Irrigated Shiraz vines (Vitis vinifera) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field

Article *in* Functional Plant Biology · January 2009

DOI: 10.1071/FP09101

CITATIONS
52

READS
152

3 authors, including:

Marisa Jain Collins
The University of Queensland
26 PUBLICATIONS **348** CITATIONS

SEE PROFILE

Victor O. Sadras
South Australian Research and Development Institute
440 PUBLICATIONS **9,916** CITATIONS

SEE PROFILE

CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb Functional Plant Biology, 2009, 36, 1–14

Irrigated Shiraz vines (*Vitis vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field

Chris J. Soar[A], *Marisa J. Collins*[B] *and Victor O. Sadras*[A,C]

[A]South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia.
[B]CSIRO Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia.
[C]Corresponding author. Email: sadras.victor@saugov.sa.gov.au

Abstract. We tested the hypotheses that (i) a short period of high maximum temperature disrupts gas exchange and arrests berry growth and sugar accumulation in irrigated Shiraz vines (*Vitis vinifera* L.), and (ii) the magnitude of these effects depend on the phenological window when stress occur. Using a system combining passive heating (greenhouse effect) and active cooling (fans) to control daytime temperature, we compared vines heated to a nominal maximum of 40°C for three consecutive days and untreated controls. Maximum air temperature in heated treatments was 7.3°C (2006–07) and 6.5°C (2007–08) above ambient. Heat episodes were aligned with the beginning of a weekly irrigation cycle and applied in one of four phenological windows, namely post-fruit set, pre-veraison, veraison and pre-harvest. Heating systems did not affect relative humidity, hence vapour pressure deficit (VPD) was increased in the heated treatments and tracked the daily cycle of temperature. Heat did not affect the dynamics of berry growth and sugar accumulation, except for a 16% reduction in berry size and sugar content in vines heated shortly after fruit set in 2006–07. Vines upregulated stomatal conductance and gas exchange in response to heat. Stomatal conductance, photosynthesis and transpiration at a common VPD were consistently higher in heated vines than in controls. We suggest that stomatal behaviour previously described as part of Shiraz anisohydric syndrome may be adaptive in terms of heat tolerance at the expense of short-term transpiration efficiency.

Additional keywords: photosynthesis, stomatal conductance, Syrah, transpiration efficiency, total soluble solids, vapour pressure deficit, *Vitis vinifera*.

Introduction

Extreme events are not unprecedented but are uncommon, and play a disproportionate role in shaping the physiology, ecology and evolution of terrestrial plants (Gutschick and Bassirirad 2003). In addition to their biological significance, extreme events including heat waves are highly relevant for the wine industry. Indirect evidence indicates that high temperature may disrupt photosynthesis and berry sugar accumulation in commercial vineyards (AWBC 2008; Retallick and Schofield 2008) and phenological windows when high temperature correlates with low wine quality have been identified (Soar *et al*. 2008). However, these interpretations are speculative in the absence of experiments where heated vines and their products are compared with unheated controls under realistic field conditions.

Many studies of heat stress in vines have been carried out in controlled environments with a dominant focus on low levels of organisation and short-time responses (Wang *et al*. 2004, 2005; Kadir 2006; Wang and Li 2006*a*, 2006*b*; Kadir *et al*. 2007; Liu *et al*. 2008; Wen *et al*. 2008; Zhang *et al*. 2008). The focus of these studies included for instance, subcellular localisation of heat shock proteins (Zhang *et al*. 2008) and short-term ($\leq$24 h) thermotolerance and related antioxidant enzyme activities (Wang and Li 2006*b*). The viticultural relevance of these studies is restricted by one or more factors including (i) the difficulties in scaling up from low (e.g. molecular or cellular) to the crop level of organisation and from short (hours) to seasonal (months) time scales (Struik *et al*. 2007; Sadras *et al*. 2009), (ii) unrealistic growing conditions, e.g. 40/35°C day/night temperature for 4 weeks (Kadir 2006); (iii) artefacts typical of pot-grown plants (Ben-Porath and Baker 1990; Wise *et al*. 1990; McConnaughay and Bazzaz 1991; Sadras *et al*. 1993*a*, 1993*b*; Passioura 2006; Sachs 2006), and (iv) other experimental manipulations, e.g. use of detached berries (Wen *et al*. 2008). Tarara *et al*. (2000) further discuss the artefacts generated from growing plants in controlled environments with a particular focus on grapevine.

Less often, heat stress has been investigated under more realistic field conditions. Tarara *et al*. (2000) used ingenious devices to heat individual bunches, Bowen *et al*. (2004*a*, 2004*b*) used clear polyethylene enclosures around canes or cordons, and Petrie and Clingeleffer (2005) used small plastic chambers to increase bud temperature. All these studies targeted specific questions, such as the separation of temperature and radiation effects, but none of them aimed at the heating of the whole canopy that is typical of heat wave conditions.

10.1071/FP09101 1445-4408/09/080001

In this study, we used closed chambers to simulate short heat episodes in established Shiraz vines (*Vitis vinifera* L.). We tested the hypotheses that (i) three consecutive days of high maximum temperature (~40°C) disrupt leaf gas exchange and arrest berry growth and sugar accumulation, and (ii) the magnitude of these effects depend on the phenological window when stress occur.

Materials and methods

Experiments were carried out over two seasons. In 2006–07, an unreplicated trial aimed at refining heating systems and collecting preliminary data on vine responses. In 2007–08, a fully replicated experiment was carried out to test our working hypotheses.

Site and vines

Experiments were established on a red brown earth (Northcote 1979) at SARDI's Nuriootpa Research Station in the Barossa Valley of South Australia (34°S, 139°E). Gladstones (1992) and Dry and Coombe (2004) described the climate, soils and viticultural practices of the region. We used 10-year old *Vitis vinifera* L. Shiraz vines grafted on Sauvignon Blanc roots (2006–07) and 3-year-old own-rooted Shiraz (2007–08). Vines were spur pruned to 40–50 buds per vine and trained to a single-wire trellis; row spacing was 3.0 m and vine spacing 2.25 m. Vines were drip-irrigated weekly from mid December at a rate of ~21 L vine^{-1} per irrigation.

Heating system

The system combined passive heating (greenhouse effect) and active cooling (fans) to control daytime temperature (Fig. 1). Each chamber enclosed a single panel of three vines and comprised a 6 × 2 × 2 m (L × W × H) steel tubular frame covered with soft PVC sheeting in 2006–07 and solid Standard-Clear-Greca polycarbonate sheeting (Suntuf, Australia) in 2007–08. This material blocks most UV radiation (200–400 nm) and has a very high (90%) and uniform transmittance between 400 and 1600 nm. Maximum temperature was thermostatically controlled by ventilation with outside cooler air circulated by four fixed fans (Nicotra DD9–9T 315W, Australia). Air was distributed through 300 mm PVC ducts with holes (100 mm diameter) at 30 cm intervals (Fig. 1). The thermostats were set to start the fans on the low setting when the temperature in the chamber reached 42°C. The fan speed was automatically increased if temperature continued to rise above the set point. Temperature and humidity both inside and outside the chambers were recorded at 15 min intervals using TinyTag Ultra2 loggers (Hastings Dataloggers, Port Macquarie, Australia) which where shielded in Stevenson type screens.

Treatments and experimental design

In both seasons, untreated (open air) controls were compared with vines heated to a nominal maximum of 40°C for three consecutive days. Comparison with long-term temperature records indicated this represents a rare (frequency: 5.6%) but realistic event in Nuriootpa (Fig. 2). Heat episodes were aligned with the beginning of the weekly irrigation cycle and applied in one of four phenological windows (Appendix 1).

A single chamber was used in 2006–07; replication of heating treatment was therefore limited to the three within-chamber vine replicates rather than actual treatment replicates. However, to increase the confidence in the comparison, four control panels, each of three vines, were included. In 2007–08 the treatments were arranged within a randomised block design with three replicates. Each block contained one timing of heat treatment (a single panel or three vines), and two controls (two panels). Treatment and control plots were arranged to allow for a minimum of one untreated buffer panel or one row between neighbouring plots.

Fig. 1. Heat chamber combining passive heating (greenhouse effect) and active cooling (fans) to control daytime temperature.

Vine measurements

Phenological development was assessed visually using the E-L scale (Coombe 1995). Foliar and bunch temperatures were measured using an Agri-therm 2 infrared thermometer (Everest Interscience; Tucson, Arizona, USA). Measurements were taken in the morning (commencing 1000 hours) and afternoon (commencing 1330 hours) on days 2 and 3 of the heating treatments. In 2006–07, temperature was measured on 15 leaves of treated and control vines. In 2007–08, temperature was measured in two sections of the canopy per replicate at 1 m from the canopy surface at 45° and 135° to the northern face. With the infrared thermometer's variable focal length set to maximum, this generated a measurement spot size ~40 cm diameter. Bunch surface temperatures were measured on 20 randomly selected bunches per replicate at each measurement time in both seasons. The thermometer was held ~5–7 cm from the

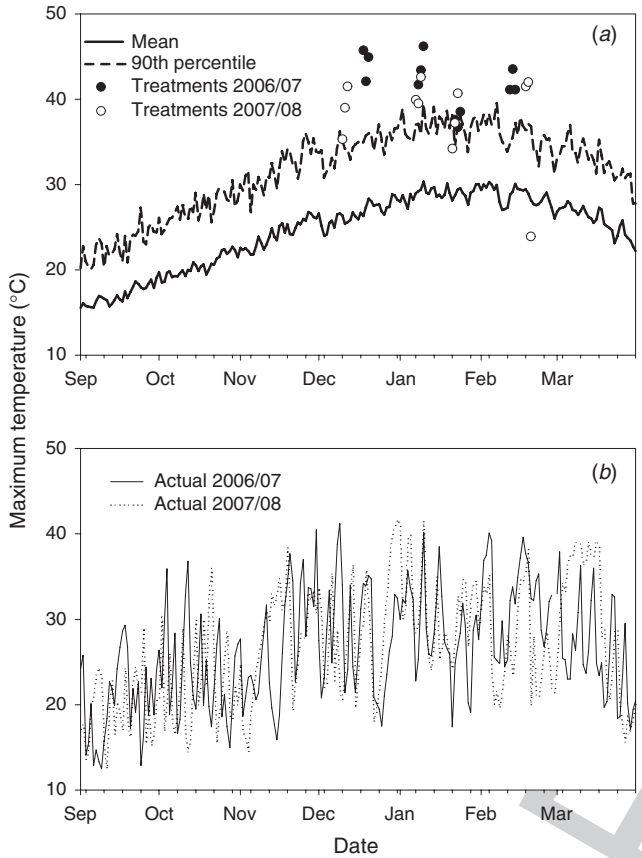

Fig. 2. (*a*) Maximum temperature in heating treatments (circles) compared with long-term average (solid line) and 90th percentile (dashed line) and (*b*) actual maximum temperature during the experiments. Long-term (50 years) records at Nuriootpa, South Australia, are from the Australian Bureau of Meteorology.

bunch, which at the maximum spot size measured a field ~3.5 cm diameter.

In 2007–08, we measured stomatal conductance and gas exchange in the morning (0900 to 1030 hours) and afternoon (1230 to 1400 hours) of the second and/or third day of the post set, pre-veraison, and veraison treatments. Stomatal conductance, transpiration and photosynthesis were measured using a Li-Cor 6400 photosynthesis system with a red-blue LED light source (Li-Cor Environmental Sciences, Lincoln, Nebraska, USA). Transpiration efficiency was calculated as the ratio of photosynthesis and transpiration. For each replicate we measured nine sun-exposed leaves at the top of the canopy; measurement conditions included: chamber temperature set at ambient temperature, saturating PAR (2000 $\mu mol\, m^{-2}\, s^{-1}$), air flow set to 500 $\mu mol\, s^{-1}$ and chamber CO_2 concentration set to 380 $\mu mol\, mol^{-1}$ using an external CO_2 injector. Air flow into Li-Cor 6400 was not scrubbed for humidity. Matching of the sample and reference chambers was performed at the beginning of each treatment replicate (every nine leaves).

Stomatal conductance measured with the Li-Cor 6400 reflects the treatments only to the extent that the conditions in the chamber enclosing the leaf during measurements reflected the environmental conditions of the corresponding treatment; this is particularly relevant for the heated treatments. To account for putative artefacts associated with gas-exchange chamber conditions, we also measured stomatal conductance using an AP4 diffusion porometer (DeltaT devices, Cambridge, UK) in 12 sun-exposed leaves per replicate. The porometer was calibrated according to the instructions in the AP4 manual using the supplied calibration plate. Calibration was repeated in the morning and afternoon and when changing from control to heat chamber readings.

Periodically throughout the season leaf chlorophyll was measured using a SPAD-502 (Minolta, Plainfield, Illinois, USA). Measurements included three spots per leaf × five mature leaves per vine × three vines per replicate.

Berry growth and sugar accumulation

Berries were sampled to determine fresh weight and total soluble solids (TSS) as explained in Sadras *et al*. (2008). Briefly, weekly samples were taken between 0800 and 1100 hours in the period between 5 weeks after full bloom and harvest. Each sample comprised 50 berries per replicate cut with scissors through the pedicel as close as possible to their point of attachment. For the entire 2006–07 season and before veraison in 2007–08, each complete sample was crushed using moderate hand pressure in a zip-lock resealable bag from which the juice fraction was recovered and centrifuged at 3000 g for 10 min. Total soluble solids were measured using a constant temperature bench refractometer. After berry softening in 2007–08 (commencing January 5th), juice + pulp and skins were separated for other analyses (not reported here). A 1 mL-aliquot of juice taken from the mixed juice + pulp sample was spun at 5000*g* in a bench top micro-centrifuge and measurement of TSS was made as previously described.

Statistical analysis

Differences between treatments in canopy and bunch temperature, stomatal conductance, photosynthesis and transpiration were tested with ANOVA (GENSTAT version 11). Associations between pairs of variables (e.g. stomatal conductance and VPD) were explored with regression analysis; statistical significance of quadratic terms was used to test departures from linearity.

Potvin *et al*. (1990) outlined statistical methods to compare response curves involving repeated-measures. Here, we assessed the effect of temperature by comparison of the functions describing the time course of berry weight (BW) and total soluble solids (TSS):

$$BW = a + \frac{BW_{max}}{1 + e^{-\left(\frac{t - t_0}{b}\right)}} \quad (1a)$$

$$TSS = a' + \frac{TSS_{max}}{1 + e^{-\left(\frac{t - t_0'}{b'}\right)}} \quad (1b)$$

where a, a' are constants, subscript max indicates maximum; t_0 and t_0' are the transition centres, i.e. the time when berry weight or total soluble solids are half-way between minimum and maximum, and b and b' are the transition width * 2.197^{-1} (SYSTAT 2002). The transition width is the time (days) it takes for berry weight or soluble solids to raise from 0.25 to 0.75 of maximum (Sadras *et al*. 2008).

Results

Control of temperature in the field

Figure 2 compares maximum temperatures in the heated treatments with climate records, Fig. 3 illustrates the quarter-hourly course of temperature, relative humidity and vapour pressure deficit in heated and untreated controls and Tables A1 and A2 (Appendix 1) summarise the treatments during the two seasons. Maximum ambient temperature rarely exceeded 40°C. Of the 12 treatment days in each season, all except one in 2007–08 were close to or above the 90th percentile for the time of year. Our target temperature was therefore a realistic representation of extreme heat events in the Barossa Valley. As expected from a passive system relying on greenhouse effect, heating was less effective on overcast days, but these were infrequent (e.g. 20 February 2008, Appendix Table A2). Occasionally, the target temperature was surpassed when ambient temperature was over 38°C in the first season; improvements in ventilation prevented this problem in the second season (Appendix 1). Chambers had negligible effects on relative humidity, and therefore vapour pressure deficit tracked temperature (Fig. 3). Chamber air flux was high while the fans were in operation with volume turnover occurring once every 13–16 s depending on fan speed. However, even when the fans were not operating (e.g. at night) relative humidity inside the chamber was not significantly different to ambient.

Canopy and bunch temperature

Inter- and intra-seasonal variation generated a range of maximum ambient temperature from 20.7 to 41.0°C during the treatment periods. Over this two-fold range, the heating treatments consistently increased foliar and bunch temperature relative to controls (Fig. 4).

Stomatal conductance and gas exchange

Heating increased stomatal conductance, leaf transpiration and leaf photosynthesis with more marked effects in the morning than in the afternoon (Fig. 5). For the pooled data, stomatal conductance accounted for 69% of the variation in leaf photosynthesis (inset Fig. 5). Heating had no detectable effect on leaf chlorophyll as measured with SPAD (not shown).

The results obtained with the Li-Cor 6400 in Fig. 5 are a true reflection of the treatments only to the extent that the conditions in the chamber enclosing the leaf during measurements reflected the environmental conditions of the corresponding treatment; this is particularly relevant for the heated treatments. Vapour pressure deficit inside the Li-Cor leaf chamber correlated well with ambient VPD ($r^2 = 0.80$ for controls and 0.77 for heated treatments, both $P < 0.0001$). Furthermore, measurements with a diffusion porometer also showed that high temperature increased stomatal conductance in comparison to controls (Fig. 6).

Effects of temperature at a common VPD

We analysed the effects of temperature at a common VPD on stomatal conductance, leaf photosynthesis and leaf transpiration (Fig. 7). Our experimental design (see Materials and methods) included two control replicates per block to increase the degrees of freedom for comparisons, and this involved a trade-off in terms of higher density of measurements in controls compared with heated treatments. Stomatal conductance and gas exchange were therefore uniformly distributed in the range of VPD from ~1.1 to 5.1 kPa in controls, whereas measurements in heated leaves were more clustered at the extremes of the range with a gap between ~3 and 4 kPa. The clustering of data in the heated treatment was, however, unrelated to morning *v.* afternoon measurements (Fig. 7).

Stomatal conductance at a common VPD was consistently higher in heated vines than in controls (Fig. 7*a*). Non-linear terms in the response of stomatal conductance to VPD were not significant, i.e. $P > 0.91$ in controls and $P > 0.31$ in heated vines. The linear rate of change in stomatal conductance with VPD was 62% higher in heated vines than in controls (-89 ± 16.5 *v.* -55 ± 9.6 mmol $H_2O\,m^{-2}\,s^{-1}\,kPa^{-1}$) but this difference was not significant ($P > 0.05$). Measurements with diffusion porometer reinforced the conclusion that heating increased stomatal conductance at a common VPD (inset Fig. 7*a*).

Photosynthetic rate at a common VPD was consistently higher in heated vines than in controls (Fig. 7*b*). Non-linear terms in the response of net photosynthesis to VPD were not significant, i.e. $P > 0.27$ in controls and $P > 0.86$ in heated vines. The linear rate of change in photosynthesis with VPD was similar in both treatments, i.e. -1.27 ± 0.154 in controls and -1.36 ± 0.215 μmol $CO_2\,m^{-2}\,s^{-1}\,kPa^{-1}$ in heated vines.

Leaf transpiration rate of controls increased with VPD at an average rate of 0.73 ± 0.138 mol $H_2O\,m^{-2}\,s^{-1}\,kPa^{-1}$ (non-linear term: $P > 0.35$) and was unrelated to VPD ($P > 0.45$) in heated vines (Fig. 7*c*). Transpiration of control leaves at a common VPD was higher in the afternoon than in the morning (dotted lines in Fig. 7*c*). Consistent with this response of transpiration and the lack of hysteresis in photosynthesis (Fig. 7*b*), the plot of transpiration efficiency as a function of VPD^{-1} revealed a strong diurnal hysteresis in controls (Fig. 8). In controls, transpiration efficiency at low VPD was higher in the morning than in the afternoon, and morning and afternoon efficiencies converged with high VPD. The response of transpiration efficiency to VPD in heated leaves was similar to that of controls in the afternoon.

Berry growth and sugar accumulation

The use of different plant material in the two seasons (see Method) did not seem to generate differences in berry responses to temperature (Fig. 9). A single model (Eqn 1a) with common parameters for all treatments accounted for 96.4% of the variance in berry weight in 2006–07 and 96.5% of the variance in 2007–08 (both $P < 0.001$). Adding a separate constant a for each treatment but maintaining a common value for BW_{max} and b improved the model significantly ($P < 0.001$). This improvement is related to the smaller berry weight for post-set treatment in 2006–07 and the veraison treatment in 2007–08. Further tests directly comparing these two treatments and their respective controls indicated significant differences ($P < 0.05$), i.e. the slopes of the regressions between berry weight in heated and control berries were lower than 1, i.e. 0.79 ± 0.059 g g^{-1} for post-set treatment in 2006–07 and 0.90 ± 0.032 g g^{-1} for the veraison treatment in 2007–08 (insets in Fig. 9).

A single model (Eqn 1b) with a common set of parameters accounted for 99% of the variation in accumulation of total

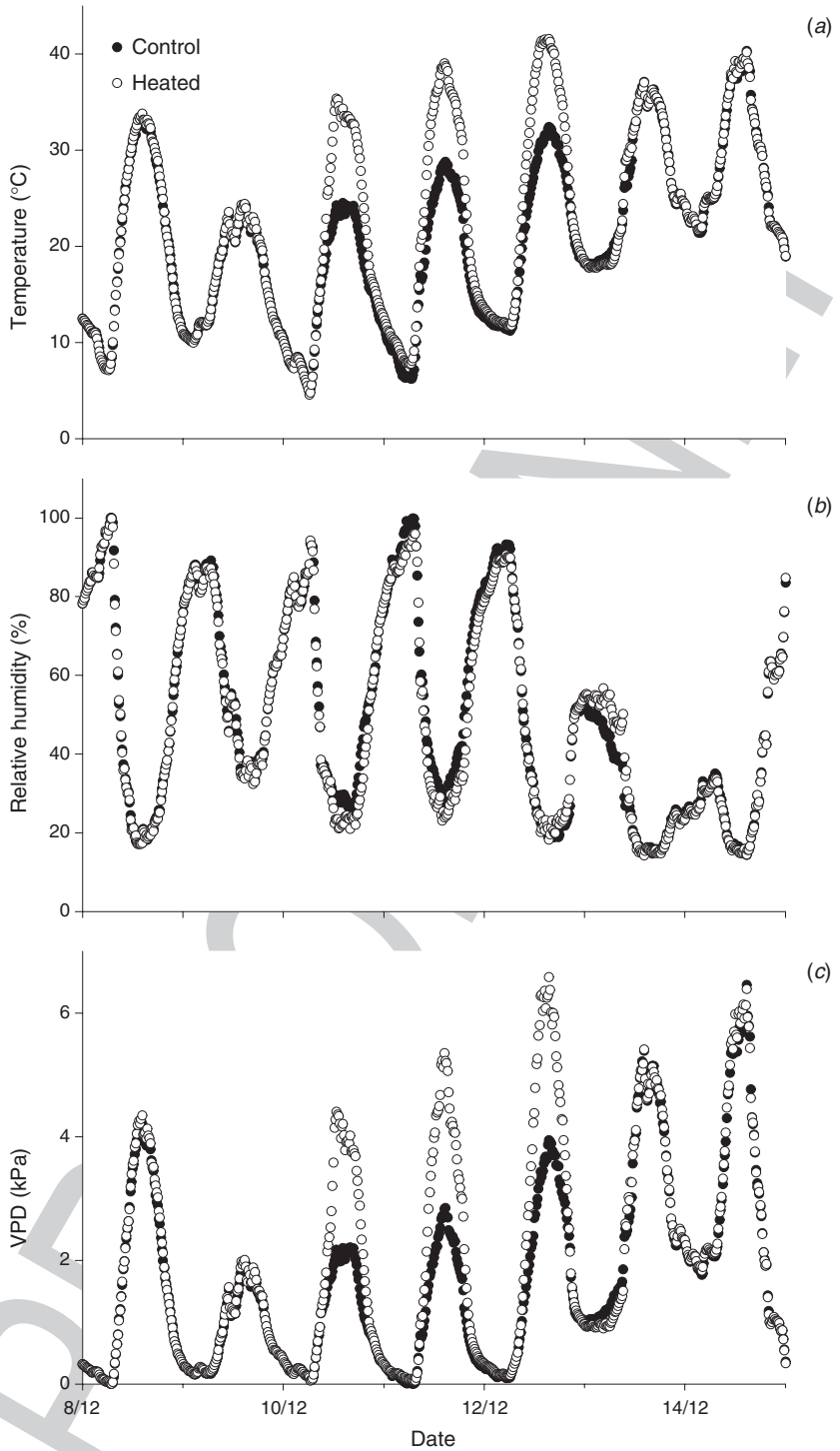

Fig. 3. Temperature, relative humidity and vapour pressure deficit inside (open symbols) and outside (closed symbols) the heating chambers during the post-set treatment in 2007. The treatment was applied from the 10th to the 12th of December inclusive. As background, trajectories are expanded by two days both sides of the treatment period.

soluble solids ($P < 0.001$) indicating no difference between treatments in 2007–08 (Fig. 9). In 2006–07, the best model included a different constant term (a') for each treatment ($P < 0.001$, $r^2 = 0.99$), allowing for a slightly lower TSS in the post-set treatment. Direct comparison of post-set and control treatments, however, indicated no significant difference in TSS

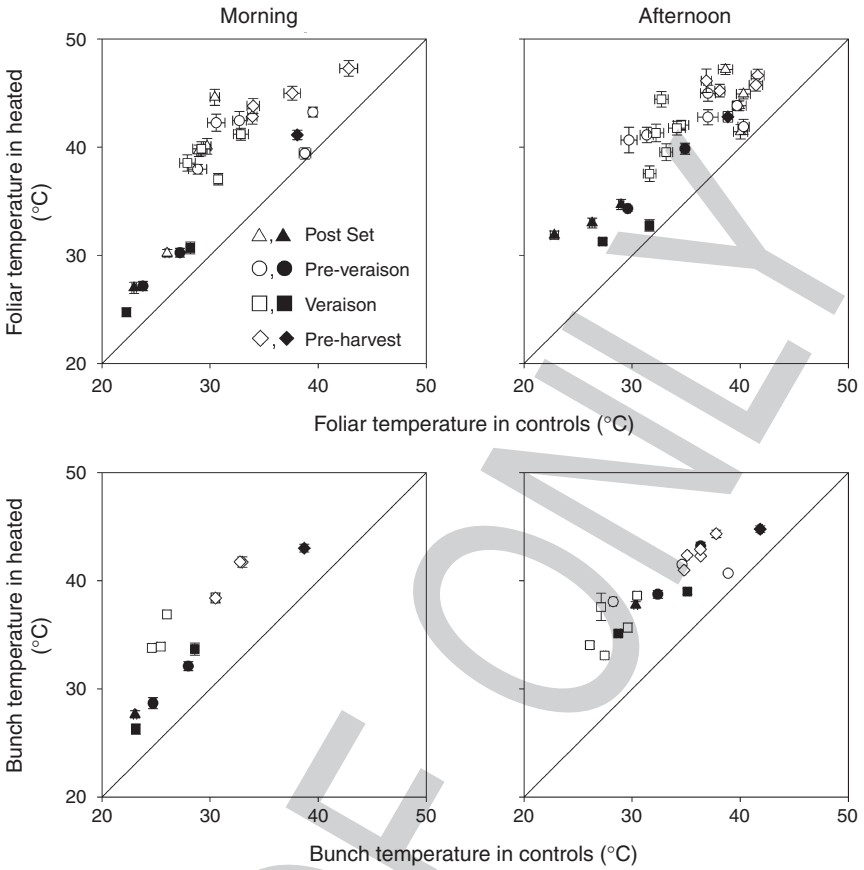

Fig. 4. Comparison of foliar temperature and bunch temperature in heated and controls vines in 2006–07 (open symbols) and 2007–08 (closed symbols). Foliar temperature is single leaf (2006–07) or canopy (2007–08). The solid line is $y = x$ and error bars are s.e.m.

($P > 0.05$) and, associated with differences in berry weight, a 16% reduction in the amount of sugar per berry ($P < 0.05$).

Discussion

Heating treatments: realism, limitations and potential artefacts

Our heating treatments generated extreme but realistic maximum temperatures (Fig. 2). To avoid unrealistic interactions between temperature and vapour pressure deficit in experiments where temperature is manipulated, vapour pressure deficit rather than relative humidity needs to be controlled (Hall and Sadras 2009). An important feature of our treatments was therefore the realistic time courses of vapour pressure deficit (Fig. 3). Similarly important, a high turnover rate, i.e. up to one chamber volume replaced every 13 s, prevented air stratification in the chamber.

The main aspect where treatments departed from real heat wave conditions is that we did not manipulate night temperature, which is normally above average during heat waves (W. Grace, pers. comm.) Night temperature can affect plant processes with consequences for crop yield and quality (Thomas and Raper 1981; Warrag and Hall 1984; Mutters and Hall 1992; Mori *et al.* 2005; Koshita *et al*. 2007; Aguirrezábal *et al*. 2009).

Other secondary effects included a slight reduction in PAR (10%), increased diffuse radiation and reduced UV radiation. Measurements and modelling support the notion that canopy and ecosystem photosynthesis increase with increased diffuse radiation (Sinclair *et al*. 1992; Roderick *et al*. 2001; Rodriguez and Sadras 2007; Urban *et al*. 2007; Wohlfahrt *et al*. 2008). Where diffuse radiation increased in association with cloudiness and atmospheric particles, three mechanisms accounted for enhancement of photosynthesis: (i) canopy changes, i.e. improved distribution of light in the canopy profile, (ii) microclimatic changes, i.e. reduction in temperature and VPD, and (iii) leaf changes, i.e. stimulation of photochemical reactions and stomatal opening via an increase of blue/red light ratio (Urban *et al*. 2007). The first mechanism is quantitatively the most important (Roderick *et al*. 2001; Urban *et al*. 2007; Wohlfahrt *et al*. 2008) but is not relevant to our measurements of individual leaf photosynthesis using a red-blue LED light source under saturating light. Likewise, microclimatic changes were not relevant, as high diffuse radiation in the chambers was paralleled with high temperature and high VPD. Changes in leaf-level photosynthesis associated with changes in blue/red ratio of light were unlikely, as the chamber has a high and uniform transmittance between 400 and 1600 nm. Long-term exclusion or enhancement of UV-B alters leaf traits including

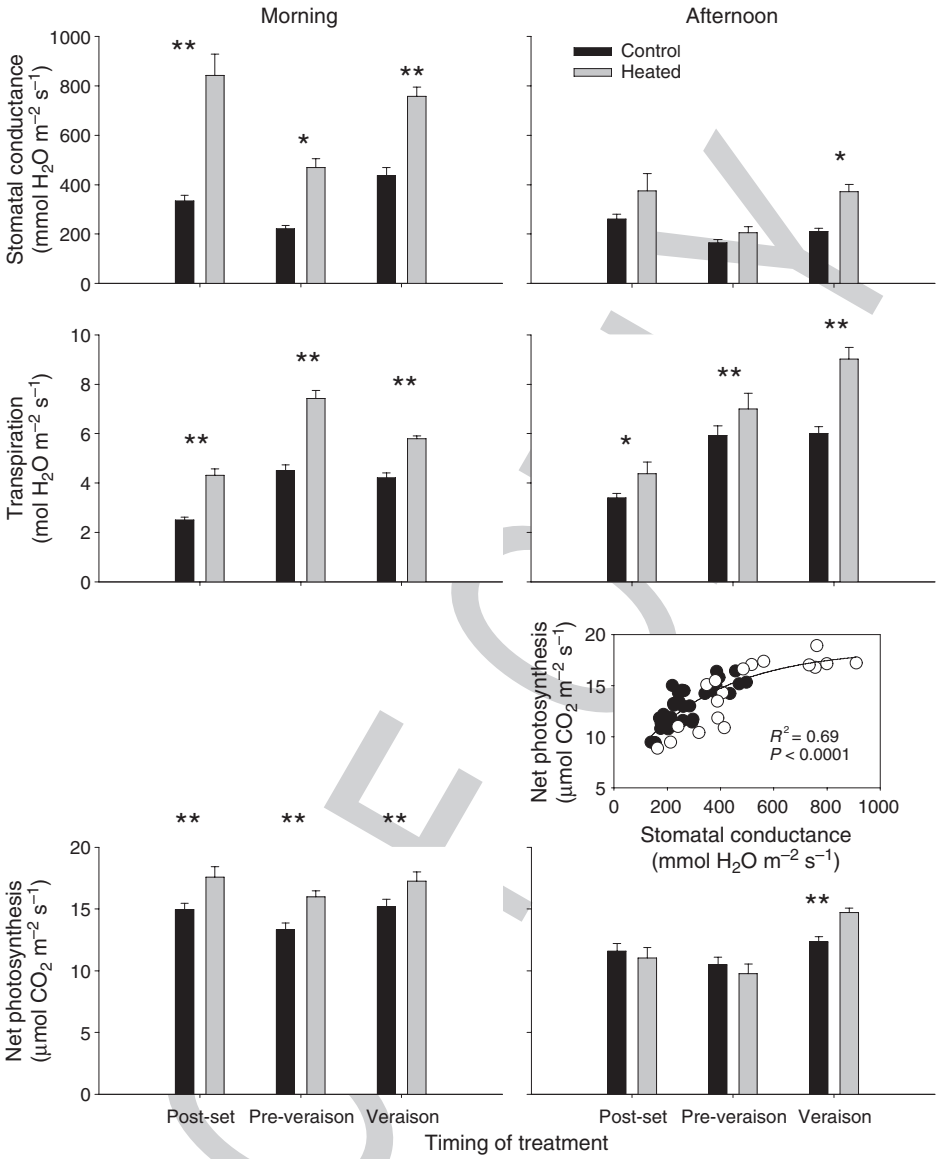

Fig. 5. Stomatal conductance, leaf transpiration and net photosynthesis of heated and control vines in the morning and afternoon of the second day of heating for the post-set, pre-veraison and veraison treatments. Error bars are standard errors of the means and asterisks indicate significant differences between heated and control vines as tested by ANOVA (*, $P < 0.05$; **, $P < 0.01$). Inset shows the relationship between net photosynthesis and stomatal conductance for the pooled data from control (closed symbols) and heated (open symbols) treatments. Data from 2007–08 using Li-Cor 6400 system.

photosynthetic pigment composition, specific leaf mass and UV-B absorbing flavonoids (Láposi *et al*. 2009). To minimise the impact of these and other secondary factors, we established treatments for only 3 days.

Canopy temperature, stomatal conductance and gas exchange

Studies with grapevine in controlled environments showed that heat stress can trigger the production and accumulation of heat-shock proteins in young leaves, reduce stomatal conductance, disrupt the photosynthetic apparatus and reduce CO_2 assimilation (Sepulveda and Kliewer 1986; Kadir 2006; Kadir *et al*. 2007; Zhang *et al*. 2008). Growing conditions in most of these studies, however, are unrepresentative of vineyard situations (see Introduction).

Our aim was to measure vine responses in realistic field conditions, while minimising secondary effects from heat chambers. We found that recently irrigated Shiraz vines responded to short duration heat shock with an increase in stomatal conductance, a corresponding increase in transpiration, a small to moderate increase in photosynthesis and no evident degradation of leaf chlorophyll in young leaves.

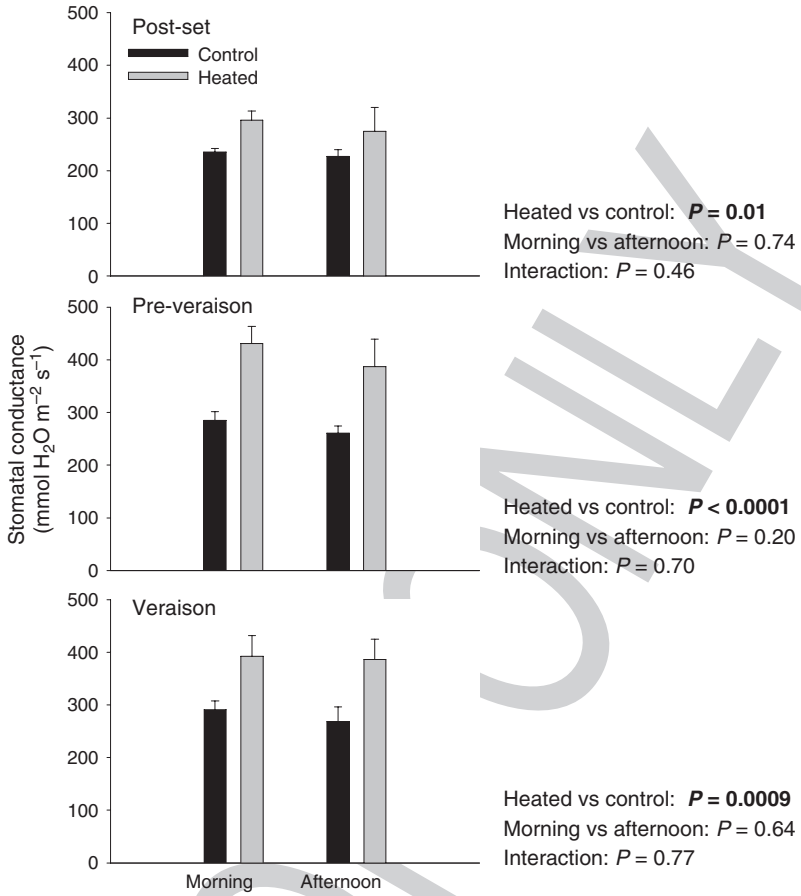

Fig. 6. Stomatal conductance measured with diffusion porometer in heated and control vines in the morning and afternoon of the second and/or third day of heating for the post-set, pre-veraison and veraison treatments. Error bars are s.e.m. *P*-values are from ANOVA. Data from 2007–08.

Bowen *et al.* (2004*a*, 2004*b*) used clear polyethylene enclosures around Merlot canes or cordons which increased maximum temperatures by 5–8°C and enhanced photosynthetic rate in association with increased mesophyll and stomatal conductance in relation to controls. Our data indicated stomatal conductance was the dominant source of variation in photosynthesis (inset of Fig. 5). Diffuse radiation inside the chamber might have contributed to the enhancement of photosynthesis in heated leaves, but this was unlikely, as discussed in the previous section. In our study relative humidity in the chambers was not significantly different from ambient and vapour pressure deficit was dramatically and realistically increased (Hall and Sadras 2009). We propose, therefore, that the increased stomatal conductance, verified with both diffusion porometer and gas exchange measurements, and enhanced gas exchange in our heated vines was a likely response to high temperature rather than an artefact of growing conditions.

Generally, stomatal responses partially counteract shifts in the balance between supply and demand of water, e.g. increased VPD shifts the hydraulic balance towards demand, and stomata respond to increased transpiration rate by reducing their apertures (Mott and Parkhurst 1991; Fredeen and Sage 1999; Buckley 2005). The coordination between stomatal conductance and water balance is generally accepted, but the underlying mechanisms are still controversial (Buckley 2005). In this context, the responses of stomatal conductance and gas exchange to increasing VPD in controls were typical (Brodribb and Jordan 2008; Pou *et al.* 2008): stomatal conductance, photosynthesis and water use efficiency decreased and transpiration increased. Against this pattern, heated vines showed qualitative and quantitative differences (Fig. 7).

Fredeen and Sage (1999) concluded that VPD and leaf temperature have independent effects on stomatal conductance of *Picea glauca* (Moench) Voss., and consolidated this notion in a two-phase model of transpiration *v.* VPD (their fig. 4). In the first phase, transpiration increased linearly with VPD up to a temperature-dependent threshold, e.g. ~2 kPa at leaf temperature of 35°C. In the second phase, transpiration was stable. In the context of stomata responses to supply and demand of water, they proposed this pattern was mediated by (i) a reduction in water viscosity and increase in plant membrane permeability with increasing temperature leading to (ii) a linear increase in water supply to guard cells, and (iii) an exponential increase in VPD with temperature eventually leading to (iv) a decline in stomatal conductance restricting transpiration (Fredeen and Sage 1999). We suggest that the lack of net response of transpiration to VPD

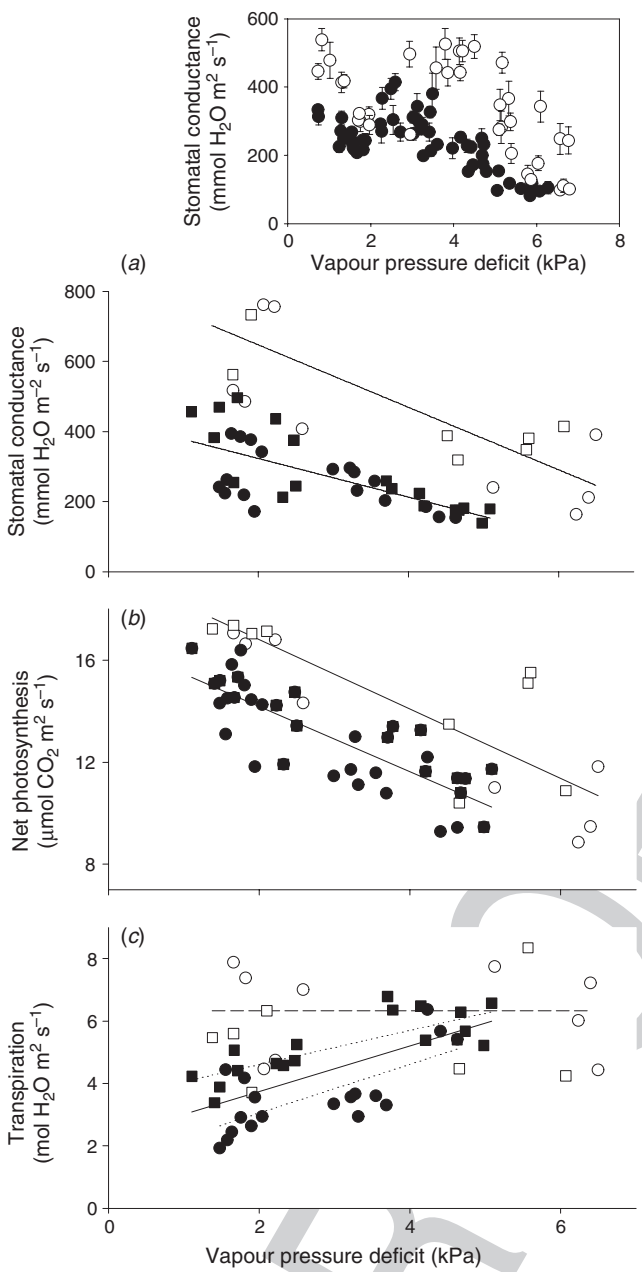

Fig. 7. Relationships between (*a*) stomatal conductance, (*b*) transpiration, and (*c*) net photosynthesis and vapour pressure deficit for heated (open symbols) and control (closed symbols) vines. Measurements were taken in the morning (circles) and afternoon (squares) of the second day of heating applied at post-set, pre-veraison and veraison; each point is the average of nine leaves. Solid lines are linear regressions for the pooled morning and afternoon data ($0.46 \leq r^2 \leq 0.71$, $P < 0.0001$). In (*c*), the dashed line represents the average transpiration of heated leaves across VPD (regression not significant, $P > 0.45$) and the dotted lines are separate regressions for morning and afternoon measurements. Inset shows the relationship between stomatal conductance measured with diffusion porometer and VPD for heated (open symbols) and control (closed symbols) vines. Data from 2007–08.

in our heated vines (Fig. 7*c*) corresponded to the second phase in the model of Fredeen and Sage (1999) where non-stomatal limitations were impacting on water loss from the leaf surface. We speculate that the first phase was not evident because of the scarcity of data for VPD <2 kPa under our experimental conditions.

Irrespective of the physiological mechanism, stomatal regulation allowed for heated, well-watered Shiraz to maintain a relatively high and steady transpiration flux independent of VPD (dashed line in Fig. 7*c*). The lack of relationship between leaf transpiration and VPD, and the maintenance of a relatively high transpiration rate in our heated vines compares with the decoupling and maintenance of a relatively low rate of transpiration in water-stressed grapevine (Pou *et al*. 2008) and olive (*Olea europaea* L.; fig. 5 in Moriana *et al*. 2002). Stomatal regulation has been often interpreted in terms of optimisation of transpiration efficiency and prevention of xylem cavitation (Kramer and Boyer 1995; Buckley 2005). We suggest that stomatal responses previously described as part of Shiraz anisohydric behaviour (Schultz 2003; Soar *et al*. 2006) may play a role in terms of heat stress tolerance. In common with previous reports in other species (Lu *et al*. 1994; Radin *et al*. 1994; Amani *et al*. 1996), our study indicates that stomatal regulation in heat-stressed Shiraz may have favoured evaporative cooling at the expense of short-term transpiration efficiency. A corollary of this is that timely pulses of water before heat waves could help mitigate the otherwise damaging effects of high daytime ambient temperature on the photosynthetic capacity of plants with anisohydric stomatal regulation. Specific studies on the interaction between water supply, heat stress and cultivar are required for a direct test of this hypothesis; viticultural implications are discussed below.

Berry growth and sugar accumulation

We characterised the dynamics of berry growth and sugar accumulation to test the hypothesis that high maximum temperature alone can disrupt berry development and ripening, and to determine whether there are phenological stages that are more vulnerable to high temperature. We found significant reductions in berry size for the post-set treatment in 2006–07 and the veraison treatment in 2007–08 (Fig. 8). A reduction in berry size associated with high temperature shortly after fruit set is consistent with the active process of cell division in fruit tissues at this developmental stage (Coombe and Iland 2004). Heating in the post-set stage raised maximum temperatures up to 42.1–45.7°C in 2006–07 whereas the corresponding treatment in 2007/08 reached 35.3–41.5°C (Appendix 1). These differences may account for the measurable effect in 2006–07 (i.e. 16% reduction in berry size and sugar per berry) and the lack of response in 2007–08. The reduction of berry size in the veraison treatment in 2007–08 was clearly associated with differences in berry size before the imposition of the treatment, so we can safely conclude this difference was related to vine-to-vine variability rather than to the treatment. The dynamics of TSS in berries were largely unaffected by heating.

Some studies supported the notion that high temperature could reduce berry growth in a process mediated by reductions in stomatal conductance and net carbon assimilation (Matsui *et al*. 1986; Sepulveda and Kliewer 1986). Kliewer (1977) reported variety-dependent reductions in berry size when vines were exposed to high temperature (32–40°C) from bloom to fruit

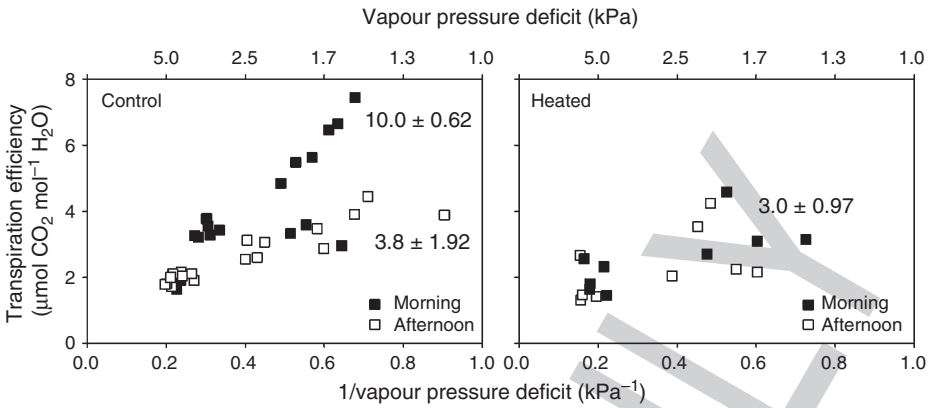

Fig. 8. Transpiration efficiency as a function of the inverse of vapour pressure deficit in leaves from control and heated vines. Measurements were taken in the morning and afternoon of the second day of heating applied at either post-set, pre-veraison and veraison; each point is the average of nine leaves. Numbers are slopes (±s.e.) of linear regressions for measurements in the morning and afternoon for controls and for the pooled data in heated leaves, as morning and afternoon responses were statistically undistinguishable ($P > 0.05$). The top *x*-axis indicates vapour pressure deficit to facilitate interpretation, but note this scale is not linear. Data from 2007–08.

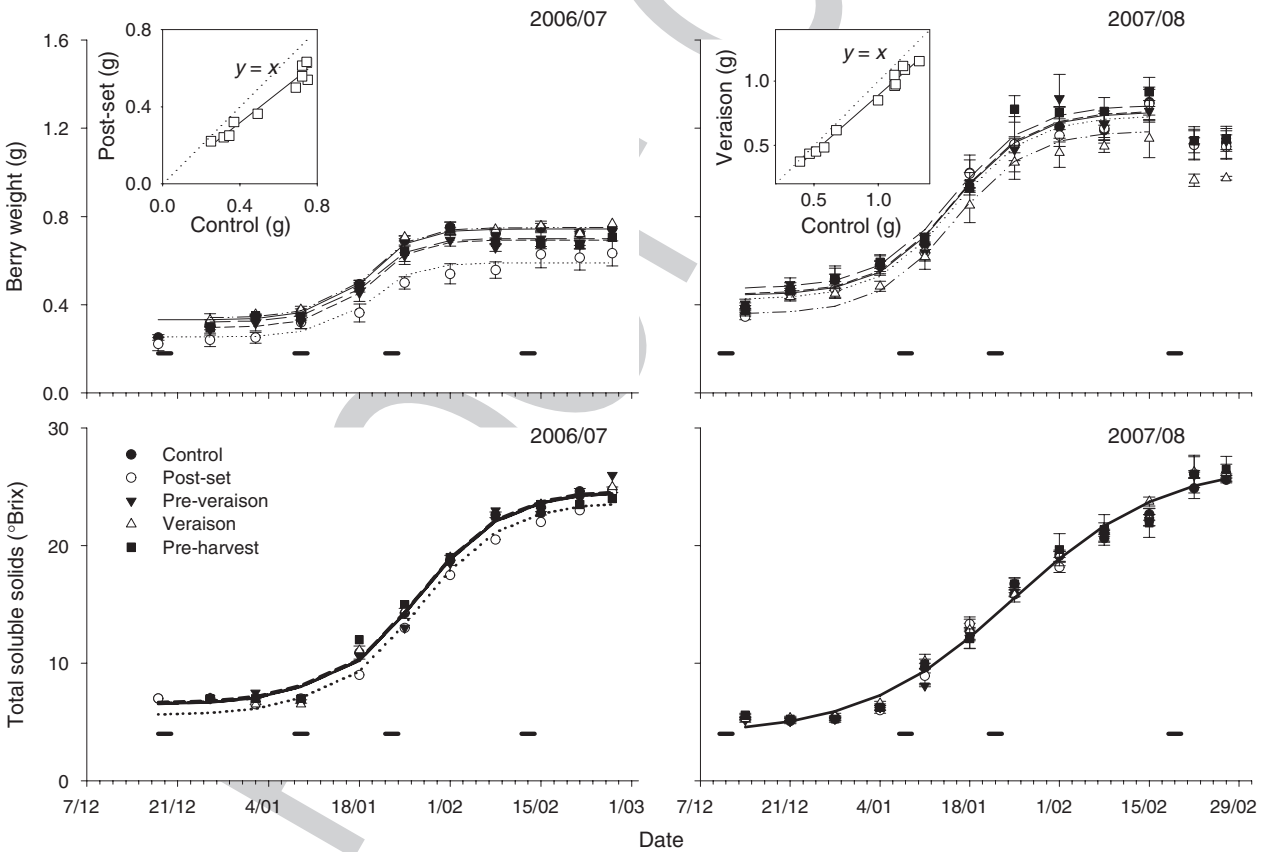

Fig. 9. Dynamics of berry growth and total soluble solids in berries from controls, and from vines heated during 3 days at one of four phenological stages (horizontal bars). Error bars represent s.e.m. Fitted curves are Eqn 1. In 2007–08, measurements of berry weight during the shrinkage period (Sadras and McCarthy 2007) were not used to fit the curves (two last dates).

set in controlled environments. Some controlled-environment experiments indicated that sugar accumulation is sensitive to temperature at early berry growth stages (Buttrose *et al*. 1971; Hale and Buttrose 1974), others pointed to post-veraison as a vulnerable stage (Jackson and Lombard 1993) and many found no effects (Radler 1965; Kliewer 1970; Spayd *et al*. 2002).

Differences in duration and intensity of heat stress, interactions with other factors (chiefly water supply and radiation), artefacts from controlled environments or a combination of these may account for the differences with our study where we found little or no alteration of berry growth in vines exposed to short episodes of heat stress at several phenological stages.

Viticultural implications

The short, extreme heat treatments imposed to field-grown irrigated Shiraz did not affect berry growth or sugar accumulation, except for the post-set treatment in 2006–07 when maximum temperature was maintained above 42°C for three consecutive days. Consistent with the ability of irrigated Shiraz to maintain berry growth and sugar accumulation, stomatal conductance and gas exchange were either maintained at the levels of controls or enhanced. In addition to the duration of heat stress, three main factors could account for the discrepancy between this response and the apparent arrest of berry growth commonly attributed to heat waves in the Australian wine industry: night temperature, wind and water supply. Heat waves in south-eastern Australia increase not only maximum but also night temperature and are associated with northerly hot and dry winds (Grace and Curran 1993). For some plant processes such as seed set and seed composition, the influence of temperature may be more prominent during the night than during the day (Warrag and Hall 1984; Mutters and Hall 1992; Aguirrezábal *et al*. 2009). In grapevine, high night temperature may alter berry composition (Kliewer 1973; Koshita *et al*. 2007). Under the experimental conditions of Greenspan *et al*. (1996), Cabernet Sauvignon berries showed marked day–night fluctuations, i.e. daytime reduction and night-time increase in diameter, that were more noticeable before veraison and under water deficit. Wind speed alters the boundary layer of the canopy and therefore influences the response of transpiration to temperature, vapour pressure deficit and stomatal conductance (Jarvis and McNaughton 1986; Aphalo and Jarvis 1993). Heating in this study was applied at the beginning of an irrigation cycle, therefore the chances of water deficit arising during treatment were reduced and the capacity for evaporative cooling was maintained. However, water deficit and heat stress often co-occur, particularly in rainfed systems or production systems with limited irrigation. Both stresses interact in complex ways at scales from molecular to whole-crop and regional (Warrag and Hall 1984; Barnabas *et al*. 2008; Dalla-Salda *et al*. 2009). The generally non-additive effect of water and heat stress has potentially damaging consequences. Crown necrosis and death of individual trees, for example, were reported following the heat and drought wave of 2003 in France (Dalla-Salda *et al*. 2009).

Extrapolation of these results to other grapevine species or varieties needs to account for genotype-dependent thermotolerance and water relations (Schultz 1997; Kadir 2006; Soar *et al.* 2006; Kadir *et al*. 2007). In the study of Kadir (2006), the reduction in quantum efficiency of Photosystem II after exposure to 40/35°C (day/night) ranked Cabernet Sauvignon > Semillon > Pinot Noir. Differences between anisohydric (e.g. Shiraz) and isohydric (e.g. Grenache) types in stomatal response to VPD and soil drying (Schultz 2003; Soar *et al*. 2006) may be relevant for heat stress tolerance. In comparison to Grenache, Shiraz maintained high stomatal conductance and transpiration in dry soil, i.e. predawn leaf water potential up to –1.4 MPa (Schultz 2003) and high VPD, i.e. up to 5 kPa (Soar *et al*. 2006). Anisohydric behaviour may contribute to heat dissipation, provided soil water content is sufficient to maintain transpiration, whereas reduction in stomatal conductance in isohydric plant types might enhance the damaging effects of high ambient temperature. Upregulation of stomatal conductance improved the tolerance to heat stress in some combinations of plants and environments (Radin *et al*. 1994; Banowetz *et al*. 2008; Natarajan and Kuehny 2008) but direct comparisons among grape varieties are required, including the probing for trade-offs between upregulation of stomatal conductance and other attributes with putative value for heat tolerance, e.g. membrane thermostability (Blum *et al*. 2001; Hong *et al*. 2003).

Depending on the relative importance of day and night temperature, wind, and the interaction between heat, water supply and cultivar, different practices may have different effectiveness in dealing with heat waves. For example, shading is likely to be less effective for physiological disruption caused by high night temperature and irrigation is less likely to be effective in isohydric plant types. Thus, disentangling the main components of heat waves and accounting for variety-dependent interactions between temperature and water are critical to devise management strategies to deal with heat stress in vineyards. Irrigated Shiraz in our study, however, showed a larger than expected capacity to cope with three consecutive days of extreme heat, partially accounted by physiological upregulation of gas exchange.

Acknowledgements

We thank Brian Loveys for input in design of the chamber and insightful comments on the manuscript, Mike McCarthy for expert advice, and Tony Condon, Peter Clingeleffer and Everard Edwards for critical comments on the manuscript. Craig Chidgey, Tim Abbot, Kerryn O'Brien, Treva Hebberman, and Tony Gerlach provided valuable technical support. The Australian Grape and Wine Research and Development Corporation (Grants SAR 05–01; CSP 05–02) and the River Murray Improvement Program funded this research. VOS is an affiliate member of The University of Adelaide.

References

Aguirrezábal L, Martre P, Pereyra-Irujo G, Izquierdo N, Allard V (2009) Management and breeding strategies for the improvement of grain and oil quality. In 'Crop physiology: applications for genetic improvement and agronomy'. (Eds VO Sadras, DF Calderini) pp. 387–421. (Academic Press: San Diego)

Amani I, Fischer RA, Reynolds MP (1996) Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *Journal Agronomy & Crop Science* **176**, 119–129. doi: 10.1111/j.1439-037X.1996.tb00454.x

Aphalo PJ, Jarvis PG (1993) The boundary layer and the apparent responses of stomatal conductance to wind speed and to the mole fractions of CO_2 and water vapour in the air. *Plant, Cell & Environment* **16**, 771–783. doi: 10.1111/j.1365-3040.1993.tb00499.x

AWBC (2008) http://www.wineaustralia.com/australia/Default.aspx?tabid=802 [Verified 23 June 2009]

Banowetz GM, Azevedo MD, Stout R (2008) Morphological adaptations of hot springs panic grass (*Dichanthelium lanigunosum* var. sericeum (Schmoll) to thermal stress). *Journal of Thermal Biology* **33**, 106–116.

Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell & Environment* **31**, 11–38.

Ben-Porath A, Baker DN (1990) Tap-root restriction effects on growth, earliness, and dry weight partitioning of cotton. *Crop Science* **30**, 809–814.

Blum A, Klueva N, Nguyen HT (2001) Wheat cellular thermotolerance is related to yield under heat stress. *Euphytica* **117**, 117–123. doi: 10.1023/A:1004083305905

Bowen PA, Bogdanoff CP, Estergaard B (2004*a*) Impacts of using polyethylene sleeves and wavelength selective mulch in vineyards. I. Effects on air and soil temperatures and degree day accumulation. *Canadian Journal of Plant Science* **84**, 545–553.

Bowen PA, Bogdanoff CP, Estergaard B (2004*b*) Impacts of using polyethylene sleeves and wavelength selective mulch in vineyards. II. Effects on growth, leaf gas exchange, yield components and fruit quality of *Vitis vinifera* cv. Merlot. *Canadian Journal of Plant Science* **84**, 555–568.

Brodribb TJ, Jordan GJ (2008) Internal coordination between hydraulics and stomatal control in leaves. *Plant, Cell & Environment* **31**, 1557–1564. doi: 10.1111/j.1365-3040.2008.01865.x

Buckley TN (2005) The control of stomata by water balance. *New Phytologist* **168**, 275–292. doi: 10.1111/j.1469-8137.2005.01543.x

Buttrose MS, Hale CR, Kliewer WM (1971) Effect of temperature on the composition of 'Cabernet Sauvignon' berries. *American Journal of Enology and Viticulture* **22**, 71–75.

Coombe BG (1995) Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research* **1**, 104–110. doi: 10.1111/j.1755-0238.1995.tb00086.x

Coombe BG, Iland PG (2004) Grape berry development and winegrape quality. In 'Viticulture. Vol. 1. Resources'. (Eds PR Dry, BG Coombe) pp. 210–248. (Winetitles: Adelaide)

Dalla-Salda G, Martinez-Meier A, Cochard H, Rozenberg P (2009) Variation of wood density and hydraulic properties of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) clones related to a heat and drought wave in France. *Forest Ecology and Management* **257**, 182–189. doi: 10.1016/j.foreco.2008.08.019

Dry PR, Coombe BG (Eds) (2004) 'Viticulture. Vol. 1. Resources.' (Winetitles: Adelaide)

Fredeen AL, Sage RF (1999) Temperature and humidity effects on branchlet gas-exchange in white spruce: an explanation for the increase in transpiration with branchlet temperature. *Trees – Structure and Function* **14**, 161–168.

Gladstones JS (1992) 'Viticulture and environment.' (Winetitles: Adelaide)

Grace W, Curran E (1993) A binormal model of frequency distributions of daily maximum temperature. *Australian Meteorological Magazine* **42**, 151–161.

Greenspan MD, Schultz HR, Matthews MA (1996) Field evaluation of water transport in grape berries during water deficit. *Physiologia Plantarum* **97**, 55–62. doi: 10.1111/j.1399-3054.1996.tb00478.x

Gutschick VP, Bassirirad H (2003) Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist* **160**, 21–42. doi: 10.1046/j.1469-8137.2003.00866.x

Hale CR, Buttrose MS (1974) Effect of temperature on ontogeny of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Journal of the American Society for Horticultural Science* **99**, 390–394.

Hall AJ, Sadras VO (2009) Whither crop physiology? In 'Crop physiology: applications for genetic improvement and agronomy'. (Eds VO Sadras, DF Calderini) pp. 541–570. (Academic Press: San Diego)

Hong S-W, Lee U, Vierling E (2003) *Arabidopsis* hot mutants define multiple functions required for acclimation to high temperatures. *Plant Physiology* **132**, 757–767. doi: 10.1104/pp.102.017145

Jackson DI, Lombard PB (1993) Environmental and management practices affecting grape composition and wine quality – a review. *American Journal of Enology and Viticulture* **44**, 409–430.

Jarvis P, McNaughton K (1986) Stomatal control of transpiration: scaling up from leaf to region. *Advances in Ecological Research* **15**, 1–49. doi: 10.1016/S0065-2504(08)60119-1

Kadir S (2006) Thermostability of photosynthesis of *Vitis aestivalis* and *V. vinifera*. *Journal of the American Society for Horticultural Science* **131**, 476–483.

Kadir S, Von Weihe M, Al-Khatib K (2007) Photochemical efficiency and recovery of photosystem II in grapes after exposure to sudden and gradual heat stress. *Journal of the American Society for Horticultural Science* **132**, 764–769.

Kliewer WM (1970) Effect of day temperature and light intensity on colouration of *Vitis vinifera* L. grapes. *Journal of the American Society for Horticultural Science* **95**, 693–697.

Kliewer WM (1973) Berry composition of *Vitis vinifera* cultivars as influenced by photo- and nycto- temperatures during maturation. *Journal of the American Society for Horticultural Science* **98**, 153–159.

Kliewer WM (1977) Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. *American Journal of Enology and Viticulture* **28**, 215–222.

Koshita Y, Asakura T, Fukuda H, Tsuchida Y (2007) Nighttime temperature treatment of fruit clusters of 'Aki queen' grapes during maturation and its effect on the skin color and abscisic acid content. *Vitis* **46**, 208–209.

Kramer PJ, Boyer JS (1995) 'Water relations of plants and soils.' (Academic Press: San Diego)

Láposi R, Veres S, Lakatos G, Oláh V, Fieldsend A, Mészáros I (2009) Responses of leaf traits of European beech (*Fagus sylvatica* L.) saplings to supplemental UV-B radiation and UV-B exclusion. *Agricultural and Forest Meteorology* **149**, 745–755. doi: 10.1016/j.agrformet.2008.10.023

Liu HT, Liu YP, Huang WD (2008) Root-fed salicylic acid in grape involves the response caused by aboveground high temperature. *Journal of Integrative Plant Biology* **50**, 761–767. doi: 10.1111/j.1744-7909.2008.00640.x

Lu Z, Radin JW, Turcotte EL, Percy R, Zeiger E (1994) High yields in advanced lines of Pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. *Physiologia Plantarum* **92**, 266–272. doi: 10.1111/j.1399-3054.1994.tb05336.x

Matsui S, Ryugo K, Kliewer WM (1986) Growth inhibition of Thompson Seedless and Napa Gamay berries by heat stress and its partial reversibility by applications of growth regulators. *American Journal of Enology and Viticulture* **37**, 67–71.

McConnaughay KDM, Bazzaz FA (1991) Is physical space a soil resource? *Ecology* **72**, 94–103. doi: 10.2307/1938905

Mori K, Sugaya S, Gemma H (2005) Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae* **105**, 319–330. doi: 10.1016/j.scienta.2005.01.032

Moriana A, Villalobos FJ, Fereres E (2002) Stomatal and photosynthetic responses of olive (*Olea europaea* L.) leaves to water deficits. *Plant, Cell & Environment* **25**, 395–405. doi: 10.1046/j.0016-8025.2001.00822.x

Mott KA, Parkhurst DF (1991) Stomatal responses to humidity in air and helox. *Plant, Cell & Environment* **14**, 509–515. doi: 10.1111/j.1365-3040.1991.tb01521.x

Mutters RG, Hall AE (1992) Reproductive responses of cowpea to high temperature stress during different night periods. *Crop Science* **32**, 202–206.

Natarajan S, Kuehny JS (2008) Morphological, physiological, and anatomical characteristics associated with heat preconditioning and heat tolerance in *Salvia splendens*. *Journal of the American Society for Horticultural Science* **133**, 527–534.

Northcote KH (1979) 'A factual key for the recognition of Australian soils.' (Rellim: Glenside)

Passioura JB (2006) The perils of pot experiments. *Functional Plant Biology* **33**, 1075–1079. doi: 10.1071/FP06223

Petrie PR, Clingeleffer PR (2005) Effects of temperature and light (before and after budburst) on inflorescence morphology and flower number of Chardonnay grapevines (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research* **11**, 59–65. doi: 10.1111/j.1755-0238.2005.tb00279.x

Potvin C, Lechowicz MJ, Tardif S (1990) The statistical analysis of ecological response curves obtained from experiments involving repeated measures. *Ecology* **71**, 1389–1400. doi: 10.2307/1938276

Pou A, Flexas J, Alsina Md M, Bota J, Carambula C, *et al*. (2008) Adjustments of water use efficiency by stomatal regulation during drought and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri* × *V. rupestris*). *Physiologia Plantarum* **134**, 313–323. doi: 10.1111/j.1399-3054.2008.01138.x

Radin JW, Lu Z, Percy RG, Zeiger E (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 7217–7221. doi: 10.1073/pnas.91.15.7217

Radler F (1965) The effect of temperature on the ripening of 'Sultana' grapes. *American Journal of Enology and Viticulture* **16**, 38–41.

Retallick M, Schofield P (2008) Grapevine management options in times of drought and water restrictions. http://www.winesa.asn.au/media/docs/vineyardmgmt.pdf [Verified 23 June 2009]

Roderick ML, Farquhar GD, Berry SL, Noble IR (2001) On the direct effect of clouds and atmospheric particles on the productivity and structure of vegetation. *Oecologia* **129**, 21–30. doi: 10.1007/s004420100760

Rodriguez D, Sadras VO (2007) The limit to wheat water use efficiency in eastern Australia. I. Gradients in the radiation environment and atmospheric demand. *Australian Journal of Agricultural Research* **58**, 287–302. doi: 10.1071/AR06135

Sachs T (2006) The plasticity of organ size and anatomy. *Israel Journal of Plant Sciences* **54**, 257–264. doi: 10.1560/IJPS_54_4_257

Sadras VO, McCarthy MG (2007) Quantifying the dynamics of sugar concentration in berries of *Vitis vinifera* cv. Shiraz: a novel approach based on allometric analysis. *Australian Journal of Grape and Wine Research* **13**, 66–71. doi: 10.1111/j.1755-0238.2007.tb00236.x

Sadras VO, Villalobos FJ, Fereres E (1993*a*) Leaf expansion in field-grown sunflower in response to soil and leaf water status. *Agronomy Journal* **85**, 564–570.

Sadras VO, Villalobos FJ, Fereres E, Wolfe DW (1993*b*) Leaf responses to soil water deficits: comparative sensitivity of leaf expansion and leaf conductance in field-grown sunflower. *Plant and Soil* **153**, 189–194. doi: 10.1007/BF00012991

Sadras VO, Collins M, Soar CJ (2008) Modelling variety-dependent dynamics of soluble solids and water in berries of *Vitis vinifera*. *Australian Journal of Grape and Wine Research* **14**, 250–259.

Sadras VO, Calderini DF, Connor DJ (2009) Sustainable agriculture and crop physiology. In 'Crop physiology: applications for genetic improvement and agronomy'. (Eds VO Sadras, DF Calderini) pp. 1–20. (Academic Press: San Diego)

Schultz HR (1997) Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Acta Horticulturae* **427**, 251–266.

Schultz HR (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant, Cell & Environment* **26**, 1393–1405. doi: 10.1046/j.1365-3040.2003.01064.x

Sepulveda G, Kliewer WM (1986) Stomatal response of three grapevine cultivars (*Vitis vinifera* L.) to high temperature. *American Journal of Enology and Viticulture* **37**, 44–52.

Sinclair TR, Shiraiwa T, Hammer GL (1992) Variation in crop radiation-use efficiency with increased diffuse radiation. *Crop Science* **32**, 1281–1284.

Soar CJ, Speirs J, Maffei SM, Penrose AB, McCarthy MG, Loveys BR (2006) Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. *Australian Journal of Grape and Wine Research* **12**, 2–12. doi: 10.1111/j.1755-0238.2006.tb00038.x

Soar CJ, Sadras VO, Petrie PR (2008) Climate-drivers of red wine quality in four contrasting Australian wine regions. *Australian Journal of Grape and Wine Research* **14**, 78–90. doi: 10.1111/j.1755-0238.2008.00011.x

Spayd SE, Tarara JM, Mee DL, Ferguson JC (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture* **53**, 171–182.

Struik PC, Cassman KG, Koorneef M (2007) A dialogue on interdisciplinary collaboration to bridge the gap between plant genomics and crop science. In 'Scale and complexity in plant systems research: gene–plant–crop relations'. (Eds JHJ Spiertz, PC Struik, HH Van Laar) pp. 319–328. (Springer: Dordrecht, The Netherlands)

SYSTAT (2002) 'TableCurve 2D.' (Richmond, California) Q1

Tarara JM, Ferguson JC, Spayd SE (2000) A chamber-free method of heating and cooling grape clusters in the vineyard. *American Journal of Enology and Viticulture* **51**, 182–188.

Thomas JF, Raper J (1981) Day and night temperature influence on carpel initiation and growth in soybeans. *Botanical Gazette (Chicago, Ill.)* **142**, 183–187. doi: 10.1086/337210

Urban O, Janous D, Acosta M, Czerny R, Markova I, *et al*. (2007) Ecophysiological controls over the net ecosystem exchange of mountain spruce stand. Comparison of the response in direct vs. diffuse solar radiation. *Global Change Biology* **13**, 157–168. doi: 10.1111/j.1365-2486.2006.01265.x

Wang LJ, Li SH (2006*a*) Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. *Plant Science* **170**, 685–694. doi: 10.1016/j.plantsci.2005.09.005

Wang LJ, Li SH (2006*b*) Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. *Plant Growth Regulation* **48**, 137–144. doi: 10.1007/s10725-005-6146-2

Wang LJ, Huang WD, Li JY, Liu YF, Shi YL (2004) Peroxidation of membrane lipid and Ca^{2+} homeostasis in grape mesophyll cells during the process of cross-adaptation to temperature stresses. *Plant Science* **167**, 71–77. doi: 10.1016/j.plantsci.2004.02.028

Wang LJ, Huang WD, Liu YP, Zhan JC (2005) Changes in salicylic and abscisic acid contents during heat treatment and their effect on thermotolerance of grape plants. *Russian Journal of Plant Physiology: a Comprehensive Russian Journal on Modern Phytophysiology* **52**, 516–520. doi: 10.1007/s11183-005-0076-8

Warrag MOA, Hall AE (1984) Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp) to heat-stress. 2. Responses to night air-temperature. *Field Crops Research* **8**, 17–33. doi: 10.1016/0378-4290(84)90049-2

Wen PF, Chen JY, Wan SB, Kong WF, Zhang P, Wang W, Zhan JC, Pan QH, Huang WD (2008) Salicylic acid activates phenylalanine ammonia-lyase in grape berry in response to high temperature stress. *Plant Growth Regulation* **55**, 1–10. doi: 10.1007/s10725-007-9250-7

Wise RR, Friederick JR, Alm DM, Kramer DM, Hesketh JD, Crofts AR, Ort DR (1990) Investigation of the limitations to photosynthesis induced by leaf water deficits in field-grown sunflower (*Helianthus annus* L.). *Plant, Cell & Environment* **13**, 923–931. doi: 10.1111/j.1365-3040.1990.tb01982.x

Wohlfahrt G, Hammerle A, Haslwanter A, Bahn M, Tappeiner U, Cernusca A (2008) Disentangling leaf area and environmental effects on the response of the net ecosystem CO_2 exchange to diffuse radiation. *Geophysical Research Letters* **35**, L16805. doi: 10.1029/2008GL035090

Zhang JH, Wang LJ, Pan QH, Wang YZ, Zhan JC, Huang WD (2008) Accumulation and subcellular localization of heat shock proteins in young grape leaves during cross-adaptation to temperature stresses. *Scientia Horticulturae* **117**, 231–240. doi: 10.1016/j.scienta.2008.04.012

Manuscript received 6 May 2009, accepted 18 June 2009

Appendix 1

Table A1. Comparison of maximum temperature, minimum relative humidity and maximum vapour pressure deficit (VPD) in control and heated treatments applied at four phenological windows in 2006–07. Each heating episode lasted 3 days

Heating window	Day	Maximum temperature (°C)			Minimum relative humidity			Maximum VPD (kPa)		
		Control	Heated	Diff.	Control	Heated	Diff.	Control	Heated	Diff.
18–20 December	1	38.2	45.7	7.5	9.0	7.2	–1.8	6.05	9.18	3.13
post-set	2	36.1	42.1	6.0	13.0	12.7	–0.3	5.19	7.20	2.01
E-L 31*	3	38.5	44.9	6.5	12.8	11.3	–1.5	5.94	8.40	2.46
8–10 January	1	28.5	41.7	13.2	25.3	17.8	–7.5	2.85	6.66	3.80
pre-veraison	2	35.4	43.4	8.0	16.3	12.3	–4.0	4.80	7.72	2.92
E-L 33	3	41.0	46.2	5.2	14.4	12.7	–1.7	6.49	8.87	2.39
22–24 January	1	28.4	37.6	9.2	38.2	40.9	2.7	2.37	3.70	1.33
Veraison	2	28.7	36.7	8.0	30.2	36.4	6.1	2.44	3.40	0.95
E-L 35	3	30.4	38.5	8.1	31.5	38.5	6.9	2.93	4.05	1.12
12–14 February	1	35.7	41.1	5.5	23.5	19.6	–4.0	4.46	6.29	1.83
pre-harvest	2	38.5	43.5	5.1	21.2	17.2	–4.0	5.34	7.39	2.05
2 weeks before E-L 37	3	36.1	41.1	5.0	28.5	23.9	–4.6	4.26	5.99	1.73

*Phenological stage in E-L scale (Coombe 1995).

Table A2. Comparison of maximum temperature, minimum relative humidity and maximum vapour pressure deficit (VPD) in control and heated treatments applied at four phenological windows in 2007–08

Each heating episode lasted 3 days; the days before and after establishment of treatments are also shown. Night differences (heated – control) are shown for minimum temperature and maximum relative humidity

Heating window	Day	Maximum temperature (°C)			Minimum relative humidity (%)			Maximum VPD (kPa)			Night difference	
		Control	Heated	Diff.	Control	Heated	Diff.	Control	Heated	Diff.	Min temp. (°C)	Max relative humidity (%)
10–12 December	Before	24.3	24.4	0.1	33.5	32.4	–1.1	1.99	2.00	0.02	–0.1	–1.5
post-set	1	24.5	35.3	10.8	25.4	20.9	–4.5	2.21	4.40	2.19	–0.3	0.7
E-L 31*	2	28.8	39.0	10.2	27.4	23.0	–4.4	2.85	5.35	2.50	1.5	–3.8
	3	32.4	41.5	9.2	18.6	18.2	–0.4	3.94	6.58	2.63	0.4	–2.6
	After	37.1	37.0	–0.1	14.6	14.2	–0.4	5.38	5.40	0.02	–0.1	5.1
7–9 January	Before	33.9	33.5	–0.3	20.5	20.7	0.3	4.14	4.10	–0.05	0.0	–3.7
pre-veraison	1	32.4	39.9	7.5	21.6	21.3	–0.3	3.77	5.72	1.96	0.0	–1.1
E-L 33	2	32.8	39.5	6.7	24.4	24.1	–0.3	3.72	5.33	1.61	0.3	–2.6
	3	37.8	42.6	4.8	15.3	18.5	3.2	5.53	6.84	1.32	0.1	–1.3
	After	44.4	45.0	0.5	9.9	9.8	–0.1	8.39	8.55	0.17	0.9	4.2
21–23 January	Before	28.6	28.2	–0.4	36.4	36.8	0.5	2.47	2.39	–0.08	0.0	–1.9
veraison	1	27.1	34.2	7.1	25.8	27.0	1.3	2.57	3.81	1.24	–0.1	–1.1
E-L 35	2	29.9	37.2	7.4	26.1	25.9	–0.1	3.07	4.49	1.42	0.3	–2.7
	3	34.7	40.7	6.0	12.0	16.9	4.9	4.64	5.92	1.28	0.2	–0.9
	After	32.4	32.5	0.1	27.2	27.1	0.0	3.53	3.55	0.01	1.1	–6.9
18–20 February	Before	37.3	37.3	–0.1	13.6	14.4	0.9	5.43	5.40	–0.03	–0.1	–1.5
pre-harvest	1	38.1	41.5	3.4	14.2	17.2	3.0	5.70	6.52	0.83	0.9	–1.1
2 weeks before E-L 37	2	39.8	42.0	2.2	15.1	16.5	1.4	6.23	6.77	0.54	0.7	4.8
	3	20.7	23.9	3.2	65.9	56.7	–9.2	0.82	1.26	0.44	0.0	–3.4
	After	30.3	29.6	–0.8	30.8	32.8	2.0	2.98	2.77	–0.21	0.4	0.4

*Phenological stage in E-L scale (Coombe 1995).

AUTHOR QUERIES

1. Author: pl. provide publisher name.