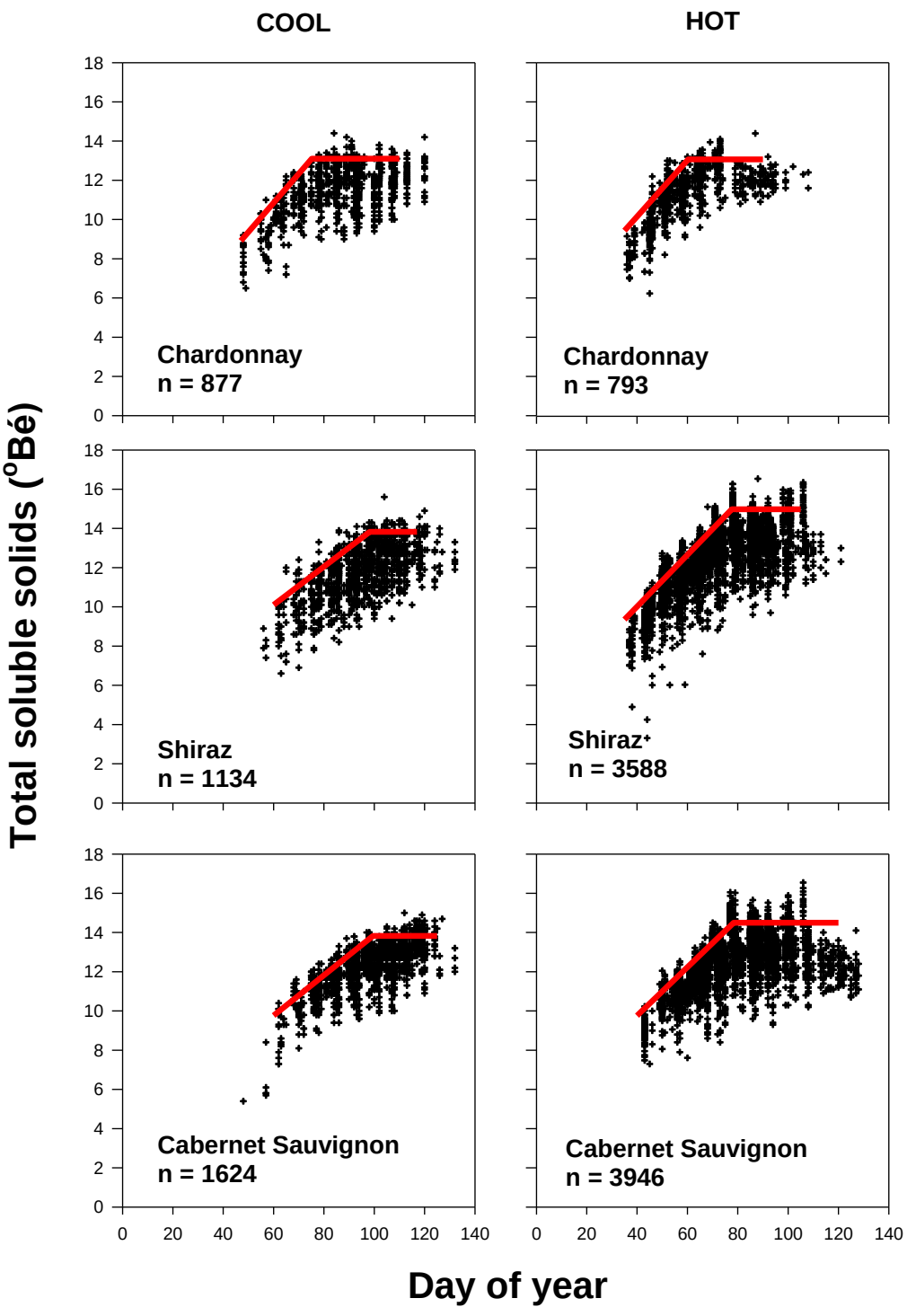

COOL
HOT
Total soluble solids (°Bé)
Chardonnay
n = 877
Chardonnay
n = 793
Shiraz
n = 1134
Shiraz
n = 3588
Cabernet Sauvignon
n = 1624
Cabernet Sauvignon
n = 3946
Day of year

Fig. 2

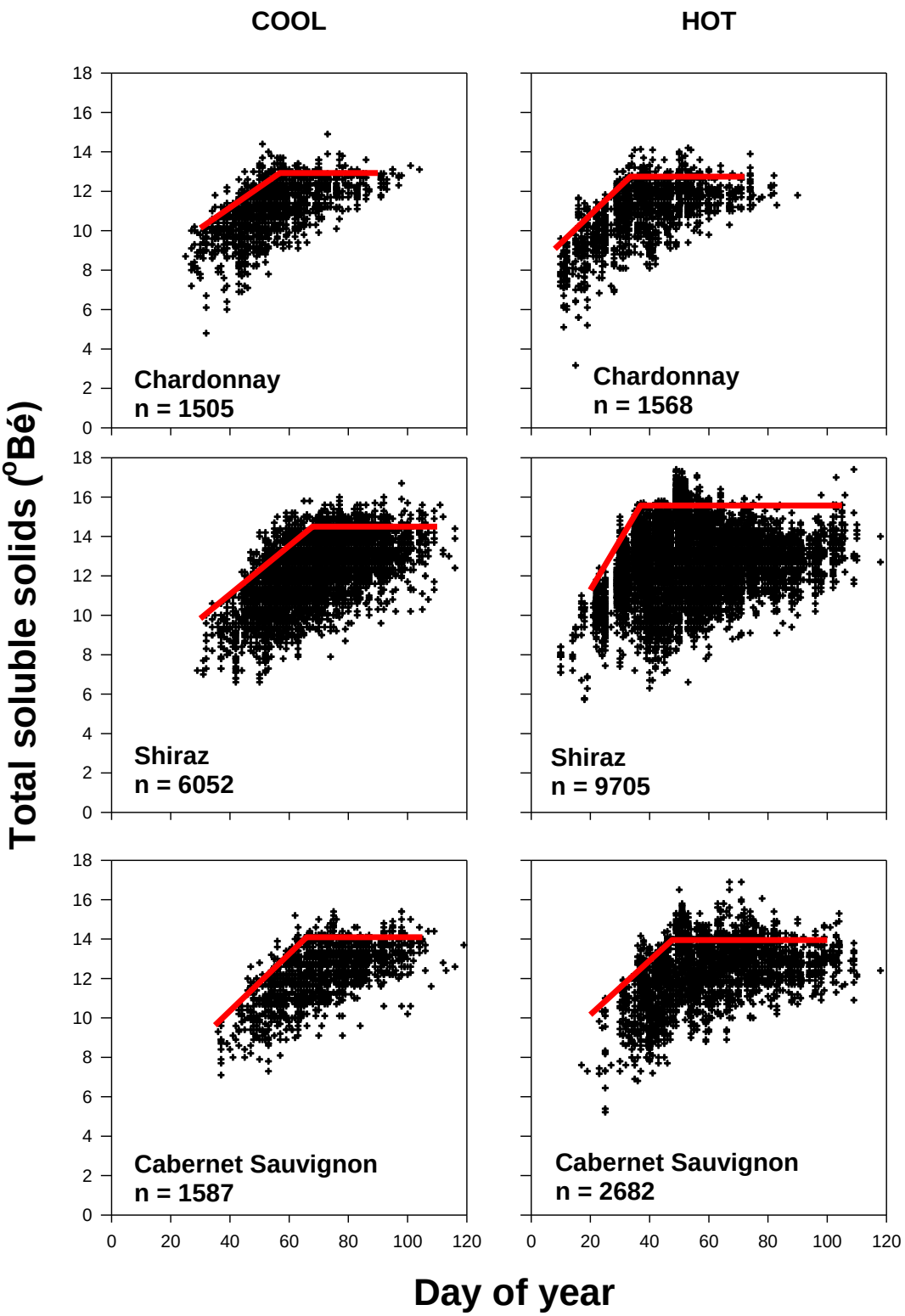

COOL
HOT
Total soluble solids (°Bé)
Day of year
Chardonnay
n = 1505
Chardonnay
n = 1568
Shiraz
n = 6052
Shiraz
n = 9705
Cabernet Sauvignon
n = 1587
Cabernet Sauvignon
n = 2682
0
2
4
6
8
10
12
14
16
18
0
20
40
60
80
100
120

Fig. 3

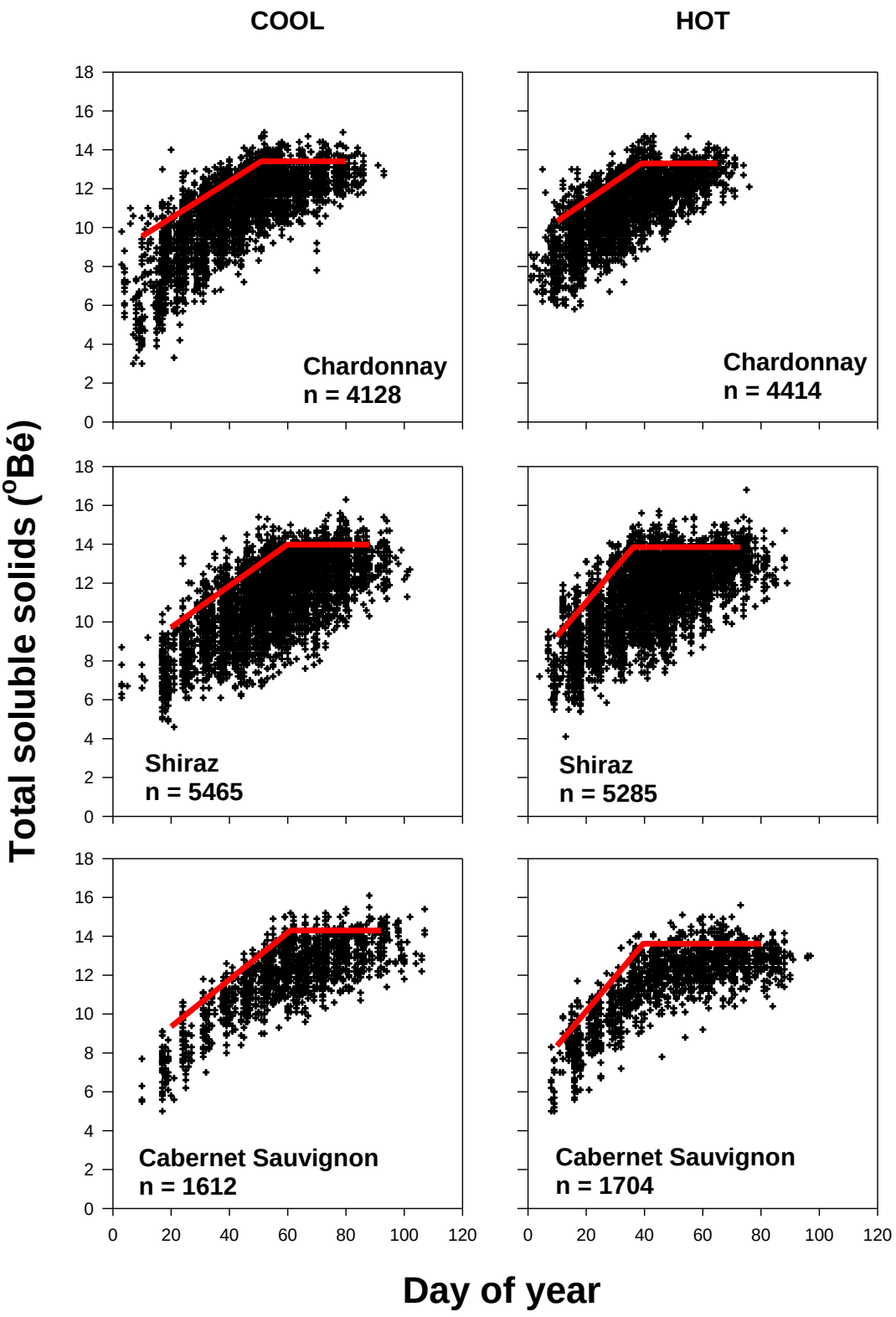

COOL
HOT
Total soluble solids (°Bé)
Chardonnay
n = 4128
Chardonnay
n = 4414
Shiraz
n = 5465
Shiraz
n = 5285
Cabernet Sauvignon
n = 1612
Cabernet Sauvignon
n = 1704
Day of year

Fig. 4

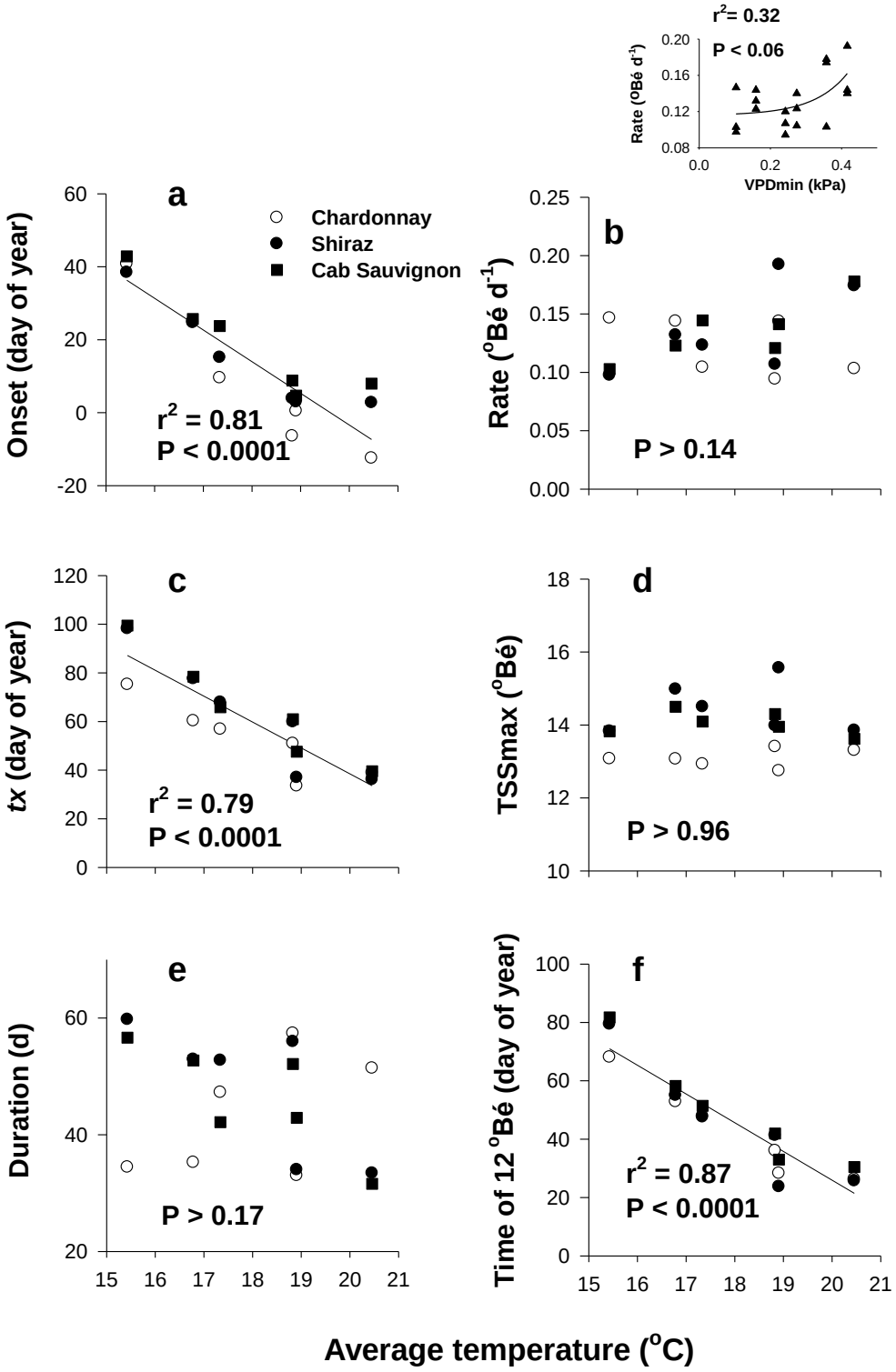

r²= 0.32
P < 0.06
Rate (°Bé d⁻¹)
VPDmin (kPa)
a
Chardonnay
Shiraz
Cab Sauvignon
Onset (day of year)
r² = 0.81
P < 0.0001
b
Rate (°Bé d⁻¹)
P > 0.14
c
tx (day of year)
r² = 0.79
P < 0.0001
d
TSSmax (°Bé)
P > 0.96
e
Duration (d)
P > 0.17
f
Time of 12 °Bé (day of year)
r² = 0.87
P < 0.0001
Average temperature (°C)

Fig. 5

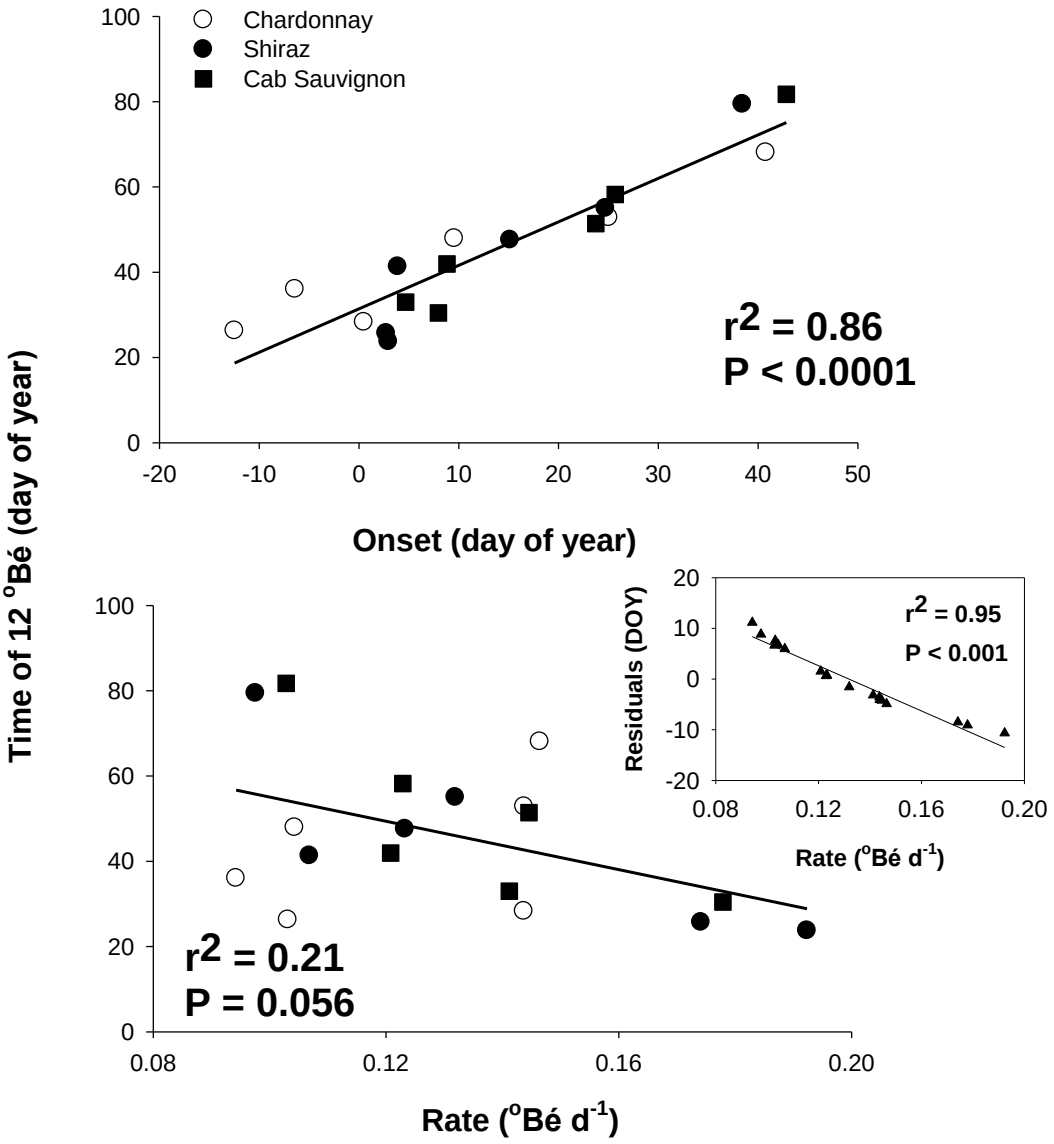

Chardonnay
Shiraz
Cab Sauvignon
r2 = 0.86
P < 0.0001
Onset (day of year)
Time of 12 °Bé (day of year)
r2 = 0.21
P = 0.056
Rate (°Bé d-1)
r2 = 0.95
P < 0.001
Residuals (DOY)
Rate (°Bé d-1)

Fig. 6

Chapter 11. Predicting the time course of grape ripening

VO Sadras, PR Petrie

Abstract

To predict harvest time for logistic applications in vineyards and wineries, measurements of total soluble solids (TSS) are combined with projections assuming TSS increases at 1 ^{o}Bé wk^{-1}. Here we ask three questions. (1) What is the magnitude and direction of the bias in estimates of ripening assuming 1 ^{o}Bé wk^{-1} across varieties, regions and seasons? (2) What are the climatic drivers of this bias? (3) Could thermal rates improve predictions?

Actual TSS of Chardonnay, Shiraz and Cabernet Sauvignon was measured in commercial vineyards in Coonawarra, Barossa Valley and Riverland. Predictions used (a) the default 1 ^{o}Bé wk^{-1}, (b) variety and location specific chronological rates between 0.8 and 1.2 ^{o}Bé wk^{-1}, and (c) variety and location specific thermal rates between 0.009 to 0.017 ^{o}Bé ^{o}Cd^{-1}. In 8 out of 9 cases, 1 ^{o}Bé wk^{-1} biased ripening predictions. Seasonal bias correlated with evaporative demand in the Riverland and Coonawarra, and with minimum temperature in the Barossa. Thermal rates were not superior to calibrated chronological rates to predict ripening.

In conclusion, locally calibrated rates significantly improved ripening predictions for major grapevine varieties in regions with contrasting climates and viticultural profiles. Improved prediction of maturity time would allow for better allocation of key resources in vineyards and wineries – labour, time, machinery, transport, analytical services, chemicals, storage.

Introduction

Time of harvest is one of the critical stages in the annual calendar of the wine industry. Grape growers and winery operators have to plan for the allocation of key resources – labour, time, machinery, transport, analytical services, chemicals, storage. Projections of time of harvest are thus required for the logistics of harvest and post-harvest handling of fruit. The time course of sugar content during ripening, complemented with assessment of other traits such as colour and sensory attributes of fruit, is the central element to define harvest time. Currently, the Australian industry combines measurements of total soluble solids and projections based on a rule of thumb of approximately 1 ^{o}Bé per week to estimate the time course of ripening for logistic purposes (Anon., 2011). Implicit in this approach is the notion that, after the onset, sugar concentration increases linearly with time, hence differences in time of maturity among regions, seasons and varieties are dependent on the onset rather than the rate of sugar accumulation. Our recent studies based on large data sets from commercial vineyards of south eastern Australia support this assumption (Sadras and Petrie, 2011a; Sadras and Petrie, 2011b).

Quantitative models of berry growth and development span a wide range of detail and approaches (Dai et al., 2010; De Cortazar-Atauri et al., 2009; Downes and Mahon, 2012; Due et al., 1993; Godwin et al., 2002; Sadras et al., 2008; Williams et al., 1985). For example, the developmental models in Godwin et al. (2002) and Williams et al. (1985) combined thermal and source-sink drivers to predict maturity. A common feature of all these models is that they have been primarily designed to integrate knowledge and answer scientific questions. For this reason, the complexity of these models and their requirements of detailed parameters and inputs make them unsuitable for practical estimates of time of maturity as required by decision makers in the vineyard and winery. Assuming that most of the differences in time of grapevine sugar maturity derive from differences in the onset of the rapid increase in sugar concentration, appropriate rates of increase in total soluble solids complemented with actual observations to estimate the onset would be sufficient to predict maturity for logistic applications (Sadras and Petrie, 2011a; Sadras and Petrie, 2011b). This is indeed the current approach used in the local industry. However, if 1 ^{o}Bé per week is a suitable rate for, say, an early ripening variety in a hot environment like Chardonnay in the Riverland, would it still be suitable for a later variety in a cooler environment like Shiraz in Coonawarra?

In this paper, we ask three questions. First, what is the magnitude and direction of the bias in estimates of ripening assuming a rate of 1 ^{o}Bé per week across regions, seasons and varieties? Second, what are the climatic drivers of this bias? Third, could we improve our projections of total soluble solids using a thermal rate instead of a chronological rate?

Method

Varieties and climates

Our focus is on Chardonnay, Shiraz, and Cabernet Sauvignon on three climatically contrasting regions: Coonawarra as representative of cool regions, Riverland as representative of hot regions, and Barossa Valley as an intermediate region (Dry et al., 2005). Daily climate records were obtained from the Australian Bureau of Meteorology for three locations central to these regions, i.e. Coonawarra (37º 18'S, 140º 49' E) for the Coonawarra region, Loxton (34^{o} 25' S, 140^{o} 35' E) for Riverland and Nuriootpa (34º 29'S, 139º 00'E) for Barossa Valley.

Actual time courses of total soluble solids

The approaches used in sampling and measurement of total soluble solids have been described in detail elsewhere (Petrie and Sadras, 2008b; Sadras and Petrie, 2011a; Sadras and Petrie, 2011b). Here we used data from six vintages, 2002 to 2006 and 2011. The data for the period between 2007 and 2010 were used to derive thermal rates of sugar accumulation tested in this paper (Sadras and Petrie, 2011b), hence were excluded in this study to avoid circularity. Briefly, company representatives or vineyard staff collected maturity samples at approximately weekly intervals yielding a minimum of four samples per block prior to harvest. Approximately 30 bunches per block from both exposed and shaded crop sections were sampled to account for the typical variation of a vineyard. In the Riverland, total soluble solids of fruit was measured on site. The fruit was usually macerated in the plastic bag used for collection, and a sample decanted for sugar analysis using an analogue refractometer or hydrometer; these measurements were normally not compensated for temperature (Bayindirli, 1993). Juice from the samples from the Barossa and Coonawarra was extracted using a roller crusher and analysed using a FOSS WineScan FT120 (FOSS, Hillerød, Denmark). The calibration of the WineScan was checked daily against a hydrometer with temperature correction.

Question 1 - bias in estimates assuming a rate of 1 ^{o}Bé per week

This question was addressed in two steps. First, we assessed the data for each block, variety and season as illustrated in Figure 1. The first point in the series, around 8 ^{o}Bé, was used as input to the model (square, Fig. 1ab); this initial point was subsequently excluded from all graphical and statistical comparisons between measured and estimated data. We aimed at ~ 8 ^{o}Bé as a starting point for projections of ripening for two reasons: this value allows for the linear phase of increase in total soluble solids for the combination of varieties and regions under study (Sadras and Petrie 2011b) and sampling for ripening projections usually starts around this value in commercial

vineyards (Anon., 2005). The mean starting points and standard errors, in ^{o}Bé, were as follows. In Riverland, 7.4 ± 0.14 for Cabernet Sauvignon, 9.2 ± 0.08 for Chardonnay and 7.3 ± 0.07 for Shiraz. In Barossa, 8.1 ± 0.18 for Cabernet Sauvignon, 10.1 ± 0.51 for Chardonnay and 8.0 ± 0.09 for Shiraz. In Coonawarra, 8.7 ± 0.14 for Cabernet Sauvignon, 7.8 ± 0.69 for Chardonnay and 8.6 ± 0.52 for Shiraz. Data on the plateau (open symbols Fig. 1a) were identified visually and excluded from the analysis; the actual plateau was specific for each variety, season and region (see Table 1 in Sadras and Petrie 2011b). However, where total soluble solids increased after a transient halt, all data were included in the analysis (Fig. 1b). We then used the first measurement of total soluble solids as input, and projected the time course assuming a progression at 1 ^{o}Bé wk^{-1} (triangles in Fig. 1a). Second, we evaluated the precision and accuracy of predictions. We compared measured (y_i) vs estimated (x_i) total soluble solids (Piñeiro et al., 2008) and calculated the correlation coefficient as a measure of precision. Statistics to assess the accuracy of the model included the root mean squared error (*RMSE*), the bias (*B*) and squared bias ($SB = B^2$) as defined by Kobayashi and Salam (2000):

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - x_i)^2} \qquad \text{eq. 1}$$

$$B = \frac{1}{n}\sum_{i=1}^{n}(y_i - x_i) \qquad \text{eq. 2}$$

RMSE and *B* are complementary aspects of the deviation: *RMSE* represents the mean distance between prediction and measurement whereas *B* represents the difference between the means of prediction and measurement (Kobayashi and Salam, 2000). In addition to estimates using a rate of 1 ^{o}Bé wk^{-1}, we estimated total soluble solids using rates 0.8, 0.9, 1.1 and 1.2 ^{o}Bé wk^{-1} and calculated the bias for each rate; the aim was to determine the rate that minimises bias for each combination of region and variety.

Question 2 - climatic sources of seasonal bias in the prediction of ripening

To address this question, we calculated the correlation coefficients between mean seasonal bias (eq. 2) for each variety and region and the seasonal mean of maximum, minimum and average temperature, rain, solar radiation, vapour pressure, relative

humidity at maximum and minimum temperature and reference evapotranspiration (Table 1).

Question 3 – can we improve predictions with thermal rates?

To address this question, the first measurement of total soluble solids was used as input and projections of ripening were based on actual temperatures and the variety and location specific thermal rate that minimises bias within the range from 0.009 to 0.017 ^{o}Bé ^{o}Cd^{-1} (Sadras and Petrie, 2011b). We then compared the bias from estimates using thermal and chronological rates.

Thermal rates combined with temperature forecasts could be used for predictive purposes. The Australian Bureau of Meteorology issues daily temperature forecasts (http://www.bom.gov.au/index.shtml) and a number of companies deliver forecasts by email (www.willyweather.com.au, www.weatherzone.com.au). To test the accuracy and precision of temperature forecasts necessary to derive berry ripening trajectories using thermal rates, we compared 5-day forecasts from commercial providers and actual records for the ripening period in 2011.

Results

What is the magnitude and direction of the bias in estimates of ripening assuming a rate of 1 ^{o}Bé per week across regions, seasons and varieties?

The number of observations ranged from 1085 for Shiraz in Riverland to 49 for Cabernet Sauvignon in Coonawarra (Fig. 2). Less intensive sampling and many blocks where the initial sample was well above the 8 ^{o}Bé required to run the model account for the lower numbers in Coonawarra. In the Riverland, the assumption of ripening progressing at 1 ^{o}Bé wk^{-1} provided a sound prediction for Shiraz, underestimated ripening of Chardonnay and overestimated ripening of Cabernet Sauvignon (Fig. 2, top row). In the Barossa Valley, the assumption of 1 ^{o}Bé wk^{-1} underestimated the ripening for all three varieties (Fig. 2, middle row). In Coonawarra, the assumption of 1 ^{o}Bé wk^{-1} underestimated ripening of Chardonnay and overestimated ripening rates of Cabernet Sauvignon and Shiraz (Fig. 2, bottom row). It is clear therefore that a single rate across regions and varieties biases the prediction of ripening.

Figure 3 shows the bias in the prediction of ripening as a function of rate of increase in total soluble solids. The rate that minimised bias for Shiraz was 1.2 ^{o}Bé wk^{-1} in Barossa, 1.0 ^{o}Bé wk^{-1} in Riverland and 0.8 ^{o}Bé wk^{-1} in Coonawarra (Table 2). The rate that minimised bias for Cabernet Sauvignon was 1.1 ^{o}Bé wk^{-1} in Barossa and 0.9 ^{o}Bé wk^{-1} in both Riverland and Coonawarra. For Chardonnay rates of 1.1-1.2 ^{o}Bé wk^{-1} minimised bias in all three regions. Comparison of Figures 2 and 4 highlights the improvement in predictions from the use of variety and region calibrated rates.

Despite the reduction in bias in relation to predictions using a single rate, a strong seasonal bias remained when using variety and region specific rates. Indeed, analysis of variance of the mean seasonal bias from projections based on rates that minimised bias for each combination of variety and region showed a significant seasonal effect for all varieties and regions ($P < 0.05$). As an example, Fig. 5 shows the seasonal variation for Shiraz in the Barossa Valley. The next question is, hence, what are the climatic sources of these seasonal effects.

What are the climatic sources of seasonal bias in the prediction of ripening?

Table 1 summarises the mean seasonal conditions during ripening and the season-to-season variation for the period under investigation. Season-to-season variation was generally larger at Coonawarra and smaller at Riverland. For example the season-to-season range for maximum temperature during Shiraz ripening was 7.0 ^{o}C in Coonawarra, 5.4 ^{o}C in Barossa Valley and 3.0 ^{o}C in Riverland.

For each variety, region and season, we calculated mean seasonal bias *B* (eq. 2), as illustrated in Figure 5b; estimations used rates that minimised bias (Table 2). Correlation analysis was used to explore the association between *B* and seasonal conditions (Table 3). Very strong and consistent associations were identified in Riverland: the difference between measured and estimated total soluble solids increased with maximum temperature, average temperature and evaporative demand, and decreased with increasing relative humidity. The deviation between measured and estimated total soluble solids increased with high radiation and high evaporative demand in Coonawarra. In Barossa, we only detected a significant association with minimum temperature.

Could we improve our projections of the time course of total soluble solids using a thermal rate?

Two conditions must be met for a thermal rate to be useful in predicting berry ripening. One is the accurate and precise prediction of ripening time course using thermal rates and actual temperature. The second is sound forecasting of temperature.

In comparison to the fixed rate of 1 ^{o}Bé wk^{-1}, the variety and region specific thermal rate selected to minimise bias improved the accuracy and precision of predictions in most cases (Table 2, Fig. 2 vs Fig. 6). However, the thermal rate that minimises bias was not superior to the chronological rate calibrated to minimise bias, except for Cabernet Sauvignon in Coonawarra. In this case, the prediction with the best chronological rate had $r = 0.66$ and RMSE = 1.12 (Fig. 4) that improved to $r = 0.77$ and RMSE = 0.84 with the calibrated thermal rate (Fig. 6). In all other cases, differences

between the thermal and chronological rates were small; SB indicates no approach was clearly superior (Table 2).

Five-day temperature forecasts compared well with actual temperatures (Fig. 7). Nonetheless, there was a region-dependent bias which increased from Coonawarra to Barossa to Riverland (Fig. 7). As the performance of the variety and region specific thermal rates using measured temperatures was already similar to the calibrated chronological rate, the inclusion of forecasted temperatures in the model was not justified.

Discussion

Predicting the time course of ripening is essential for the logistics of harvest and post-harvest operations. Currently, total soluble solids are measured periodically and the time course of ripening is projected by assuming a progression rate of 1 ^{o}Bé wk^{-1}. Qualitatively, this approach is scientifically sound: early maturity of Chardonnay relative to Shiraz, early maturity of grapes in Riverland relative to Coonawarra, or early maturity in a hotter relative to a cooler season in a locality are all primarily related to the onset, rather than the rate of ripening (Sadras and Petrie, 2011a; Sadras and Petrie, 2011b). Quantitatively, we expected a bias of unknown size and direction from a fixed rate applied across varieties, regions and seasons. Hence, our first aim was to quantify this bias. Averaged across regions and varieties, the rate that minimised bias was 1.0 ± 0.05 ^{o}Bé wk^{-1}. Thus, in the absence of any additional information, using a default rate of 1 ^{o}Bé wk^{-1} is justified. However, in 8 out of 9 cases resulting from the combination of varieties and regions, 1 ^{o}Bé wk^{-1} significantly biased the estimates of ripening (Figure 2 vs 4; Fig. 2, Table 2). The only exception was Shiraz in the Riverland, for which 1 ^{o}Bé wk^{-1} minimised the prediction bias. The rate that minimised bias ranged from 0.8 to 1.2 ^{o}Bé wk^{-1} and no pattern was apparent in relation to variety or region (Table 2). This makes it difficult to generalise, and reinforces the importance of local calibration of rates for practical purposes.

Strong seasonal effects remained after calibration for rates to minimise bias (Fig. 5, Table 3). Hence our second aim was to examine the sources of this bias. The rate of increase in total soluble solids is relatively stable in response to source:sink ratios as affected by yield, fruit thinning, defoliation and nitrogen supply (Just et al., 2003; Nuzzo and Matthews, 2006; Petrie and Clingeleffer, 2006). Changes in onset rather than rate of ripening thus explain the shifts in maturity associated with these factors (Sadras and Petrie, 2011a; Sadras and Petrie, 2011b). In the Riverland, there was a strong and consistent correlation between seasonal bias in prediction of ripening and several meteorological variables: using the best chronological rate, progression of total soluble solids was underestimated under conditions of high evaporative demand (Table 3). Likewise, the deviation between measured and estimated total soluble solids increased

with high evaporative demand and high radiation in Coonawarra. These empirical correlations are consistent with our understanding of the physiology of berry ripening, whereby sugar accumulation is linked to berry transpiration rate as affected by bunch microclimate (Rebucci et al., 1997). In the Barossa Valley, we only detected a significant association between seasonal bias and minimum temperature.

Sadras and Petrie (2011b) proposed that an onset-rate model could be use to improve the accuracy and precision of predictions of grapevine ripening. In common with the current approach, this model requires an observed starting point for sugar concentration, whereas the putative improvement would derive from using a site and cultivar specific thermal rate driven by mean daily temperature. This approach would only be justified in practical terms if a thermal rate produces better estimates than chronological rates. Hence the third aim of this study was to compare both approaches. Using the calibrated chronological rates that minimised bias as a benchmark, a thermal rate did not improve predictions except for Cabernet Sauvignon in Coonawarra (Table 3). This is consistent with the dominant role of evaporative demand as a major source of bias in predictions using a chronological rate and the minor and likely indirect effect of temperature (Table 2). Irrespective of the causes, for our extensive data set a thermal rate was not superior to a chronological rate in most cases. Furthermore, for a thermal rate to be practically used in predicting the evolution of total soluble solids, forecasts of mean daily temperature would be required which would add noise to the predictions (Fig. 7), making them inferior to the simplest chronological rate approach.

In conclusion, the Australian grape and wine industry relies on a simple method combining observations of total soluble solids in the field and projections based on a fixed rate of 1 ^{o}Bé wk^{-1}. Qualitatively, this approach is consistent with our current understanding of ripening physiology: variety, region and seasonal differences in maturity are primarily associated with differences in onset of ripening. Qualitatively, the default rate is justified when no additional information is available. Locally calibrated rates, in the range from 0.8 to 1.2 ^{o}Bé wk^{-1} significantly improved ripening predictions for major grapevine varieties in a wide range of environments, with no additional complications in terms of implementation. Using these rates, the model applies within the range of total soluble solids from ~8 ^{o}Bé to the actual plateau which is variety, region and season specific; it is typically lower for Chardonnay (~13 ^{o}Bé) than for Shiraz and Cabernet Sauvignon (~15 ^{o}Bé) and is lower in the hot Riverland environment than in Barossa and Coonawarra (see Table 1 in Sadras and Petrie 2011b). No further improvement would be expected from thermal rates, as temperature on its own had little direct effect on the time course of ripening. Evaporative demand was an important source of seasonal bias when using chronological rates, particularly in Riverland and Coonawarra.

Acknowledgements

We thank the growers, vineyard and weight bridge staff from Treasury Wine Estates Ltd. for collecting the maturity and yield data, Harry Driscoll for help with the database, and the Grape and Wine Research and Development Corporation of Australia and the Department of Agriculture, Forestry and Fisheries for financial support. Use of temperature forecasts from commercial sites does not mean SARDI or Treasury Wine Estates endorse these services.

Table 1. Weighted mean and *range* of climatic elements during the period of rapid increase in total soluble solids of three grapevine varieties in three contrasting climatic regions of Australia. Maximum temperature (Tmax, ^{o}C), minimum temperature (Tmin, ^{o}C), mean temperature (Tav, ^{o}C), Rain (mm d^{-1}), solar radiation (MJ m^{-2} d^{-1}), vapour pressure (VP, hPa), relative humidity calculated using 9am VP and either maximum temperature (RHM, %) or minimum temperature (RHm, %), and reference evapotranspiration (ETo, mm d^{-1}). Calculated from daily records by the Australian Bureau of Meteorology from Loxton for Riverland, from Nuriootpa for Barossa Valley and from Coonawarra for the Coonawarra region.

Region	**Variety**	**Tmax**		**Tmin**		**Tav**		**Rain**		**Radiation**		**VP**		**RHM**		**RHm**		**ETo**	
Riverland	Chardonnay	31.9[a]	*3.6*[b]	15.2	*5.5*	23.6	*4.6*	0.1	*0.2*	25.0	*2.3*	12.6	*3.2*	28.0	*4.1*	73.2	*16.4*	6.3	*0.8*
	Cab Sauvignon	30.0	*4.4*	13.3	*2.3*	21.6	*3.1*	0.3	*0.7*	23.3	*4.8*	12.5	*2.6*	30.5	*9.3*	81.0	*12.7*	5.5	*1.5*
	Shiraz	30.7	*3.0*	13.7	*2.8*	22.2	*2.2*	0.1	*0.4*	23.8	*3.3*	12.4	*3.6*	29.2	*9.5*	78.5	*13.9*	5.8	*0.8*
Barossa	Chardonnay	28.2	*6.3*	13.5	*4.7*	20.9	*5.5*	0.2	*0.6*	22.5	*1.8*	12.1	*2.1*	32.9	*9.5*	77.5	*15.6*	5.2	*1.1*
	Cab Sauvignon	25.8	*4.7*	11.9	*1.6*	18.9	*1.6*	0.3	*0.9*	20.7	*2.7*	12.1	*3.2*	38.0	*15.4*	84.5	*14.8*	4.4	*0.9*
	Shiraz	26.4	*5.4*	12.3	*2.0*	19.4	*2.6*	0.2	*0.5*	21.2	*3.2*	11.9	*2.7*	36.0	*16.1*	81.6	*7.7*	4.7	*1.1*
Coonawarra	Chardonnay	25.3	*5.6*	10.4	*9.2*	17.9	*6.9*	0.9	*2.6*	18.2	*3.9*	13.3	*3.7*	42.3	*10.1*	95.0	*13.5*	3.9	*1.3*
	Cab Sauvignon	23.7	*4.3*	9.9	*6.2*	16.8	*4.7*	6.3	*24.5*	17.2	*3.8*	13.8	*3.6*	48.2	*7.3*	98.1	*4.0*	3.3	*0.9*
	Shiraz	23.3	*7.0*	9.1	*3.1*	16.2	*2.6*	8.0	*24.5*	14.6	*5.3*	12.8	*4.2*	47.9	*30.7*	95.3	*12.7*	3.0	*1.3*

[a]Weighted mean, i.e. $\bar{x} = \frac{\sum_{i=1}^{n} x_i d_i}{\sum_{i=1}^{n} d_i}$ where x_i is the value of a variable on day i, and d is the number of times that date i is represented in the sampled time course of total soluble solids. This calculation reduced the undue weight given by the arithmetic mean to very early and very late dates in the total period of sampling. [b]Range, i.e. difference between the minimum and maximum seasonal mean in the period 2002-2006 and 2011. For example, the maximum seasonal average of Tmax during ripening of Chardonnay in Riverland was 33.9 ^{o}C (2011) and the minimum was 30.3 ^{o}C (2002), hence the range = 3.6 ^{o}C.

Table 2. Squared bias (SB) in estimating total soluble solids using three rates: the default rate 1^{o}Bé wk^{-1}, and the chronological and thermal rates that minimise bias for three varieties in three climatically contrasting regions of Australia. The rates that minimise SB are also shown. Derived from data for seasons 2002-2006 and 2011.

Region	Variety	SB			Rate that minimises SB	
		1^{o}Bé wk^{-1}	Chronological	Thermal	Chronological (^{o}Bé wk^{-1})	Thermal (^{o}Bé Cd^{-1})
Riverland	Chardonnay	0.123	0.011	0.026	1.1	0.011
	Cab Sauv	0.052	0.030	0.122	0.9	0.009
	Shiraz	0.007	0.007	0.089	1.0	0.009
Barossa	Chardonnay	0.197	0.001	0.002	1.2	0.017
	Cab Sauv	0.041	0.011	0.005	1.1	0.015
	Shiraz	0.391	0.000	0.012	1.2	0.017
Coonawarra	Chardonnay	0.062	0.000	0.082	1.1	0.019
	Cab Sauv	0.229	0.016	0.000	0.9	0.019
	Shiraz	0.552	0.003	0.000	0.8	0.017

Table 3. Correlation coefficient between mean seasonal bias in estimates of total soluble solids and seasonal conditions in three climatically contrasting regions of Australia. Deviations are the difference between measured and actual total soluble solids. Maximum temperature (Tmax, ^{o}C), minimum temperature (Tmin, ^{o}C), mean temperature (Tav, ^{o}C), Rain (mm d^{-1}), solar radiation (MJ m^{-2} d^{-1}), vapour pressure (VP, hPa), relative humidity calculated using 9am VP and either maximum temperature (RHM, %) or minimum temperature (RHm, %), and reference evapotranspiration (ETo, mm d^{-1}). Bold type emphasises $P < 0.05$.

Variable	Riverland		Barossa		Coonawarra	
	Correlation	P	Correlation	P	Correlation	P
Tmax	0.66	**0.002**	-0.05	0.84	0.47	0.09
Tmin	0.23	0.36	0.51	**0.03**	0.28	0.34
Tav	0.49	**0.040**	0.19	0.47	0.43	0.13
Rain	0.25	0.33	-0.34	0.19	0.38	0.19
Radiation	0.32	0.20	0.02	0.93	0.63	**0.01**
VP	-0.40	0.10	0.43	0.08	0.17	0.58
RHM	-0.77	**0.0001**	0.26	0.32	-0.20	0.50
RHm	-0.72	**0.0004**	0.02	0.94	-0.08	0.80
ETo	0.68	**0.001**	-0.04	0.89	0.58	**0.03**

Captions to figures

Figure 1. Method used in data selection and estimates of trajectories of total soluble solids. (a) Actual data from a particular block were used provided the first sampling was close to the onset of linear phase, around 8 oBé (square). Comparisons between measured (closed circles) and estimated (triangles) total soluble solids were restricted to the linear stage; data in the plateau (open symbols) were excluded from comparisons. (b) Where total soluble solids increased after a transient halt (first week in February in this example), all data were included in the analysis.

Figure 2. Comparison of measured and estimated total soluble solids assuming a rate = 1 ^{o}Be wk^{-1} for three grapevine varieties in three climatically contrasting regions of Australia. The lines represent y = x.

Figure 3. Squared bias (SB) in the estimate of ripening trajectories as a function of rate of increase in total soluble solids for Shiraz, Cabernet Sauvignon and Chardonnay in three climatically contrasting regions of Australia. Note the scales for SB have been adapted to emphasise differences with rate, rather than comparisons among regions.

Figure 4. Comparison of measured and estimated total soluble solids using cultivar and region specific chronological rates that minimised bias. The lines represent y = x.

Figure 16. (a) Comparison of measured and estimated total soluble solids emphasising seasonal effects and (b) Mean seasonal difference between measured and estimated total soluble solids. Error bars are one standard error of the mean and P is seasonal effect from analysis of variance. Data are for Shiraz in the Barossa Valley with estimates based on a rate of 1.2 ^{o}Bé wk^{-1} .

Figure 17 Comparison of measured and estimated total soluble solids using cultivar and region specific thermal rates that minimised bias. The line is y = x.

Figure 18. Comparison of actual and forecasted temperature between 20 Jan and 31 March 2011 in three contrasting locations: Loxton for Riverland, Nuriootpa for Barossa Valley and Coonawarra for the Coonawarra region. The line is y = x. Forecasts are from www.willyweather.com.au and actual temperature from the Australian Bureau of Meteorology.

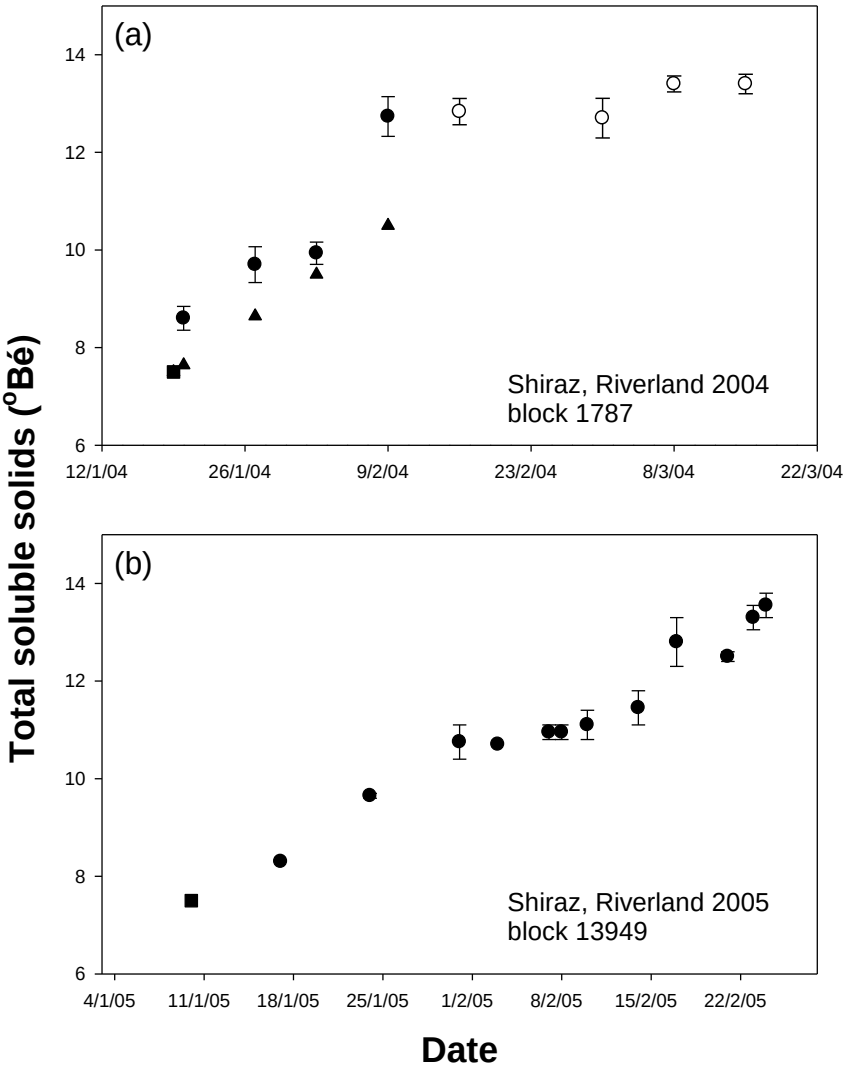

(a)
Shiraz, Riverland 2004
block 1787
(b)
Shiraz, Riverland 2005
block 13949
Total soluble solids (°Bé)
Date
14
12
10
8
6
12/1/04
26/1/04
9/2/04
23/2/04
8/3/04
22/3/04
4/1/05
11/1/05
18/1/05
25/1/05
1/2/05
8/2/05
15/2/05
22/2/05

Fig. 1

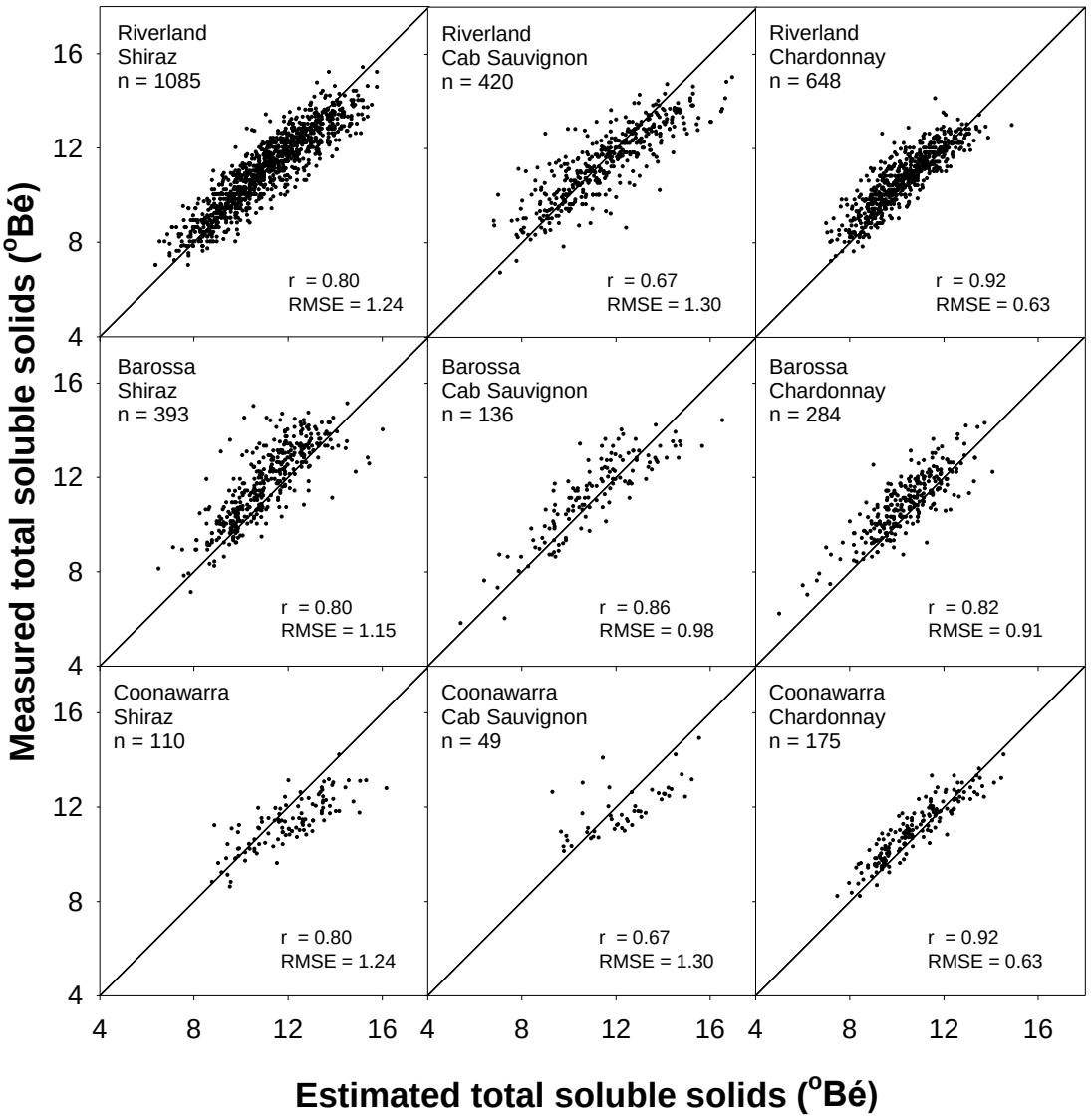

Riverland
Shiraz
n = 1085
r = 0.80
RMSE = 1.24
Riverland
Cab Sauvignon
n = 420
r = 0.67
RMSE = 1.30
Riverland
Chardonnay
n = 648
r = 0.92
RMSE = 0.63
Barossa
Shiraz
n = 393
r = 0.80
RMSE = 1.15
Barossa
Cab Sauvignon
n = 136
r = 0.86
RMSE = 0.98
Barossa
Chardonnay
n = 284
r = 0.82
RMSE = 0.91
Coonawarra
Shiraz
n = 110
r = 0.80
RMSE = 1.24
Coonawarra
Cab Sauvignon
n = 49
r = 0.67
RMSE = 1.30
Coonawarra
Chardonnay
n = 175
r = 0.92
RMSE = 0.63
Measured total soluble solids (°Bé)
Estimated total soluble solids (°Bé)
4
8
12
16

Fig. 2

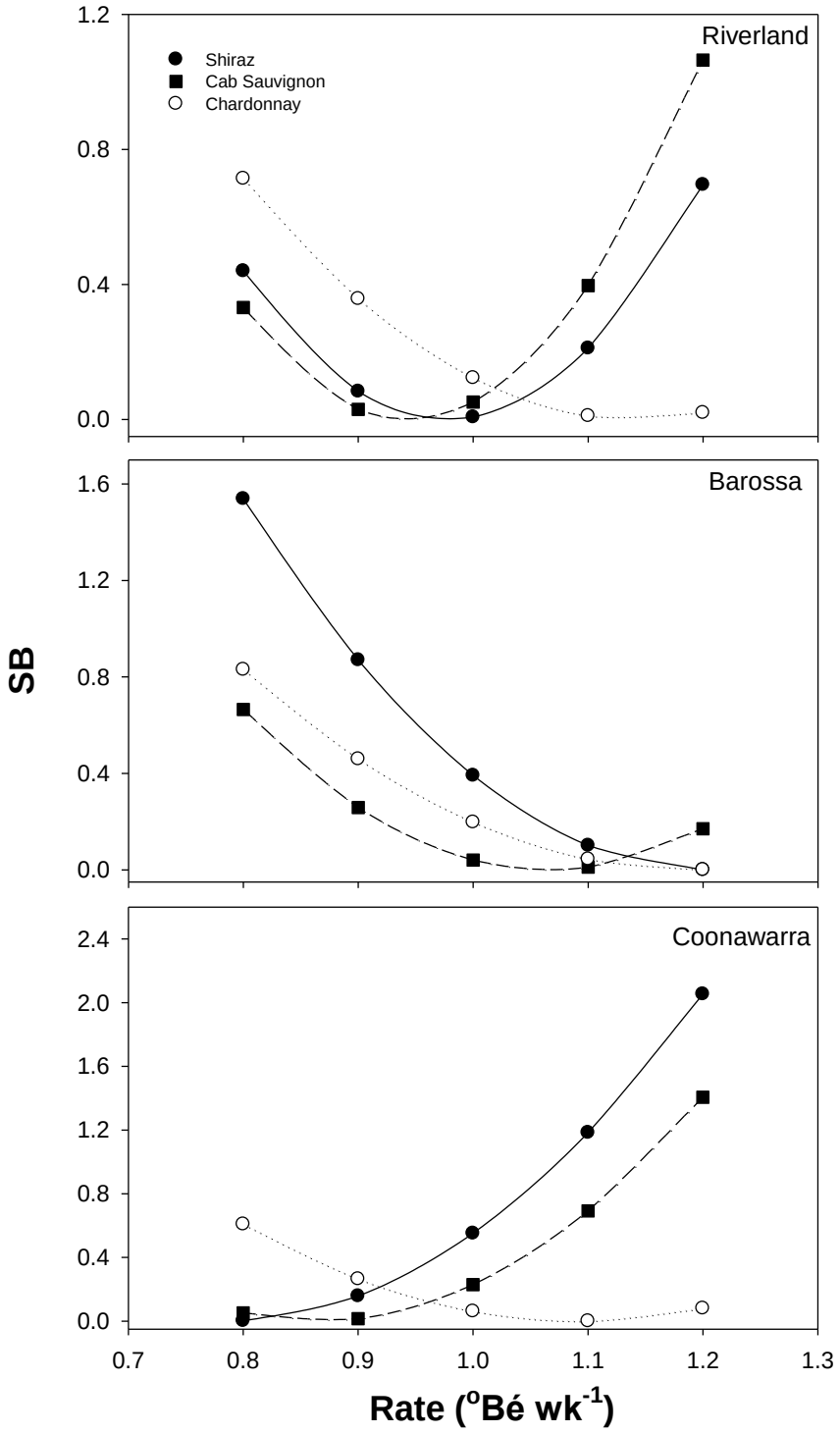

Riverland
Shiraz
Cab Sauvignon
Chardonnay
Barossa
Coonawarra
SB
Rate (°Bé wk-1)
1.2
0.8
0.4
0.0
1.6
1.2
0.8
0.4
0.0
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0.7
0.8
0.9
1.0
1.1
1.2
1.3

Fig. 3

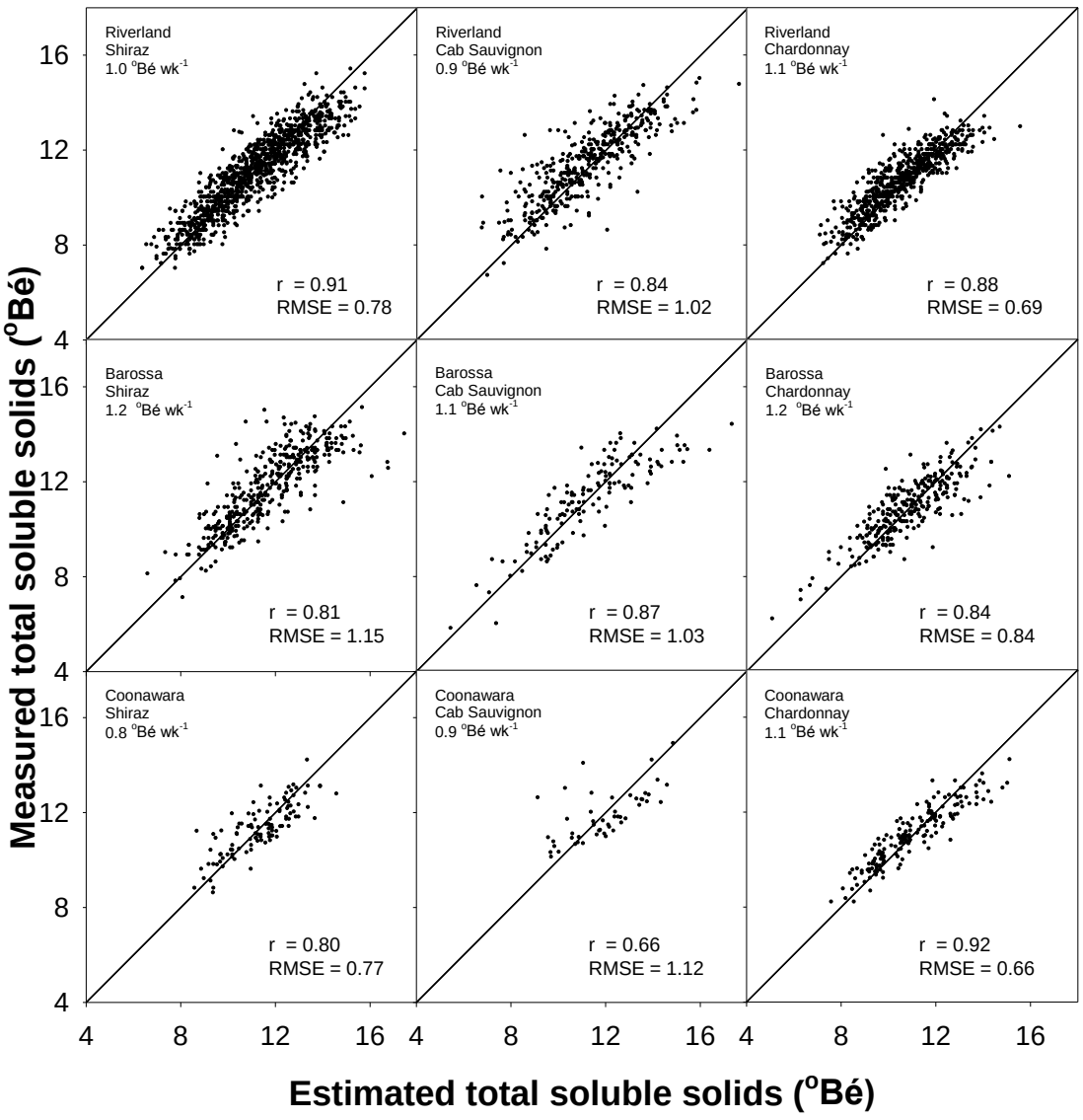

Riverland
Shiraz
1.0 °Bé wk-1
r = 0.91
RMSE = 0.78
Riverland
Cab Sauvignon
0.9 °Bé wk-1
r = 0.84
RMSE = 1.02
Riverland
Chardonnay
1.1 °Bé wk-1
r = 0.88
RMSE = 0.69
Barossa
Shiraz
1.2 °Bé wk-1
r = 0.81
RMSE = 1.15
Barossa
Cab Sauvignon
1.1 °Bé wk-1
r = 0.87
RMSE = 1.03
Barossa
Chardonnay
1.2 °Bé wk-1
r = 0.84
RMSE = 0.84
Coonawara
Shiraz
0.8 °Bé wk-1
r = 0.80
RMSE = 0.77
Coonawara
Cab Sauvignon
0.9 °Bé wk-1
r = 0.66
RMSE = 1.12
Coonawara
Chardonnay
1.1 °Bé wk-1
r = 0.92
RMSE = 0.66
Measured total soluble solids (°Bé)
Estimated total soluble solids (°Bé)
4
8
12
16

Fig. 4

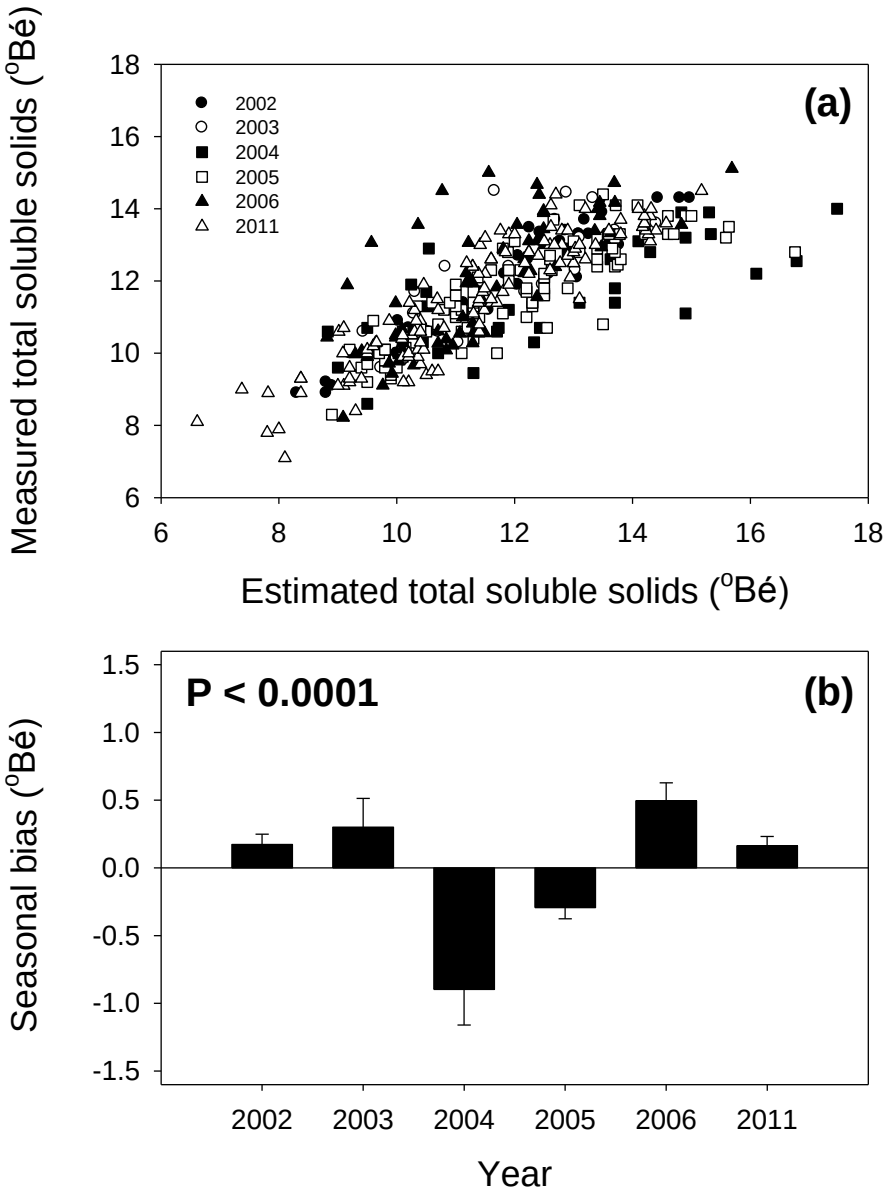

Measured total soluble solids (°Bé)
Estimated total soluble solids (°Bé)
2002
2003
2004
2005
2006
2011
(a)
P < 0.0001
(b)
Seasonal bias (°Bé)
Year

Fig. 5

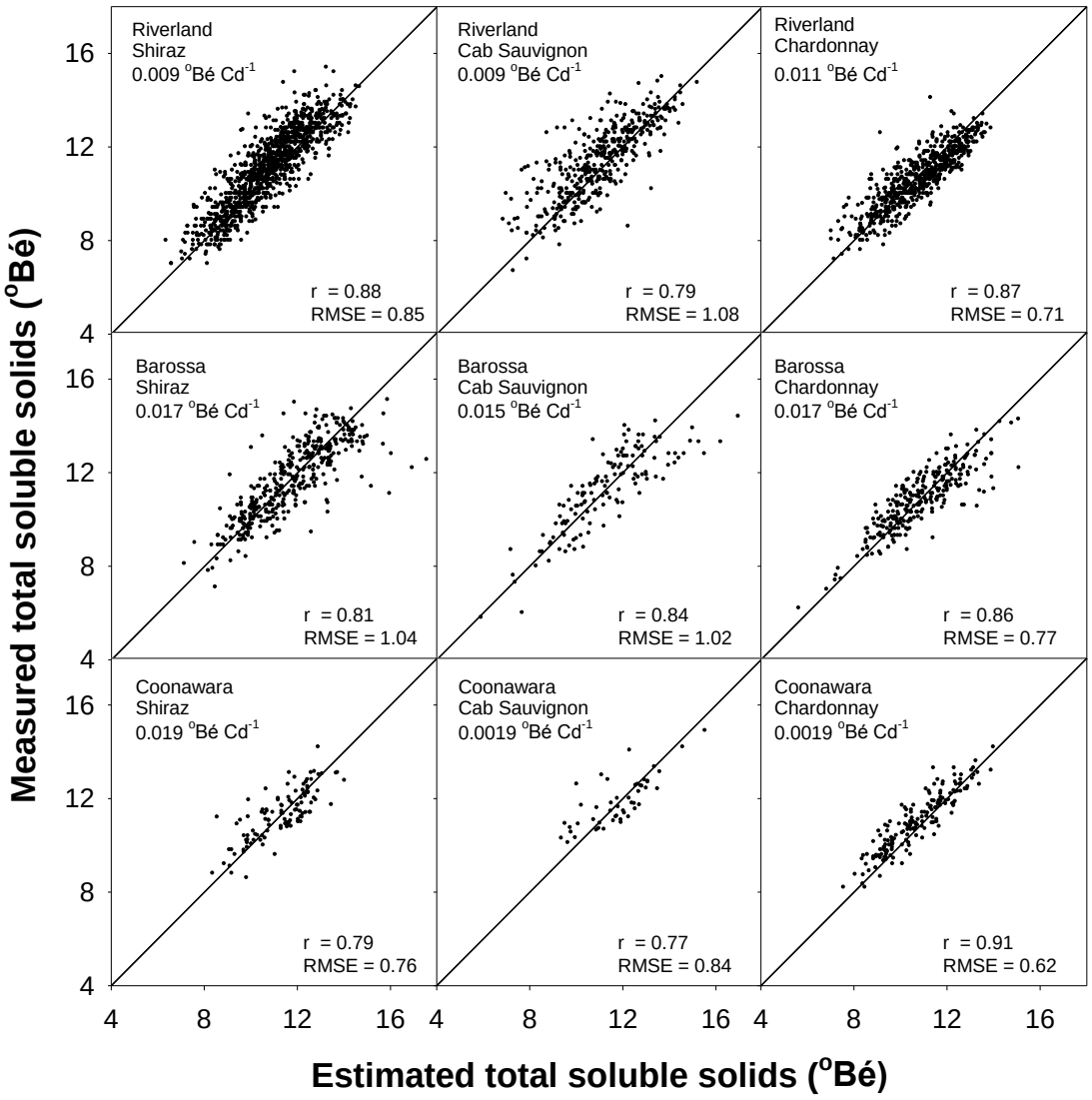

Riverland
Shiraz
0.009 °Bé Cd-1
r = 0.88
RMSE = 0.85
Riverland
Cab Sauvignon
0.009 °Bé Cd-1
r = 0.79
RMSE = 1.08
Riverland
Chardonnay
0.011 °Bé Cd-1
r = 0.87
RMSE = 0.71
Barossa
Shiraz
0.017 °Bé Cd-1
r = 0.81
RMSE = 1.04
Barossa
Cab Sauvignon
0.015 °Bé Cd-1
r = 0.84
RMSE = 1.02
Barossa
Chardonnay
0.017 °Bé Cd-1
r = 0.86
RMSE = 0.77
Coonawara
Shiraz
0.019 °Bé Cd-1
r = 0.79
RMSE = 0.76
Coonawara
Cab Sauvignon
0.0019 °Bé Cd-1
r = 0.77
RMSE = 0.84
Coonawara
Chardonnay
0.0019 °Bé Cd-1
r = 0.91
RMSE = 0.62
Measured total soluble solids (°Bé)
Estimated total soluble solids (°Bé)
4
8
12
16

Fig. 6

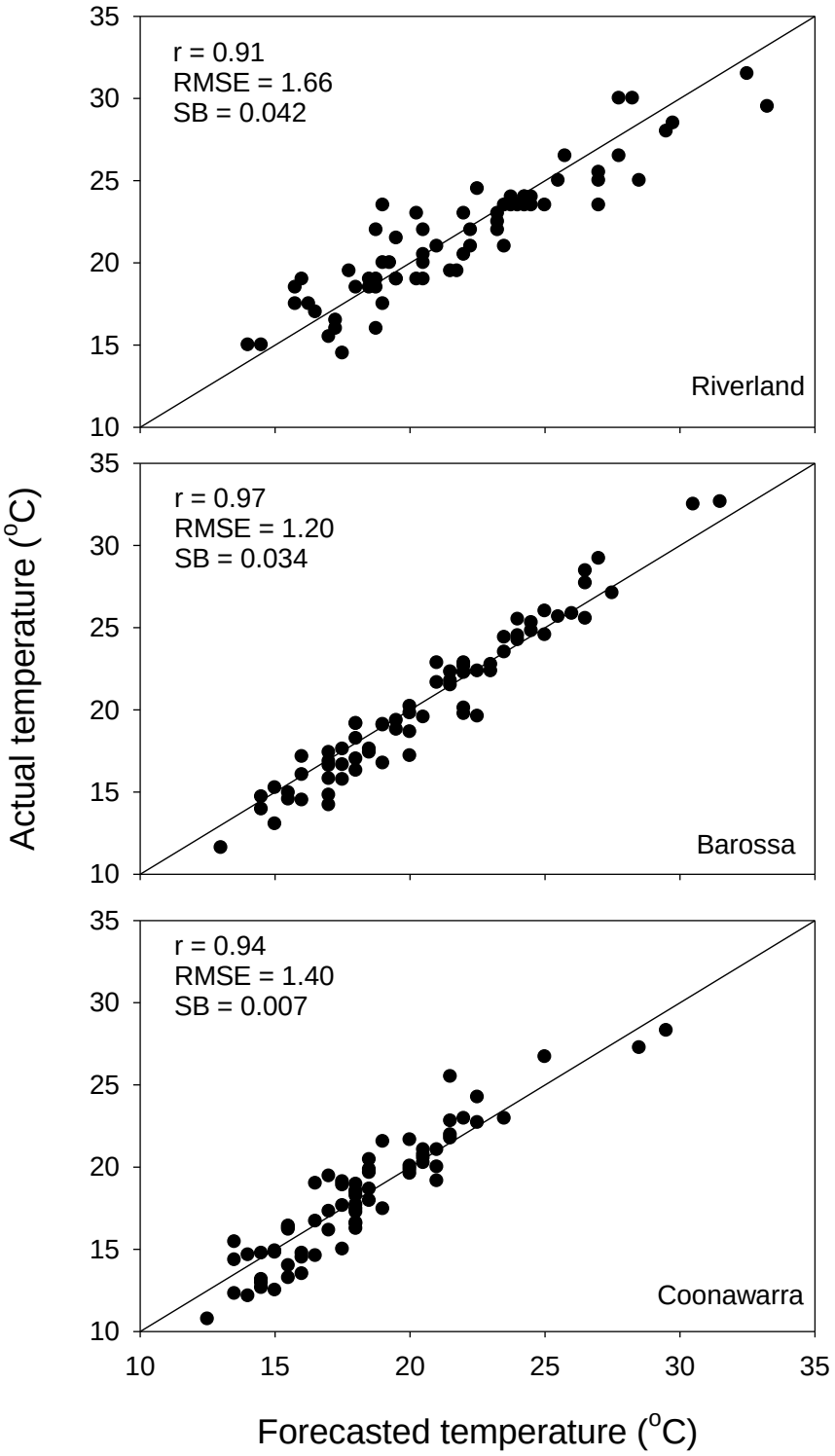

r = 0.91
RMSE = 1.66
SB = 0.042
Riverland
r = 0.97
RMSE = 1.20
SB = 0.034
Barossa
r = 0.94
RMSE = 1.40
SB = 0.007
Coonawarra
Actual temperature (°C)
Forecasted temperature (°C)
10
15
20
25
30
35

Fig. 7

Chapter 12. Communication and delivery

This project established an active program of communication and delivery including industry wine workshops, industry and scientific publications and presentations in conferences and seminars. Whenever feasible, we first exposed our findings to peer-review in scientific journals. Far from perfect, peer-review is an effective tool for quality control. At early stages of the project, we communicated our findings and sought feedback from industry in the regular meetings under the Climate Change initiative. Our state-of-the-art heating facilities attracted international interest. Researchers from Argentina and Spain joined our experimental program, added value and allowed for new international links. The project delivered two wine tasting workshops and 39 industry, conference and scientific publications. This chapter lists the communication activities of this project.



Scientists from the Chinese Academy of Science visit the GWRDC-funded vine heating facilities at Nuriootpa.

Wine Tasting Workshops



We held two workshops, which were free and open to grape growers, winemakers and other industry and media interested parties. Both were held at Jacob's Creek Visitor Centre in the Barossa Valley. The first workshop was on 15th October 2010 and the second was on 15th December 2011. Formal and informal feedback was sought with dominantly positive responses. All participants in 2010, for example, found the experience was useful and worth repeating. The list below, from 2011 workshop, highlights that the project reached not only the large corporate component of the industry, but also small and family operations.

Adam Clay	**Penfolds**
Alex Macclelland	**Schild Estate**
Alistair Dinnison	**Orlando**
Allistar Ashmead	**Grapegrower**
Andrew Downs	**Phylloxera & Grape Industry Board SA**
Andrew Miller	**Pernod Ricard**
Andy Zhon	**CAE**
Angus Seabrook	**BUE**
BL Dunlevy	**Grapegrower**
Cameron Ashmead	**Grapegrower**
Chris Brodie	**Wingara wine group**
Chris Rogers	**St Hallet**
Daniel Newson	**Yalumba**
Daniel Swincer	**Orlando Wines**
David Matthews	**Treasury wines**
Fleur Davis	**Craneford Wines**
Gemma West	**AWRI**
Huanmei	**Wine Industry Researcher (ECIC, UniAd)**
Hugh Amstrong	**Bayer**
James Rosenzweig	**Rosenzweig Vineyards**
Jen Barwick	**ANZ Magazine**
Kay Dunlevy	**Grapegrower**
Leon Deans	**Orlando Wines**
Mark Gishen	**SA Wine Industry Association**
Paul Gorell	**Orlando Wines**
Peter Leske	**La Linea**
Philip Deveen	**PWB**
Philip Laffer	**Orlando Wines**
Rob Gibson	**Gibson Wines**
Sasha Bown	**Jacobs Creek**
Scott Hazeldine	**Schild Estate**
Sonya	**ANZ Magazine**
Steve Meyer	**Orlando Wines**
Syd Kyloh	**Grapegrower**
Taras Ochota	**La Linea**
Tony Hooper	**Orlando Wines**
Troy Fischer	**Orlando Wines**
Will Thompson	**Barossa winery state**

Industry papers, conferences and workshops

2012 Petrie PR, Brooke S, Moran MA, Sadras VO 2012. Delayed pruning of grapevines: A tool to manage the effects of climate change on fruit quality and harvest compression. Climate Change Research Strategy for Primary Industries Conference, Melbourne 27-29 November 2012.

2012 Moran MA, Petrie PR, Sadras VO. Effects of elevated temperature in grapevine on juice pH, titratable acidity and wine sensory attribute. *Crush 2012, Grape and wine science symposium*, Adelaide, 15-16 November 2012

2012 Sadras VO, Moran MA. Effects of elevated temperature on vine phenology, physiology and berry composition. *Crush 2012, Grape and wine science symposium*, Adelaide, 15-16 November 2012

2012 Petrie PR, Sadras VO. 2012. Climate change: a viticulturalist's perspective. *Climate Change Adaptation in the Primary Industries Master Class. Nuriootpa, 12 Nov 2012*

2012 Sadras VO, Moran MA, Petrie PR. 2012. A window into hotter futures: measuring vine responses in realistic field conditions. *Climate Change Adaptation in the Primary Industries Master Class. Nuriootpa, 12 Nov 2012*

2012 Sadras VO, Montoro A, Moran MA and Aphalo PJ. 2012. Warming enlarges stomata in Shiraz. *Australian and New Zealand Grapegrower and Winemaker*, 584:41

2012 Sadras VO and Moran MA 2012. Study explores warming effect on grapevine yield in the Barossa. *Australian and New Zealand Grapegrower and Winemaker*, 584:42-43

2012 Petrie PR, Sadras VO. Climate Change – a Viticulturist's Perspective. CSIRO Waite Campus Seminar Series, 4 September 2012

2012 Montoro A, Sadras VO (2012) Elevated temperature increased stomata size and leaf photosynthesis in Shiraz grapevine *VII International Symposium on Irrigation of Horticultural Crops*, July 16-20 2012, Geisenheim (Germany)

2012 Sweetman C, Jenkins CLD, Sadras VO, Ford CM, Soole KL Warming effects on malic acid metabolism in field-grown *Vitis vinifera* winegrapes ComBio 2012. 26-27 September, Adelaide

2012 Sadras VO. Effects of elevated temperature on grapevine physiology, berry and wine attributes. INTA Mendoza, Argentina, 4 April 2012.

2011 Sweetman C, Sadras VO, Soole K, Hayes M, Melino V, Burbidge C,

Contreras D, Wong D, Dunlevy J, Ford C. Grape berry metabolism of organic acids – developmental and environmental control. Paper 24, *Crush 2011, Grape and wine science symposium*, Adelaide, 28-30 September 2011

2011 Sadras VO, Petrie PR. Early maturity of grapevines in south-eastern Australia between 1995 and 2009 was associated with early onset rather than faster ripening. *Climate Change Research Strategy for Primary Industries*, Melbourne 15-17 February 2011, p. 27

2011 Sadras VO, Petrie PR. Predicting harvest time. *Australian and New Zealand Grapegrower and Winemaker*, 574: 21-22

2011 Moran MA, Sadras VO, Liebich B, Bubner RA. High temp traits differ across varieties. *Australian and New Zealand Grapegrower and Winemaker* 571, 34-35

2011 Moran MA, Sadras VO, Liebich B, Bubner RA. Water supply can offset high temp effects. *Australian and New Zealand Grapegrower and Winemaker* 570, 32-33

2011 Collins C, McKay S, James A, Sadras VO 2011. Grapevine reproductive performance in a hotter climate. 17th International Symposium GiESCO. August 29th-September 2nd Asti Alba, Italy

2010 Bubner RA, Moran M, Sadras VO. Effects of elevated daytime temperature on berry sensory attributes of shiraz, cabernet franc, semillon and chardonnay. *The Australian and New Zealand Grapegrower and Winemaker* 560: 41-44

2010 Sadras VO. Biological and technological adaptations will be part of the hotter picture. *The Australian and New Zealand Grapegrower and Winemaker* 561: 19-24

2010 Sadras VO. Agricultural opportunities and challenges associated with rising temperature: A crop physiologist's viewpoint. *7th Annual Australia-China Symposium. Agriculture and food security relating to health*. Rowland Flat, Australia. 14-16 November. AAS-ATSE-CAS, p.44

2010 Sadras VO. Grapevine responses to temperature: biological and technological adaptations. In: Blair, R., Lee, T., Sakkie, P. (Eds.), *14th Australian Wine Industry Technical Conference*, Adelaide, pp. 132-135

2010 Bubner RA, Moran MA, Sadras VO. Effects of elevated daytime temperature on berry sensory attributes of Shiraz, Cabernet Franc, Semillon and Chardonnay. In: Blair, R., Lee, T., Sakkie, P. (Eds.), *14th Australian Wine Industry Technical Conference*, Adelaide, pp. 383-384

2010 Sadras VO, Bubner RA, Moran M. Interaction between background temperature and heatwaves: fruit damage in four grapevine varieties in the Barossa Valley. *Australian and New Zealand Grapegrower and Winemaker* 556:22-24

2010 Sadras VO. Vine and wine responses to temperature: current research and gap. *GWRDC Workshop, Adelaide 6 October 2010*.

2009 Sadras VO. Grapevine maturity as affected by temperature, variety and canopy-to-fruit ratio. *Australian and New Zealand Grapegrower and Winemaker* August 550: 29-31

2009 Sadras VO. A window into hotter futures. *GWRDC Climate Change Workshop*. Adelaide 12 October 2009

Scientific publications

2013 Bonada M, Sadras VO, Fuentes S. Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Australian Journal of Grape and Wine Research* in press

2013 Sadras VO, Moran MA, Bonada M. Effects of elevated temperature in grapevine. I Berry sensory traits. *Australian Journal of Grape and Wine Research* in press

2013 Sadras VO, Petrie PR, Moran MA. Effects of elevated temperature in grapevine. II Juice pH, titratable acidity and wine sensory attributes. *Australian Journal of Grape and Wine Research* in press

2012 Sadras VO, Moran MA. Nonlinear effects of elevated temperature on grapevine phenology *Agricultural and Forest Meteorology*

2012 Ortega-Farias S, Fereres E and Sadras VO. Special issue on water management in grapevines. *Irrigation Science*, 30:335-337

2012 Sadras VO, Montoro A, Moran MA, Aphalo PJ Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine. *Agricultural and Forest Meteorology* 165:35-42

2012 Sadras VO, Moran MA. Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Australian Journal of Grape and Wine Research* 18:115–122. **The Australian Society of Viticulture and Oenology awarded this paper the title of AJGWR Viticulture paper**

of the year for the most significant impact on the Australian wine industry.

2012 Sadras VO, Bubner RA, Moran MA A large-scale, open-top system to increase temperature in realistic vineyard conditions. *Agricultural and Forest Meteorology* 154–155, 187– 194

2012 Sadras VO, Petrie PR Predicting the time course of ripening in grapes. *Australian Journal of Grape and Wine Research* 18, 48-56

2012 Fereres E, Goldhamer DA, Sadras VO. 2012. Yield response to water of fruit trees and vines: guidelines. In: Steduto P, Hsiao TC, Fereres E, Raes D, eds. *Crop yield response to water*. Roma: FAO, Irrigation and Drainage Paper 66, 246-295

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2011 Sadras VO, Petrie PR Quantifying the onset, rate and duration of sugar accumulation in berries from commercial vineyards in contrasting climates of Australia. *Australian Journal of Grape and Wine Research* 17, 190-198

2011 Sadras VO, Petrie PR Climate shifts in south-eastern Australia: early maturity of Chardonnay, Shiraz, and Cabernet Sauvignon is associated with early onset rather than faster ripening. *Australian Journal of Grape and Wine Research* 17, 199-205

Chapter 13. Recommendations for further research

Our field experiments characterised a number of traits related to the dynamics of carbon and water of grapevines, including stomatal conductance, leaf photosynthesis, vegetative and reproductive growth, and starch reserves. We also characterised the phenology of vines and attributes of berries relevant to winemaking. The responses of all these traits to elevated temperature (and interactions with water, source:sink ratio and variety) were measured under realistic field conditions. Our experiments explored a wide range of seasonal conditionsin the Barossa Valley.

Both the integration of the information of individual traits, and the extrapolation to other environmental conditions require simulation models. Models are essential tools for the research of crop adaptation to major environmental drivers, and are used as a routine in other industries. Worldwide, simulation models of grapevine have been underdeveloped and underused for strategic applications. There is an urgent need to update vine simulation models to account for the effects of warming and the interactions of temperature and other factors, chiefly water supply.

Elevated temperature in the Barossa Valley showed significant effects on berry composition and wine attributes. Management practices at the vineyard and winery are required to counteract undesirable warming effects. Used on a rotational basis, late pruning is envisaged as a practice with potential to mitigate negative effects of warming on wine balance and compressed harvests.

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