Optimizing irrigation for different cultivars.



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSP 05/02**

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Research Organisation: **CSIRO Plant Industry**

Date: **November 2010**

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GWRDC Project No CSP 05/02

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CSIRO Plant Industry

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Table of Contents

1. Abstract 1

2. Executive Summary 2

3. Background 4

4. Project Aims and Performance Targets 9

5. Methods 13

6. Results/Discussion:

A) The relative performance of a range of winegrape varieties and some simple tests to differentiate them 20

B) Influence of climatic conditions on the performance of isohydric and anisohydric cultivars 41

C) Response of vines to elevated temperature 52

D) Effect of variety on response to elevated temperature 66

E) Influence of irrigation pre-history on isohydric / anisohydric responses 78

F) Effects of irrigation prehistory on the response of Cabernet Sauvignon vines to environmental change. 88

7. Outcome/Conclusion 100

8. Recommendations 101

9. Appendix 1: Communication 103

10. Appendix 2: Intellectual Property 105

11. Appendix 3: References 106

12. Appendix 4: Staff 109

13. Appendix 5. Acknowledgements 110

14. Appendix 6. Budget Reconciliation 112

1. Abstract

Tests designed to probe the short and long term response of different varieties of grapevine to increased evaporative demand or soil moisture deficit allowed the varieties to be broadly classified as showing either isohydric or anisohydric characteristics. Isohydric varieties were conservative in their use of soil water through early stomatal closer when faced with increased evaporative demand whereas aniosohydric varieties were less sensitive to stress and used more soil water before exhibiting responses such as stomatal closure. Exposure of Cabernet Sauvignon, a variety generally showing more anisohydric characters, was insensitive to short term changes in evaporative demand when supplied with abundant water but long term exposure to mild soil water deficit caused it to become more sensitive to changes in evaporative demand and to behave with more isohydric characters. The expression of these characters is therefore inducible through irrigation management. The degree of expression of these characters was also influenced by seasonal differences in climate (e.g. low spring rainfall) driving differences in growth (e.g. canopy size, yield) which in turn affected the sensitivity of vine responses to changes in evaporative demand. We also studied vine response to heat stress and showed that if Shiraz (an anisohydric variety) vines had adequate water they responded by up-regulating stomatal conductance, thereby to some extent mitigating the effects of high temperature through increased leaf cooling. On the other hand when heat and water deficit stress was combined Semillon vines showed reduced stomatal conductance and virtually zero rates of photosynthesis. Under these conditions of high temperature and water deficit stress nocturnal transpiration becomes a very significant component of daily total water loss, particularly affecting the vine's ability to rehydrate overnight. Knowledge of the potential of a grape variety to exhibit either isohydric or anisohydric characters will contribute to more effective irrigation management by allowing these characters to be selectively expressed.

2. Executive Summary.

The performance of vines in the field is driven by a range of morphological and physiological characteristics which are determined by the genetic background of individual varieties. We have assessed a number of grape varieties for their response to changes in their environment. Most tests were carried out in the field on nine varieties growing at one location under uniform conditions and these varieties included many of those more commonly grown in Australia. While the variety mix could not be exhaustive, it did represent over 77% (on an area basis) of the major wine grape varieties currently grown in Australia. Where necessary field tests were supplemented by experiments in an air-conditioned glasshouse. In order to assess varietal response to environmental challenges we subjected the vines to a number of tests ranging from simply measuring the rate at which water is lost from excised leaves to more complex measurements to assess the performance of vines over a whole season such as carbon isotope discrimination. These tests allowed a ranking of varieties in terms of their ability to perform in the face of challenges imposed by the weather and by exposure to soil water deficit. Grenache, which is generally considered to exhibit isohydric characters, was a consistently strong performer in most of the tests while Cabernet Sauvignon and Merlot, examples of vines exhibiting more anisohydric characters, generally performed poorly when exposed to stressful conditions. However, all varieties were capable of good performance in an environment which posed no challenges. By modelling the dynamics of soluble solids and water in 12 grape varieties we showed (Sadras *et al.* 2008) that traits related to carbon assimilation were important drivers of maturity date and these were in turn, a direct function of stomatal conductance. Modulation of stomatal conductance also plays an important role in the response of Shiraz vines to a short period of high temperature. We exposed well watered Shiraz vines to 3 day periods of high temperature at 4 phenological stages: post fruit-set, pre-veraison, veraison and pre-harvest (Soar *et al.* 2009). The temperature of the vines was controlled by a combination of passive heating in a plastic covered enclosure combined with fans to introduced ambient air. Maximum air temperatures were 6 to 7 ^{o}C above ambient. The heat events 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. The heated vines up-regulated their stomatal conductance during the

high temperature episodes. This may be an adaptive response which could to some extent mitigate the effects of high temperature through evaporative leaf cooling. However, it would be achieved at the expense of more rapid depletion of soil moisture reserves. We also studied the effect of combined heat and water stress by subjecting Semillon vines experiencing a water deficit to elevated temperatures during the day and night. Under these conditions heated vines showed very low values of stomatal conductance and photosynthesis. The up-regulation of conductance seen in the Shiraz vines therefore did not occur. Daily water use of the heated + deficit vines was greater than the deficit alone but this was due mainly to water loss during the night. Over 40% of daily water use occurred during the night. This trait may further differentiate isohydric from anioshydric grape varieties as Rogiers *et al.* (2009) showed that nocturnal transpiration of Semillon was about 4 times higher than that of Grenache. Variability amongst varieties in response to a changing environment suggested that the vines are able to modify their behaviour and that the extent of this may vary depending on their isohydric/anisohydric character. To test this idea we have studied the gas exchange characteristics of Cabernet Sauvignon, a variety that generally displayed more anisohydric characters, when challenged with long term soil water deficits and short term changes in evaporative demand. All vines showed increased transpiration as VPD was increased up to about 2kPa. As VPD increased further control vines receiving luxury amounts of water (about 6ML/ha/y) showed a more or less linear increase in transpiration whereas vines grown with increasing water deficit (50% and 25% of control) became increasingly more sensitive to VPD with transpiration in the 25% vines being reduced approximately 3-fold compared with the controls at high VPD. These data show that the Cabernet Sauvignon vines have adapted to their irrigation regime through changes in stomatal response to evaporative demand. This adaptation is probably driven by increased concentration of abscisic acid (ABA) in the xylem sap in the vines experiencing water deficit. A statistical analysis of the ability of leaf water potential and xylem sap ABA to predict vine response to increased transpiration demand (VPD) showed that if vines received adequate water neither variable had any predictive ability but as water deficit intensified, first xylem ABA then leaf water potential assumed greater importance in predicting water loss.

3. Background

The Australian winegrape industry is increasingly operating in an environment where resource use is under scrutiny and this has resulted in the need for better information about vine water requirements so that water use efficiency can be improved. There is also a growing realisation that water management can be used as a tool to manipulate vine vigour, fruit yield and quality. However, across-the-board reductions in water application rates are not without risk and high management skill levels may be required to reduce the risk of damage to vine health and fruit quality. This is particularly pertinent in regards to extreme thermal weather events associated with a changing climate if the buffer provided by high levels of soil moisture is not present. There is considerable evidence that the frequency of extreme weather events will increase under the influence of climate change, emphasising the need for the best information on which to base irrigation-related decisions.

The evolution and continued existence of most terrestrial plants takes place in what is essentially an extremely hostile environment. The aerial parts of the plant are frequently subject to high evaporative demand, especially during the day when gas exchange with the environment is required to sustain photosynthesis. Diffusion of CO_2 into the leaves from the atmosphere is driven by the relatively small gradients generated by a reduced level of CO_2, relative to that in the atmosphere, caused by photosynthetic activity in mesophyll cells but the loss of water vapour from leaves is driven by potentially huge gradients in water potential that exist at the leaf/air boundary. As a consequence, stomatal adjustments which may enhance the diffusion of CO_2 into the leaf inevitably lead to a disproportionate increase in transpiration. Effective stomatal control is thus an essential attribute in optimizing plant performance under potentially stressful conditions. Grapevines (*Vitis vinifera* L.) are generally considered to have origins in a mesic to semi-arid environment and may be classified as “drought avoiders”. That is, they rely on early stomatal closure when exposed to potentially stressful conditions to conserve reserves of soil moisture and maintain a relatively constant level of leaf hydration. However, this broad generalization is not always appropriate as we can now recognize that different grapevine cultivars have adopted a range of mechanisms to deal with water stress. In response to diminishing reserves of soil moisture it can be imagined that purely

hydraulic considerations will force the closure of stomata with a consequent reduction in transpiration, thus preventing damaging leaf water deficits. Research over that last 30 years or so has presented us with a much more complex picture in which hydraulic drivers and various naturally occurring bio-active chemicals interact to exert fine control over stomatal function. In the late 1960s and early 1970s it was shown that the concentration of the plant hormone abscisic acid (ABA) increased dramatically in water stressed leaf tissue and that ABA was capable of inducing stomatal closure at very low concentrations (Kriedemann *et al.* 1972). It was a logical assumption that a fall in leaf turgor associated with water stress was the trigger for ABA synthesis in leaves and it was this that induced stomatal closure. However, this seemingly simple scheme was called into question when it was shown that in grapevine diurnal changes in leaf ABA and associated changes in stomatal conductance were largely explained by uptake of ABA from the xylem rather than *in situ* synthesis (Loveys 1984), changing the focus of attention from the leaves to the roots as the primary organs sensing the onset of water stress. This view was reinforced by experiments with plants where the roots were divided between two containers, one of which could be fully irrigated and the other subject to varying degrees of water deficit. Under these conditions the water relations of the leaves were not adversely affected due to the unrestricted access to water from the wet roots but they were still subject to changes induced by chemical signals originating in the drying roots. Gowing *et al.* (1990) used this experimental system to demonstrate that leaf growth was reduced when only part of the root system was exposed to drying soil and that the effect was lost when the drying roots were severed from the plant, implying that the reduction in leaf growth was due to a chemical substance produced in the drying roots. There is now strong evidence that abscisic acid (ABA) is the primary substance able to signal changes in root moisture status (Wilkinson and Davies 2002) although other chemical signals may also be involved (Borel *et al.* 2001). This focus of attention on the roots as a source of chemicals able to modify the gas exchange performance of the canopy may lead to an over simplification since we also know that the aerial parts of the plant are important in determining stomatal response to environmental change and we can anticipate that different grapevine cultivars will display a range of responses to changes in soil moisture and evaporative demand. Holbrook *et al.* (2002), for example used a range of tomato genotypes with varying abilities to synthesise ABA to show that it was the genotype of the leaves, not the roots, that determined response to stress,

and in grapevines the aerial parts of the plant are able to modify the ABA signal present in xylem sap (Soar *et al.* 2004). A picture is now emerging of complex interactions between root and shoot often mediated by the supply of chemical messages generated in drying roots and transported to the shoots.

Irrigation management and cultivar differences

When grapevines are grown in soil which is maintained at or close to field capacity they will develop large canopies. Williams *et al.* (2003) grew Thompson Seedless (Sultana) vines in a large weighing lysimeter at Fresno in the San Joaquin Valley of California over three seasons to assess water use of fully irrigated vines By replacing transpired water with up to seven irrigation events per day the soil was maintained close to field capacity. The vines developed large canopies of approximately $25m^2$ per vine. Over the three years of the experiment seasonal water use was 844mm (6375L per vine). Since there was no drainage from the lysimeter and the drip irrigation system minimized soil evaporation, it can be assumed that most of this water was actually transpired by the vine. Maximum daily transpiration was 6.6mm (50L per vine). Under similar environmental conditions in the Sunraysia irrigation district of Australia, Yunusa *et al*. (2000) used heat pulse sap flow sensors to determine transpiration of Sultana vines and found that transpiration was only about half that of the California vines. Furthermore, there was little difference in transpiration when two comparatively cool days in November were compared with two hot days in February when vapour pressure deficit (VPD) and net radiation were considerably higher. Similarly, Lu *et al.* (2003), also working with Sultana vines in Sunraysia but using different methodology, showed the same phenomenon. The vines were able to regulate their canopy conductance to achieve virtually constant water use under days of highly contrasting evaporative demand.

So what was the difference between the California vines using 2 $L.m^{-2}$ canopy $area.day^{-1}$ (Williams *et al.* 2003) and the Sunraysia vines using 0.65 $L.m^{-2}$ canopy $area.day^{-1}$ (Lu *et al.* 2003)? We can discount the possibility that the methodologies used by Lu *et al*. (2003) and Yunusa *et al*. (2000) were in error by that degree since both authors presented convincing arguments to support the validity of their data. The differences could be explained by the influence of the irrigation environment on the expression of innate stress response mechanisms. In California the vines were grown

in a lysimeter, the soil of which was maintained at or near field capacity by frequent water application. Under these conditions the roots would never be exposed to soil matric potentials lower than perhaps -10 kPa. The Sunraysia vines, on the other hand, were grown with a more conventional drip irrigation system in which water was applied less frequently. The roots in these vines would have been subject to cyclical wetting and drying with an average soil matric potential of c.-30 kPa (Lu *et al*. 2003). Furthermore, roots outside of the zone immediately influenced by the drip emitters would have experienced soil matric potentials considerably lower than this and these too may influence canopy performance. These vines, which would be typical of irrigated vines in much of Australia, would have had the opportunity to express their innate biochemical mechanisms to optimise their efficiency of water use under a range of environmental conditions. These experiments used natural variation in environmental conditions to probe the mechanisms operating in field-grown grapevines to control water use. Taking a similar approach, Schultz (2003) showed that by comparing the gas exchange and water relations characteristics of irrigated and non-irrigated Syrah (Shiraz) and Grenache during a growing season and during selected days, it was possible to differentiate between these varieties. Schultz described Shiraz as anisohydric or 'optimistic' because when faced with declining soil water it maintained relatively high rates of gas exchange, using more current resources to maintain a higher rate of carbon assimilation. Grenache on the other hand behaved in an isohydric or 'pessimistic' fashion by exhibiting early stomatal closure when it encountered soil drying, thereby conserving resources at the expense of the immediate ability to fix carbon. Schultz concluded that differences in xylem cavitation and associated differences in hydraulic conductivity were the cause of these contrasting patterns of behaviour, although this interpretation needs to be clarified and extended to other cultivars. These cultivar differences may lead the experienced vineyard manager to devise different irrigation schedules for different vines (eg. Cabernet Sauvignon may be handled differently to Shiraz, especially in a hot climate). This appreciation of differences between cultivars is based mostly on anecdotal experience, but is a vital part of an integrated irrigation program.

We are now beginning to understand that these distinctions between cultivars are driven by very real physiological characteristics and this project aims to differentiate the major winegrape varieties grown in Australia on the basis of their physiology and

expression of their isohydric/anisohydric behaviour. This knowledge will help in the development of variety-specific irrigation schedules, especially in an environment where extreme temperature events may become more frequent.

4. Project aims and performance targets

1. Major red and white varieties categorized into isohdyric / anisohdyric behaviour
2. Influence of climatic conditions on performance of isohydric and anisohydric cultivars compared and related to fruit quality outcomes
3. Response of vines to elevated temperatures
4. Effect of variety on response to elevated temperatures
5. Influence of irrigation pre-history on isohydric / anisohydric responses
6. Determine influence of canopy genotype on the transmission of root-sourced signals which determine response to high VPD
7. Guidelines for more effective irrigation of varieties based on their isohydric/anisohydric behaviour developed including planning for extreme weather events

Outputs and Performance Targets 2005-06

Outputs	Performance Targets
1.simple test(s) for stress response type developed	June 2006
2. major red and white winegrape varieties categorized	Shiraz, Grenache, Chardonnay, Semillon by June 2006
Post-Doctoral Fellow appointed	June 2006
Framework for collaborative work with SAR05/01	Meetings held and major areas for collaboration identified by June 2006

Outputs and Performance Targets 2006-07

Outputs	Performance Targets
1 equipment to induce thermal shock built and tested	October 2006

2. tests for type of stress response confirmed	Major red and white winegrapes tested and categorized June 2007
3. vines exposed to thermal shock	June 2007
4. initial measurements of vine response to thermal shock	June 2007

Outputs and Performance Targets 2007-08

Outputs	Performance Targets
1 modify thermal shock equipment	Meet with SARDI project staff and agree/implement redesign. Oct 2007
2. extend thermal shock treatments to isohydric/anisohydric varieties	June 2008
2. Influence of irrigation pre-history understood for isohydric varieties	June 2008
3. Influence of irrigation pre-history understood for anisohydric varieties	June 2008

Outputs and Performance Targets 2008-09

Outputs	Performance Targets
1. Determine the influence of canopy genotype on the transmission of root-sourced chemical signals which determine response to high evaporative demand	June 2009

2. influence of extreme climatic conditions on performance of isohydric and anisohydric cultivars compared and related to fruit quality outcomes	June 2009
3. Trade and scientific publications prepared	Published by 2009

Outputs and Performance Targets 2009-10

Outputs	Performance Targets
1. guidelines for more effective irrigation of varieties based on their isohydric/anisohydric behaviour developed including planning for extreme weather events	June 2010
2. Trade and scientific publications prepared	Published by June 2010
4.Final report submitted	June 2010

Project variation 1, approved

*Original objective***:** Influence of extreme climatic conditions on performance of isohydric and anisohydric cultivars compared and related to fruit quality outcomes.

Proposed new objective: Determine the influence of extreme climatic conditions on overall vine performance of isohydric and anisohydric vines.

*Performance target***:** Measurements of whole vine performance under varying climatic conditions

Target date: June 2010

Justification:

It was the original intention to do these experiments in the glasshouse but difficulties in getting good fruit set and berry maturation under these conditions has suggested that a better result will be gained from using field vines. We therefore propose using the tents constructed for project SAR 05/01 and CSP 05/02 to impose a 4 to 5 day heat stress treatment (similar to that experienced in the field in many districts this year) to examine the effects on fruit development and leaf gas exchange. This will be a more realistic approach than originally intended and will be more easily related to conditions experienced in the field.

Project variation 2, approved.

Original objective: final report submitted by June 2010

Proposed new objective: submit final report by September 30th 2010

Justification: final analysis of data and alignment with approved project end date.

5. Methods

Plant material

Many of the experiments described in this report were carried out at the SARDI research station at Nuriootpa during seasons 2006/7 and 2007/8 and compared a number of winegrape varieties (Table 1). These seasons provided strong contrasts in terms of temporal variability in evaporative demand and rainfall. Season 2006/7 was considered a serious drought year, with grape production severely affected in most winegrowing regions of Australia. There was a 27% reduction in grapes crushed compared with the 2005/6 season and the decline was most severe in the cooler growing regions due to very low winter and spring rainfall and frosts. An improvement in seasonal conditions in 2007/8 resulted in a national increase in production of 19% compared with 2006/7.

Table 1. Variety, clone and age of wine grape varieties characterized in trial.

Variety	Abbreviation	Clone	Year of planting
Shiraz	**S**	BVRC12	1995
Chardonnay	**CH**	I10V1	1974
Semillon	**SE**	D10V12	1993
Riesling (Steinberg)	**R**	I10V14	1989
Sangiovese	**SA**	H6V9	1988
Merlot	**M**	8R	1997
Cabernet Sauvignon	**CS**	LC 84	1988
Verdelho	**V**	SA 168	1995
Grenache	**G**	139 HT 401-1	1991

This distinction between seasons is well illustrated by the rainfall at the Nuriootpa station, with severely restricted amounts during spring 2006 (Table 2).

Table 2. Environmental conditions and irrigation in seasons 2006/7 and 2007/8. Seasonal temperature maximum and minimum, total rainfall is compared with the long term (LT) averages for the trial site.

	Average maximum temperature (°C)			Average minimum temperature (°C)			Rain & Irrigation (mm)				
Month	**2006/7**	**2007/8**	**LT**	**2006/7**	**2007/8**	**LT**	**2006/7 Rain**	**2006/7 Irrig**	**2007/8 Rain**	**2007/8 Irrig**	**Rain LT**
Sep	20.2	19.4	*16.9*	6.1	7.2	*6.0*	20	0	40	0	*61*
Oct	23.9	22.5	*20.3*	7.1	8.0	*8.0*	0	9.6	21	0	*47*
Nov	27.0	27.5	*24.1*	10.5	12.1	*10.1*	25	0	24	0	*31*
Dec	28.0	28.7	*26.5*	12.3	12.9	*12.0*	21	57.6	23	89.6	*24*
Jan	29.4	31.0	*28.9*	14.6	14.9	*13.7*	59	12.8	3	115	*18*
Feb	32.4	26.9	*28.7*	15.6	13.3	*14.0*	0	38.4	0	76.8	*20*
Mar	25.9	29.8	*25.7*	12.9	12.7	*11.8*	33	0	7	0	*23*
TOTAL							158	118	118	281	224

Average vapour pressure deficit (calculated from maximum daily temperatures and corresponding humidity) was, on average, higher during the 2006/7 season, especially during spring and late summer (Fig 1). The dry conditions during the early part of the 2006/7 season were therefore the main driver of seasonal differences we have noted. Irrigation plus rainfall for the period November to February equated to 220mm and 330mm for the 2006/7 and 2007/8 seasons respectively (Table 2).

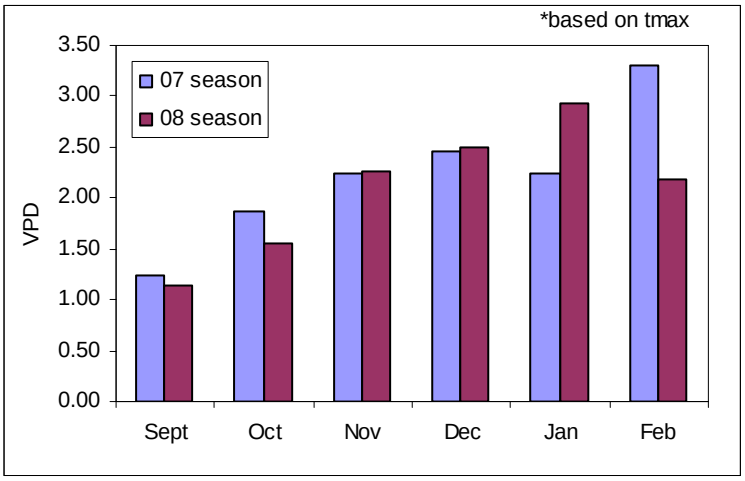


Figure 1. VPD (kPa) at the SARDI Nuriootpa field station calculated using daily maximum temperatures and averaged on a monthly basis.

Leaf drying

An assessment of the hydraulic properties of varieties during short term leaf drying may constitute a simple test to differentiate varieties with more isohydric characteristics from those with more anisohydric characteristics. Such a test might be

as simple as following the progression of water loss from excised leaves held in a moderately desiccating environment.

Leaves were excised from the cane by cutting the petioles with a razor blade at the point of insertion on the cane. Leaves were placed on a flat surface, abaxial surface uppermost and the surface was uniformly illuminated with ambient light. In the field this was achieved by placing the leaves on a plywood surface in the tray of a utility vehicle with a layer of 50% shadecloth placed over the top of the tray. In the glasshouse similar irradiance levels were maintained by covering the exterior glass surface with 50% shadecloth. Numbers of leaves varied but where simple 2 variety comparisons were made 20 leaves per variety were used and when multiple varieties were tested a minimum of 6 leaves per variety were used. Initial leaf weight was recorded and the leaves were weighed individually at approximately 10 to 20 minute intervals for the duration of the experiment. Leaves were weighed in the same order at each sample time. Weight loss as a percentage of initial weight was calculated for up to 100 minutes but for the field variety comparison averaged 70 minutes. This was done several times during the season as well as before and after midday to assess the effect that phenological stage and changing plant water status had upon drying rates.

Varietal differences in response to environment

In order to compare the responses of a range of varieties to changes in the environment a single row variety block at the SARDI research station in Nuriootpa was examined in seasons 2006/07 and 2007/08. All vines were within a single block and irrigation was uniformly applied over the block for both seasons. To reduce the confounding effect of soil moisture physiological measurements occurred within two days of weekly irrigation events. Vines measured were mature (10-20 years old) with the exception of the Chardonnay vines which were over 30 years old (Table 1). Varieties examined were Shiraz, Chardonnay, Semillon, Riesling, Sangiovese, Merlot, Cabernet Sauvignon, Verdelho and Grenache, with details of clones and year of planting being listed in Table 1. The physical attributes (leaf area, shoot number, yield and pruning weights) and physiological responses of ten vines per variety were measured in both seasons. For each vine leaf area was estimated by multiplying shoot number by average leaf number (based on 10 shoots per vine) by leaf area of the leaf at the 4^{th} internode (average of 100 leaves per variety). In season 2006/07

physiological measurements occurred on 11 days in January – February and for each variety the following measurements were taken for all varieties within a two hour period: average canopy temperature (ten vines) measured with an infrared thermometer (Fluke model 574), stomatal conductance (Delta T porometer; fifteen leaves over five replicate vines), leaf water potential (six leaves per variety) and xylem sap ABA (three leaves per variety). On some days measurements were replicated in both the morning and afternoons (18 measurements periods overall). With the exception of canopy temperature the same physiological measurements were repeated in season 2007/08, on 10 days between November to February. The time over which measurements occurred in the second season was extended to attempt to capture some lower VPD responses and on some days measurements occurred in both the morning and afternoon (13 measurement periods overall). For both seasons the differences between morning and afternoon measurements were analysed by ANOVA (SPSS) for statistical differences and were not significant so measurement periods were analysed as independent occasions rather than being averaged across the day. VPD was calculated from temperature and humidity measurements every 15 mins from the field weather station located within the vineyard.

Varietal differences in chemical signalling of water deficit

A droplet of xylem sap was sampled by over-pressurization (0.1 MPa greater than leaf water potential) of leaf and petiole using a Scholander pressure bomb for three leaves per variety, snap frozen in liquid nitrogen and stored at -40°C until analysis could be completed. ABA analysis was completed on 13 occasions across the measurement periods for both seasons.

Analysis of xylem sap ABA

Analysis of ABA abundance in xylem sap was undertaken by liquid chromatography/mass spectrometry (LC MS/MS) using a stable isotope dilution assay. Xylem sap samples were thawed, dried in a vacuum centrifuge and dissolved in 10% acetonitrile containing 0.05% acetic acid containing deuterated internal standards D3 - 7', 7', 7'- PA and DPA, D5 – 4, 5, 8', 8', 8'- ABA-GE and D6 - 3', 5', 5', 7', 7', 7'- ABA). These samples were separated by HPLC. Column temperature was set at 40°C and the column used was a *Phenomenex* C18(2) 75mm *x* 4.5mm *x*

5µm. Solvents used were nanopure water and acetonitrile, both with 0.05% acetic acid. Samples were eluted with a linear 15 minute gradient starting at 10% acetonitrile at time 0 and ending with 90% acetonitrile. Compounds were identified by retention times and MRM. Parent and product ions are shown in Table 3.

Table 3. Analytes, retention times and m/z values for parent and product ions of native analytes and deuterated internal standards.

Compound	RT (mins)	MRM for native and deuterated ions (m/z)
DPA	7.25-7.75	281/284 → 171/174
ABA-GE	8.25-8.75	425/430 → 263/268
PA	9.0-9.5	279/282 → 139/142
ABA	10.5-11.0	263/269 → 153/159

Stable isotope discrimination

In both seasons total carbon and nitrogen levels in leaves were measured in leaves sampled at harvest. The analysis of samples was performed by CSIRO analytical services (Plant Industry, Canberra) using a Europa 20-20 (electronics rebuilt by Sercon in 2004) isotope ratio mass spectrometer with an ANCA preparation system. In addition to total carbon, results also include ^{13}C isotope levels. For each variety five leaves per vine were randomly sampled at harvest in five replicate vines, oven dried and then ground into a fine powder using a mortar and pestle. A sub-sample from each replicate vine was taken, with two samples being repeated in triplicate to account for analytical variability. Values were averaged across the five samples for each variety.

Hydraulic conductivity

In season 2007/08 hydraulic conductivity of cane sections was measured with a Dynamax hydraulic conductance flow meter. This was a destructive technique whereby water was forced to flow into sections of cane to give maximum hydraulic conductivity values. We used the transient ramp technique in the hydraulic conductance flow meter, in which pressure was ramped up to 0.5 MPa at a rate of

approximately 7 kPa s^{-1} while simultaneously recording the flow through the cane section. Because of the high pressures and the direction of water flow imposed, this technique will not detect changes in hydraulic conductance as the result of xylem embolisms. In the field four shoots per variety were removed from the vine by cutting at the base and put into water immediately. Shoots were stored overnight in a darkened cool room to ensure they were fully hydrated when measurements were completed. Canes were then cut above the fourth node and below the seventh node and maximum hydraulic conductivity of the stem section measured. Stems were flushed for 15-20 minutes before conductivity readings were taken. All measurements were repeated in triplicate and flow rates averaged. The total leaf area of the cane above the 4^{th} node, length and cross-sectional area of stem section were recorded.

Exposure of Shiraz and Semillon vines to short term heat events.

The construction of the heat chambers is detailed in Section C of this report but to allow night time heating 5kW of electric heaters were operated from sunset to dawn. The Shiraz vines were in the same block at the Nuriootpa research station as used previously and all other variables were maintained as close as possible to those of the previous year (described in Section C of the report). The heat chambers were installed for 6 three day periods during the 2008/9 season to cover the following phenological stages: budburst (24-27 Sept), flowering (10-13 Nov), post-set (9-12 Dec), pre-veraison (6-9 Jan), veraison (20-23 Jan) and pre-harvest 17-20 Feb).

The effect of elevated day and night temperature in combination with a water deficit was studied in Semillon vines on Ramsey rootstock growing at the Yalumba Oxford Landing Estate vineyard at Waikerie in the South Australian Riverland. The heating enclosures were the same as described previously. The treatments were (1) control vines irrigated according to a schedule dictated by the vineyard manager equivalent to about 5ML/ha, (2) water deficit induced by withholding irrigation water for 14 days before heat enclosures were installed. 14mm of rain fell 7 days after the water deficit was initiated which slowed the vine response, (3) water deficit as (2) with a 3 day heat event applied between days 14 and 17. Treatments were replicated 3 times with 9 vines per replicate. The heat enclosures were erected over the central 3 vines of each replicate. Sap flow sensors were installed in three vines of each treatment 2 days before the water deficit was initiated. Leaf water-potential (Scholander pressure

bomb) and gas-exchange (LiCor 6400) were measured throughout the day for both experiments.

Response of Shiraz, Cabernet Sauvignon and Grenache vines to water deficit at McLaren Vale

Vines were five year old own-rooted Shiraz, Grenache and Cabernet Sauvignon. Rows were oriented east-west. The vines were setup in a randomised block design, with two treatments (control and water deficit) with six replicate blocks of nine vines for each variety. In the deficit treatment irrigation was withheld from pre-veraison (late December) to after harvest. Soil moisture at 4 depths was monitored in the Grenache and Shiraz vines, with stomatal conductance and leaf water potentials measured throughout the season. Yield and quality data were collected at the end of the season.

Effects of irrigation prehistory on the response of Cabernet Sauvignon vines to environmental change.

Field grown Cabernet Sauvignon vines were used for these experiments. They were growing in the South Australian Riverland at the Yalumba Oxford Landing vineyard. They have been previously described (Speirs *et al.,* 2010). The vines received irrigation according to a frequency determined by the vineyard manager. All treatments received irrigation at this standard rate until fruit set, after which various deficit treatments were applied and these were sustained until after harvest in February. There were five irrigation rates applied but only three will be reported in these experiments. These were **control** (approximately 2x standard rate and equivalent to about 6ML/ha. This rate at this site produced vigorous vines showing no signs of water stress), **T50** (50% of control) and **T25** (25% of control). Measurements of basic vine performance (leaf water potential, stomatal conductance) were made throughout the season as described previously and detailed gas exchange measurements were made during January 2009. Gas exchange was measured with a LiCor 6400. VPD in the chamber was controlled by supplying water-saturated air and mixing this with dry air to achieve the required level of humidification. Leaf area index was measured with a LiCor LAI2000 canopy analyser.

6. Results and Discussion

This section of the report is sub-divided into six self-contained chapters which are structured to broadly address the stated project aims.

A) The relative performance of a range of winegrape varieties and some simple tests to differentiate them

Introduction

The work of Schultz (2003) clearly showed that Grenache and Shiraz could be differentiated on the basis of their response to soil drying. Grenache exhibited characteristics described as isohydric whereas Shiraz showed responses more like an anisohydric plant. The properties of isohydric and anisohydric plants have been discussed above in the general introduction. Schultz (2003) attributed at least some of the difference between Grenache and Shiraz to hydraulic factors such as the water conducting capacity of the stems, and particularly the petioles, and their resistance to cavitation. Grenache was more susceptible to cavitation, thus reducing the rate of water loss and possibly acting as a trigger for stomatal closure. These hydraulic characteristics may act in concert with differing sensitivity to chemical signals to differentiate varieties in their response to drying soil. We have assessed a range of physical and biochemical attributes to build a picture of varietal response to their aerial and soil environment. We have therefore documented the performance of a number of winegrape varieties growing at the same site over two seasons. The aim being to determine consistent patterns of vine behaviour under a range of environmental conditions, which will allow prediction of expression of isohydric or aniosohydric characteristics.

Results and Discussion

Vine performance

Measures of vine vigour such as total canopy area and pruning weights were significantly higher in all varieties in 2007/8 than in the previous season (Table 4) although there was a tendency for there to be fewer shoots associated with this more

vigorous growth, suggesting more intense expression of shoot apical dominance under the better conditions of the 2007/8 season.

Table 4. Canopy leaf area (LA), shoot number, yield (Tonnes per hectare) and pruning weight for varieties in seasons 2006/7 and 2007/8. Different letters indicate significant differences ($P < 0.05$) between varieties within seasons and bold lettering indicates differences between seasons ($P < 0.001$).

	Total LA m^2		Shoot number		Yield T ha^{-1}		Pruning weight kg	
	2006/7	**2007/8**	**2006/7**	**2007/8**	**2006/7**	**2007/8**	**2006/7**	**2007/8**
Cabernet Sauvignon	4.5 ^{a}	**11.8** ^{ab}	51 ^{ab}	48 ^{a}	0.7 ^{a}	**5.5** ^{a}	0.81 ^{a}	**1.84** ^{a}
Chardonnay	7.4 ^{b}	**11.7** ^{a}	59 ^{ab}	**43** ^{ab}	1.9 ^{ab}	1.8 ^{b}	0.60 ^{b}	**0.84** ^{b}
Grenache	8.9 ^{c}	**12.0** ^{ab}	55 ^{ab}	**40** ^{a}	16.5 ^{e}	15.2 ^{c}	1.22 ^{c}	**1.91** ^{a}
Merlot	5.0 ^{a}	**15.8** ^{c}	61 ^{ac}	60 ^{b}	1.2 ^{ab}	**5.7** ^{a}	0.61 ^{a}	**1.61** ^{c}
Riesling	3.9 ^{a}	**11.7** ^{a}	62 ^{ac}	**50** ^{ab}	2.9 ^{b}	**8.9** ^{d}	0.63 ^{a}	**1.10** ^{d}
Sangiovese	3.8 ^{a}	**13.0** ^{ab}	55 ^{ab}	52 ^{ab}	5.4 ^{c}	**7.6** ^{de}	0.68 ^{ab}	**1.38** ^{c}
Semillon	4.6 ^{a}	**11.6** ^{a}	47 ^{b}	46 ^{a}	4.0 ^{bc}	**7.1** ^{ae}	0.79 ^{b}	**1.00** ^{bd}
Shiraz	7.4 ^{b}	**13.8** ^{b}	49 ^{ab}	48 ^{ab}	1.8 ^{ab}	**9.4** ^{d}	0.97 ^{d}	**1.47** ^{c}
Verdelho	6.8 ^{b}	**12.8** ^{ab}	80 ^{c}	**43** ^{a}	12.1 ^{d}	**8.8** ^{d}	0.80 ^{b}	**1.26** ^{cd}

Fruit yield was generally higher in the 2007/8 season although it was obvious that some varieties had been more severely affected by the poor 2006/7 conditions than others. Cabernet Sauvignon, Merlot and Shiraz in particular had very low yields in 2006/7 whereas Grenache and Verdelho were relatively unaffected by the changed conditions in the two seasons.

Leaf resistance to drying and other physical characteristics

Initial experiments in the field clearly differentiated Grenache and Shiraz on the basis of their rate of water loss (Fig 2).

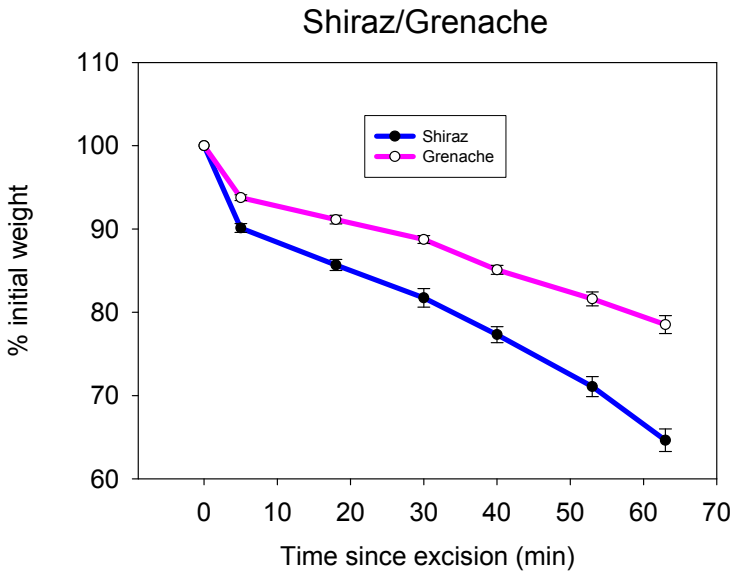


Figure 2. Weight loss of excised Grenache and Shiraz leaves in the field.

Semillon and Chardonnay also displayed different rates of water loss under similar conditions (Fig 3).

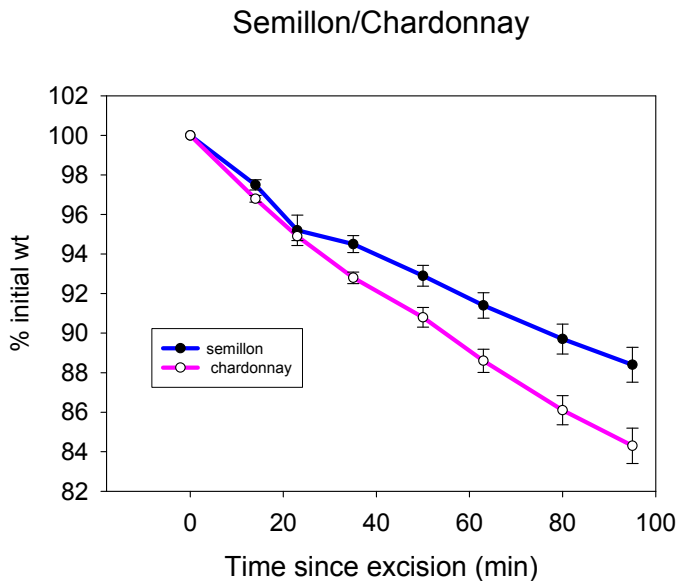


Figure 3. Weight loss of excised Semillon and Chardonnay leaves in the field

More exhaustive tests on an extended range of varieties showed that there were significant differences between many of the varieties. However, the relative order of the varieties, in terms of their ability to resist water loss after excision, varied greatly both within and between days (Figures 4-7), but it was possible to make some broad generalisations, with Riesling, Red Globe and Grenache tending to be more resistant and Cabernet Sauvignon, Verdelho and Merlot least resistant.

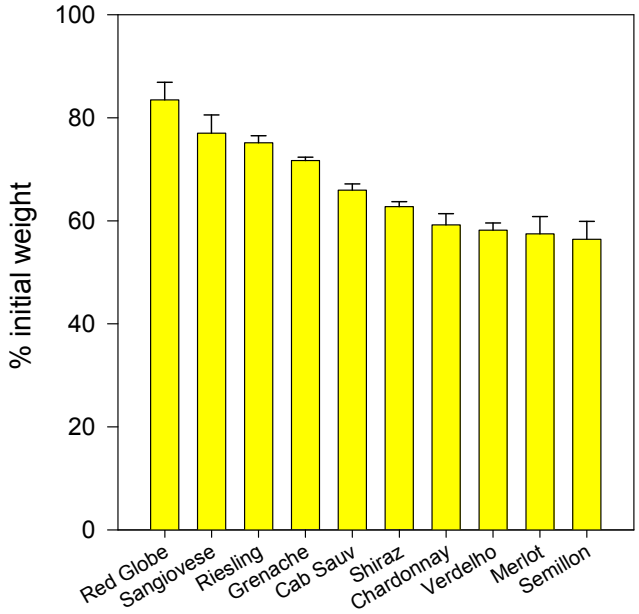


Figure 4. Weight loss of leaves from a number of grape varieties in the field at the end of a 70 min drying period. Readings made before midday 31/01/2007.

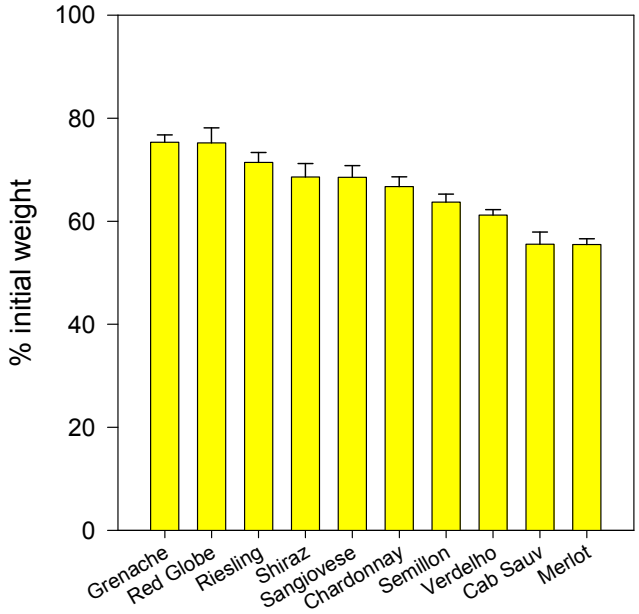


Figure 5. Weight loss of leaves from a number of varieties in the field at the end of a 70 min drying period. Readings made after midday 31/01/2007. Leaves taken from the same vines as in Figure 4.

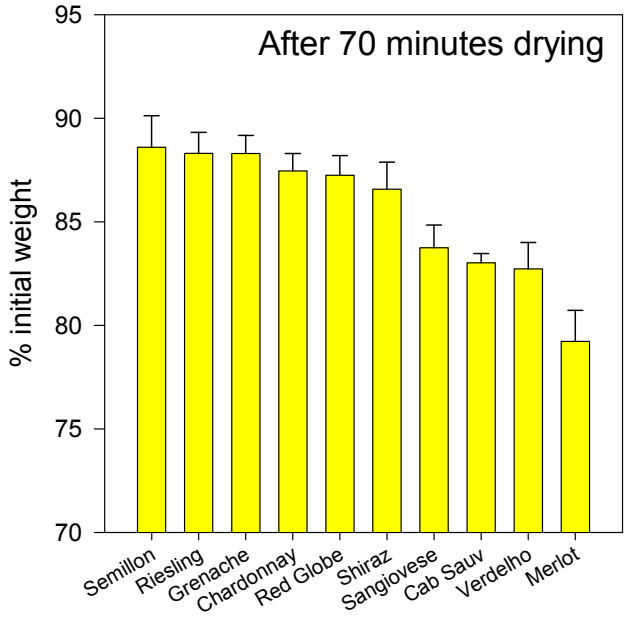


Figure 6. Weight loss of leaves from a number of varieties in the field at the end of a 70 min drying period. Readings made after midday 08/01/2007. Leaves taken from the same vines as in Figure 4.

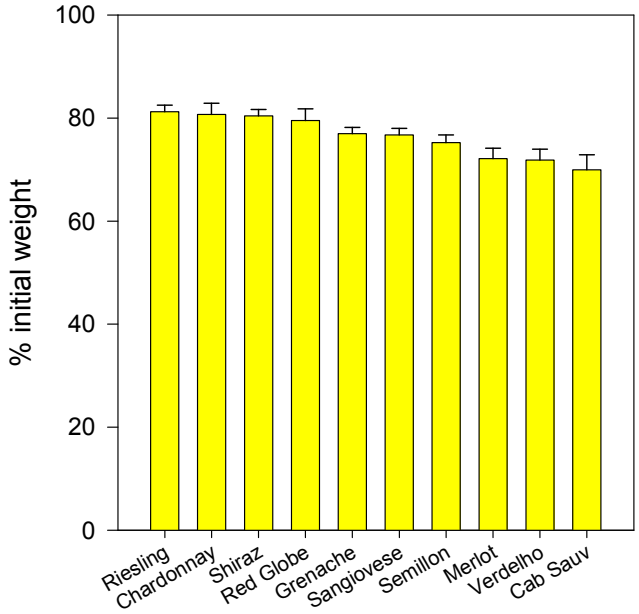


Figure 7. Weight loss of leaves from a number of varieties in the field at the end of a 70 min drying period. Readings made after midday 01/03/2007 . Leaves taken from the same vines as in Figure 4

Clearly, factors other than genotype are dictating the rate of water loss from these leaves. Current environmental conditions and recent irrigation history are the most likely drivers of this response and in an effort to reduce their likely impact we repeated these experiments with well watered vines in an air conditioned glasshouse.

Six varieties were available to test, namely: Grenache, Shiraz, Semillon, Chardonnay, Cabernet Sauvignon and Merlot. Five separate experiments were carried out. By assigning a score of 1 to 6 to each variety based on the ability of leaves to resist water loss it was possible to obtain a mean ranking. Grenache was most resistant to water loss as it had been in the field, ranking 1 in all experiments and Merlot was a clear least resistant winner, ranking on average 5. The tests could not readily differentiate between the other varieties, however Shiraz, for example, ranked second most resistant in three experiments but least resistant in two experiments and in this respect behaved similarly to Semillon. Chardonnay and Cabernet Sauvignon tended to be less resistant, behaving more like Merlot. Considering both the field and glasshouse experiments, Grenache stood out as being most resistant to desiccation and Merlot least resistant. Shiraz, the variety identified by Schultz (2003) as an example of an anisohydric variety, generally had an intermediate resistance to desiccation. Nevertheless, this simple test does suggest that leaf level hydraulic and morphological varietal differences do exist that are part of response to stressful environmental conditions. It is however unlikely that a simple test such as weight loss of excised leaves could reliably predict whole vine response and we have to look more closely at physiological adaptations to more fully understand the process. Interestingly, leaf fresh mass of the field vines closely reflected the average rankings obtained in the tests described above. Grenache and Red Globe had the largest leaf mass and Merlot, Cabernet Sauvignon and Riesling had the smallest. Verdelho, Semillon, Sangiovese, Shiraz and Chardonnay were not significantly different to each other but were different to Grenache and Red Globe at one extreme and Merlot, Cabernet Sauvignon and Riesling at the other (Figure 8).

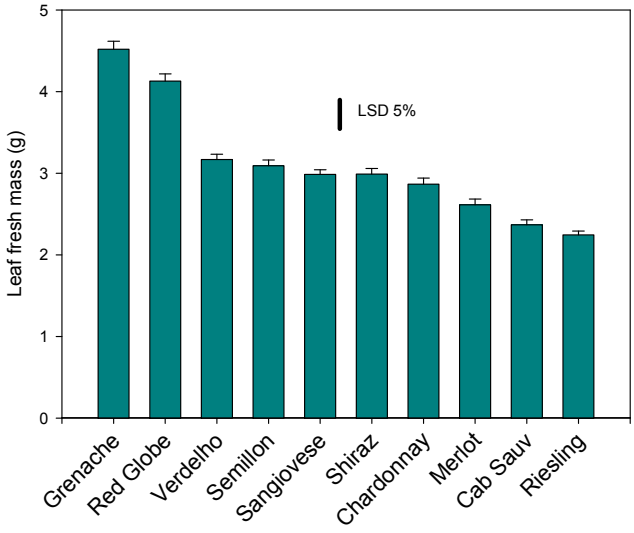


Figure 8. Mean fresh leaf mass measured 18/01/2009. The vines were the same as used in the weight loss experiments (Figures 4-7). Figures are means of 100 measurements and bars are standard errors.

Riesling appeared not to fit this relationship, having the smallest leaf mass but generally ranking as resistant to desiccation (Figs 4-7). Whatever the hydraulic property that confers resistance to desiccation of excised leaves, it presumably allows accumulation of more leaf mass in Grenache and Red Globe and conversely less accumulation for Merlot and Cabernet Sauvignon. Pruning weights in 2007 told much the same story (Table 4) with Grenache at one extreme and Merlot and Riesling at the other. In 2008 pruning weights were generally higher reflecting the better growing condition of that year. Grenache again had the highest pruning weight but the distinction between the remaining varieties did not follow the trend set by the leaf desiccation, leaf weight and pruning weight seen in previous years. Merlot, for example, had the third highest pruning weight, suggesting that additional water availability allowed this variety to perform quite well, whereas it coped less well when faced with water deficit.

Varietal differences in response to environment

Vapour pressure deficit (VPD) is the main environmental driver of leaf transpiration and in high VPD conditions it may be an advantage to the vine to conserve water

through stomatal closure. It would be anticipated that vines with isohydric characteristics will express this trait more highly than more anisohydric vines which tend to be less sensitive to VPD change. To assess a range of vine varieties for this ability to effect stomatal closure under the influence of high VPD we measured canopy temperature by infrared thermometry under a range of temperature and humidity conditions on 11 occasions during a 4 week period (Jan/Feb 2007). The difference between canopy temperature and ambient temperature is indicative of the rate of transpiration. For each grape variety the data were sorted according to whether VPD was high (>3kPa) or low (< 3kPa) and the 11 sample days were averaged, giving an indication of response over an extended time interval and range of environmental conditions. The varieties responded quite differently to changes in VPD with Verdelho showing no response and at the other extreme Grenache showing a very strong stomatal response to increased VPD (Figure 9) as we have noted previously (Soar *et al.* 2006).

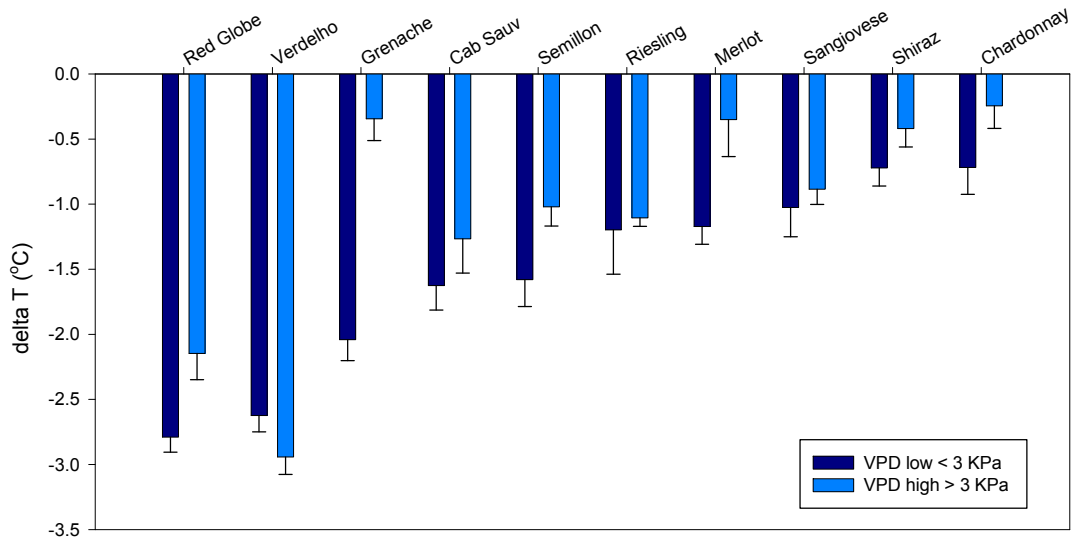


Figure 9. Difference between canopy and ambient air temperature under low VPD (less than 3kPa) and high VPD (greater than 3kPa).

Another way of looking at these data to allow a ranking of varieties, is to show the varietal differences by subtracting the high VPD figure from the low VPD figure. This should reflect the response intensity. Figure 10 shows that Grenache again stands out from the other varieties. The response intensity of the other varieties was less, with Shiraz, Riesling and Verdelho being virtually unresponsive to VPD change.

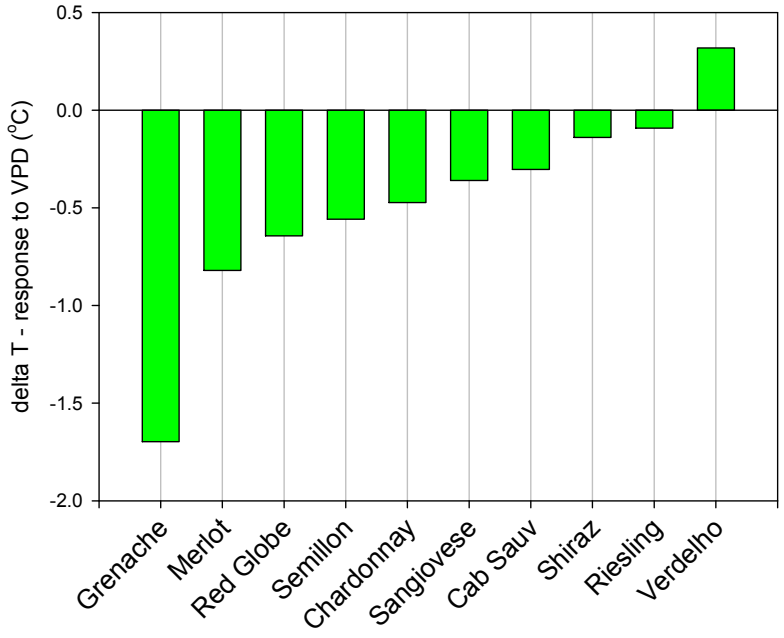


Figure 10. Magnitude of the influence of VPD on canopy temperature. A value close to zero suggests no response to VPD. Figures obtained by subtracting the high and low VPD figures from shown in Figure 9.

Since response to VPD is really at the heart of this distinction between isohydric and anisohydric varieties we looked in more detail at the response over several seasons. As suggested by the canopy temperature data, the quality of the VPD/gs relationship varied greatly between varieties and in most cases, there was a stronger relationship in the 2008 season than in 2007. Chardonnay, Verdelho and Semillon did not show a significant relationship in either season whereas Grenache, Riesling, and Shiraz did in both seasons (Fig 11). These data can to some extent be summarised by considering the response of stomatal conductance to either high or low VPD. This allows for a more defined ranking of varieties. In Table 5 we show this ranking for season 2007. Grenache stands out as having the most sensitive response to change in VPD with Cabernet Sauvignon and Merlot showing the least response.

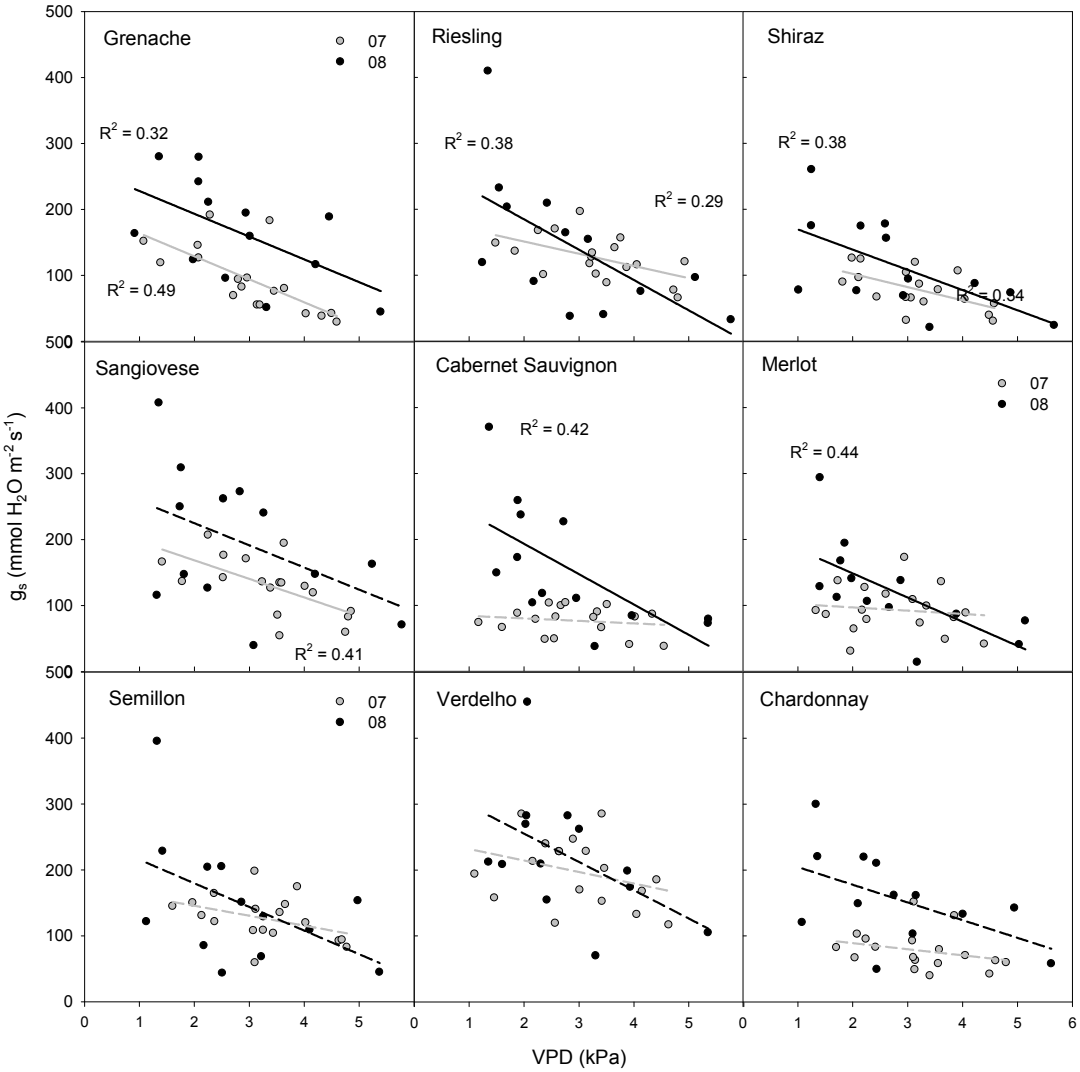


Figure. 11. Relationship between g_s and VPD in nine grapevine varieties. VPD and g_s was measured in the morning and afternoon on eight days during January and February 2007 and 2008. solid lines: relationship between VPD and g_s is significant ($p < 0.001$), dotted lines, relationship is not significant.

Table 5. Stomatal conductance under low and high VPD conditions in nine wine grape varieties. Stomatal conductance (g_s) was measured in the morning and afternoon on eight days between January 25th and February 22nd 2007. Values shown are means of these days. Letters indicate significant differences between varieties within low and high VPD conditions ($p < 0.05$); * indicates significant decrease in g_s in response to high VPD ($p < 0.05$); ** ($p < 0.001$).

Variety	Stomatal conductance (mmol H_2O $m^{-2}s^{-1}$)			
	Low VPD	High VPD	Δ g_s	% decrease
G	125 [a]	68 [a]	57**	46
SA	161 [d]	113 [c]	48**	30
R	149 [ad]	117 [c]	32**	21
SH	88 [bc]	70 [a]	18**	20
SE	142 [ad]	119 [c]	23**	16
CH	85 [bc]	73 [a]	12	14
V	204 [e]	182 [d]	22*	11
M	100 [c]	91 [b]	9	9
CS	79 [b]	73 [a]	6	8

A more rigorous assessment of stomatal response to VPD was suggested by a method used by Oren et al. (1999) to survey intra- and inter-specific variation in stomatal sensitivity to VPD. For a detailed description of the theoretical background of the analysis refer to Oren et al. (1999). Briefly, the analysis is based on the hypothesis that stomatal sensitivity is proportional to the magnitude of g_s at low VPD (≤ 1 kPa). Using the equation $g_s = gs_{ref} – m.\ln VPD$ where m is the stomatal sensitivity and $g_s$ref = g_s at VPD = 1 kPa, the authors concluded that individuals, stands and species with high stomatal conductance at low VPD show a greater sensitivity to VPD (Oren *et al.* 1999). This equation or a modified version of it appears throughout the literature (Breda *et al.* 1993; Granier and Loustau 1994; Lu *et al.* 1995; Arneth *et al.* 1996; Granier and Breda 1996; Granier *et al.* 1996). This relationship is a direct function of stomatal regulation of leaf water potential.

To apply this analysis to the inter-varietal variation in stomatal sensitivity of grapevines, g_s and VPD data collected through the 2006/7 season was regressed (gs / lnVPD) to get m (slope of the regression line). To obtain $g_s$ref VPD < 1 kPa, the value of g_s ref (b) is taken to be y0, where the regression line intercepts the y-axis. In this function -m quantifies the sensitivity of g_s to VPD and is equal to $– dg_s / d \ln VPD$ and is constant across the entire range of VPD, unlike $d g_s / d$ VPD, this definition of sensitivity allows comparisons independent of a specific VPD range (Oren *et al.* 1999).

There was a large amount of variability in the sensitivity of stomata of different grapevine varieties (Fig. 12 and Table 6). The baseline response of dg_s/dlnVPD to $g_s$ref had a shallow slope of 0.35 and 50% of the variation in dgs/dlnVPD is accounted for by $g_s$ref (R^2 0.48; Fig. 12A). Based on the high boundary layer conductance/stomatal ratio (due to using a porometer) and the VPD range (~ 1 to 5 kPa) the theoretically expected slope (sensitivity line) for isohydric plants is 0.52 (Fig. 12A and B). The regression line of the varieties is well below the sensitivity isoline expected for the VPD range in this experiment, indicating lower sensitivities and a varying degree of anisohydric response (Fig. 12A).

Across all varieties surveyed the sensitivity of g_s to VPD did not increase with $g_s$ref, for example Chardonnay and Shiraz had similar $g_s$ref values (180 and 175 mmol H_2O

m^2s^{-1}, respectively), yet very different slopes (26 and 61, respectively; Table 2). This relationship held up at both high and low $g_s$ref values with Merlot and Verdelho having similar values for $g_s$ref (107 and 112 mmol H_2O m^2s^{-1}, respectively) but very different slopes (19 and 33, respectively; Table 6).

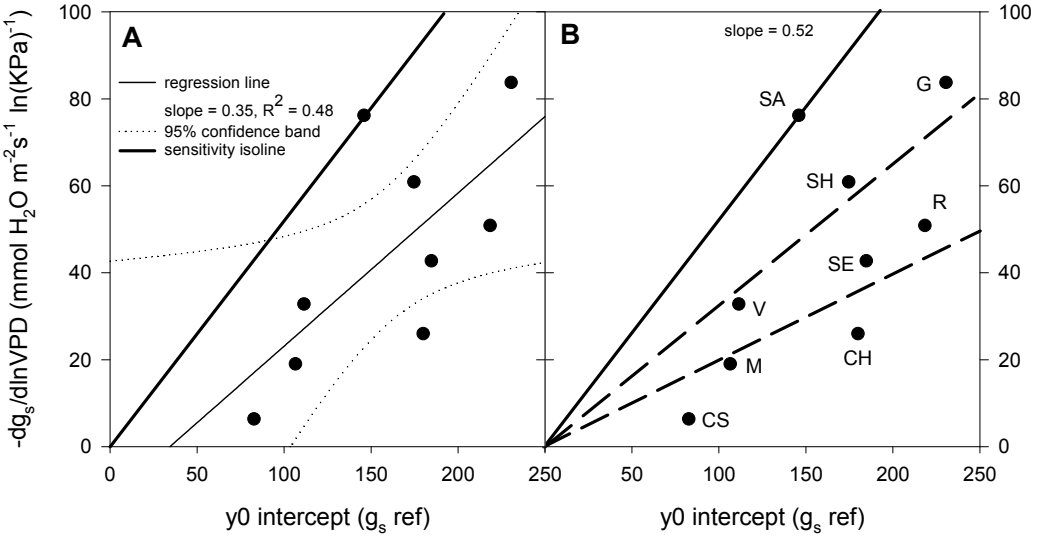


Figure 12. The sensitivity of stomatal conductance (g_s) of grapevine varieties to increasing VPD (-dg_s / d lnVPD) as a function of the stomatal conductance at VPD = 1 KPa in the 2006/7 season. The two axes represent the slope and the intercept of the relationship g_s = -m . ln(VPD) – b. The solid line (slope = 0.52) represents an isoline of sensitivity, a direct regulation of Ψ_L by stomatal control, such as occurs in isohydric plants. **(A)** Regression relationship for data set. Lines — regression slope (slope 0.35, R^2 = 0.48), ... 95% confidence interval, — sensitivity isoline (slope 0.52); **(B)** — sensitivity isoline (slope 0.52); – – dashed lines are isoline groups below the sensitivity isoline.

Table 6. The sensitivity of stomatal conductance (g_s) of grapevine varieties to increasing VPD (-dg_s / d lnVPD) as a function of the stomatal conductance at VPD = 1 kPa. For each variety the slope (-m) and the intercept (g_s ref, y0) of the relationship gs = -m . ln(VPD) – b are listed.

Variety	-m (slope)	g_s ref (y0)
G	84	231
SA	76	146
SH	61	175
R	51	219
SE	43	185
V	33	112
CH	26	180
M	19	107
CS	6	83

If varieties are grouped by isolines below the sensitivity isoline, they can be grouped into four groups; (1) Sangiovese, sitting exactly on the sensitivity isoline, (2) Shiraz

and Grenache, (3) Riesling, Verdelho and Semillon and (4) Chardonnay, Merlot and Cabernet Sauvignon (Fig. 12B). Moving up and down the sensitivity isoline slopes indicates similar sensitivity because along the slope sensitivity is proportional to the conductance.

Varietal differences in chemical signalling of water deficit

Considerable evidence links stomatal response to environment with the plant hormone abscisic acid (ABA) [see GWRDC report on project CSP06/03, Speirs, Loveys and Binney (Speirs *et al.* 2010) for a discussion on this subject]. We have therefore looked for varietal differences in the concentration of ABA in xylem sap. This may be analogous to the rootstock differences reported by Speirs et al in the project report noted above. In some varieties (Grenache, Riesling, Shiraz and Sangiovese) there was a strong negative relationship between xylem sap ABA concentration and stomatal conductance in both 2006/7 and 2007/8 seasons whereas in others (Cabernet Sauvignon, Chardonnay, Semillon, Merlot and Verdelho) a significant relationship was evident only in 2007/8 (Figure 13). The mean xylem ABA content, measured on 13 occasions in January and February, tended to reflect this grouping, with varieties that had a significant relationship between ABA and conductance having higher mean ABA (Figure 14). At first sight these data seem to contradict the ranking of varieties in terms of their conductance at low VPD shown in table 5 (gs ref, y(0)). Although there is some variation in the intermediate varieties, Grenache is shown to have the highest conductance at low VPD and Cabernet Sauvignon the lowest whereas Figure 14 shows that when measured over the whole season, under a range of environmental conditions, Grenache has the highest xylem sap ABA and Cabernet Sauvignon was significantly lower. These data strongly suggest that there is variation in the ability of the varieties to change their xylem ABA in response to VPD. This response is likely to occur in the leaves rather than it being a result of the roots responding to lower soil moisture since we know that the more isohydric varieties tend to conserve soil water under high VPD conditions. Furthermore, Soar *et al.* (2006) have shown that the genes responsible for ABA synthesis in leaves respond to VPD change and the genes responsible for ABA breakdown have also been shown to be sensitive to VPD (Okamoto *et al.* 2009; Speirs *et al.* 2010) A combination of increased ABA synthesis and changed patterns of ABA breakdown therefore occur as VPD increases, resulting in higher ABA in sensitive varieties.

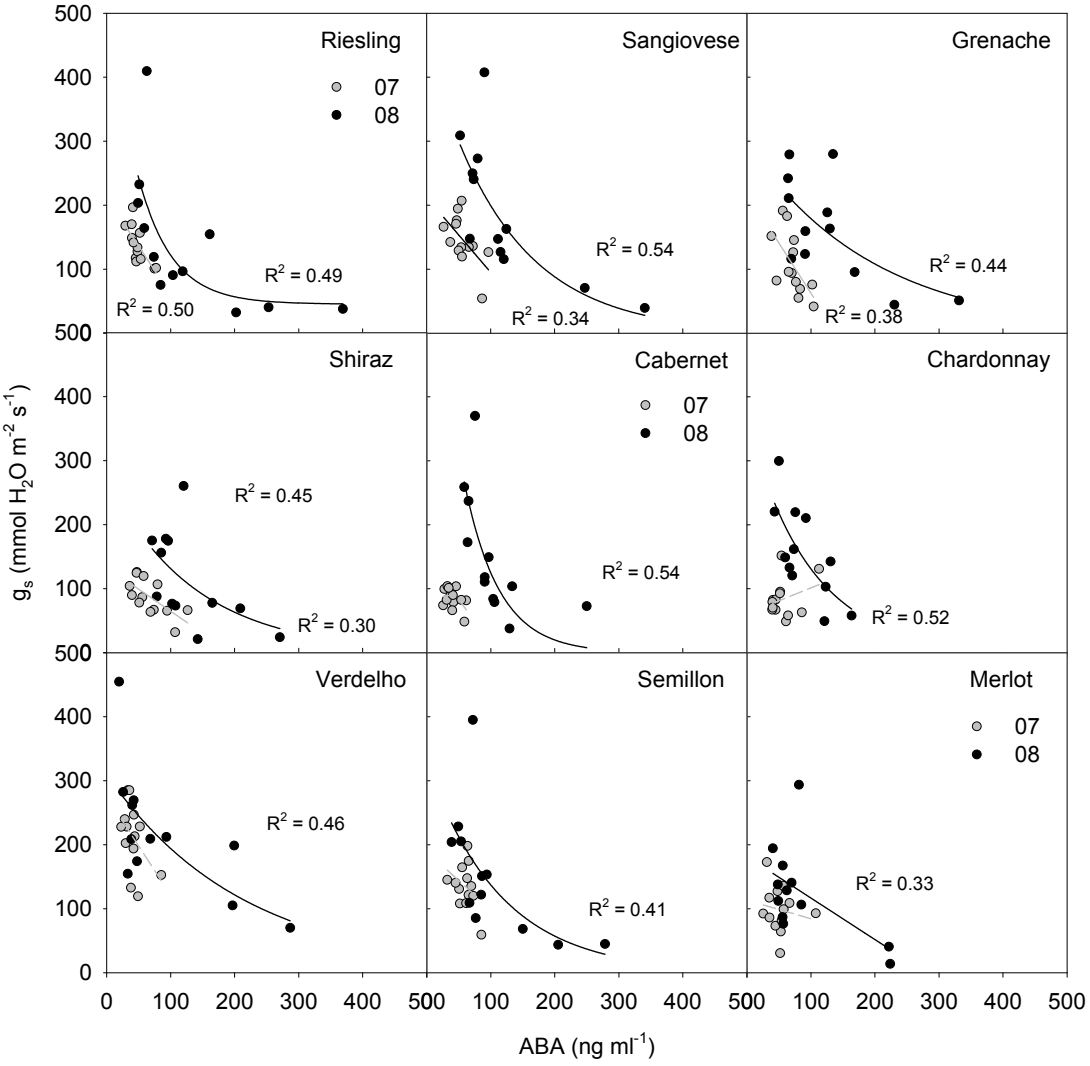


Figure 13 . Relationship between gs and ABA for nine varieties in seasons 2007 and 2008. Significant regression relationships ($p < 0.05$) are in solid lines and R^2 values are indicated on figures. All curves are single exponential decay with two parameters, except for Riesling in 2008, which is single exponential decay with three parameters.
Dashed lines indicate non-significant interactions.

Differences in stable isotope discrimination among varieties

There are many conventional indicators of plant function and response to water availability and other environmental factors. Many of these such as leaf water potential, stomatal conductance and to some extent soil water, change quite rapidly and provide a snapshot of plant performance over a time scale of minutes (stomatal conductance) to days (soil water). A measure that integrates vine performance in terms of its water use efficiency over a whole season is available in measures of the ratio of the carbon isotopes ^{12}C and ^{13}C. Discrimination between these isotopes is a property of the primary carboxylating enzyme in C3 photosynthesis, RUBISCO.

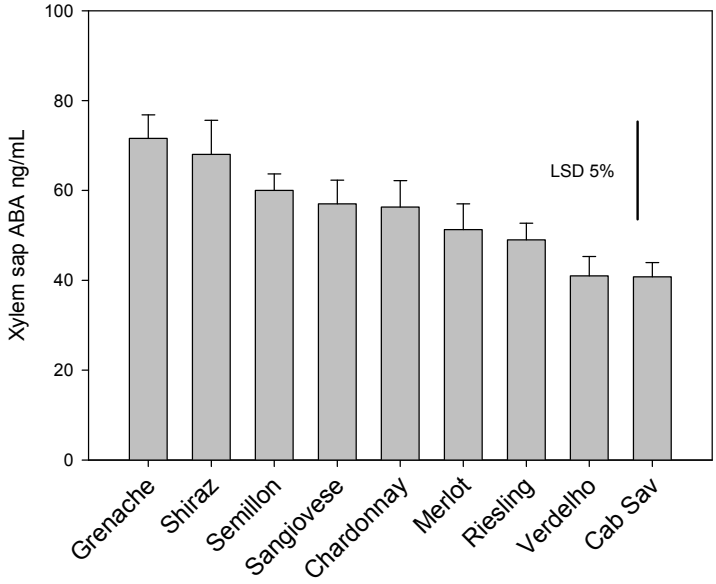


Figure 14. Mean (+ one SE) xylem sap ABA sampled on 13 occasions during January and February 2007.

This discrimination is reduced when the concentration of CO_2 available to the enzyme (Ci) is reduced due to partial stomatal closure. Thus, the relative amounts of the isotopes, expressed as parts per thousand relative to a standard ($\delta\ ^{13}C$) in a plant part will reflect the average Ci over the period that carbon was acquired during the formation of that plant part. $\delta\ ^{13}C$ was generally higher (less negative) in season 2007 than in 2008 (Figure 15A) reflecting the poorer growing conditions and generally lower stomatal conductances in that year. Riesling and Cabernet Sauvignon were the only varieties not to show this trend. In the year 2008 there was a significant relationship between $\delta\ ^{13}C$ and yield per vine (Figure 15B). At first sight it would therefore seem that measurement of $\delta\ ^{13}C$ is a useful predictor of potential yield because it integrates the influence of many factors on stomatal conductance and therefore Ci, but Ci is also influenced by photosynthetic capacity which in turn is strongly influenced by factors such as leaf nutrition. When leaf nitrogen is plotted against $\delta\ ^{13}C$ (Figure 16) the relationship between the varieties largely mirrors that shown in Figure 15. In other words, leaf nitrogen, through its effect on photosynthetic capacity, may be having a large effect on $\delta\ ^{13}C$. However, the leaf nitrogen figures are from leaves at harvest and may be influenced by the amount of nitrogen removed in the crop whereas that $\delta\ ^{13}C$ figures are derived from an integrated effect over the whole season.

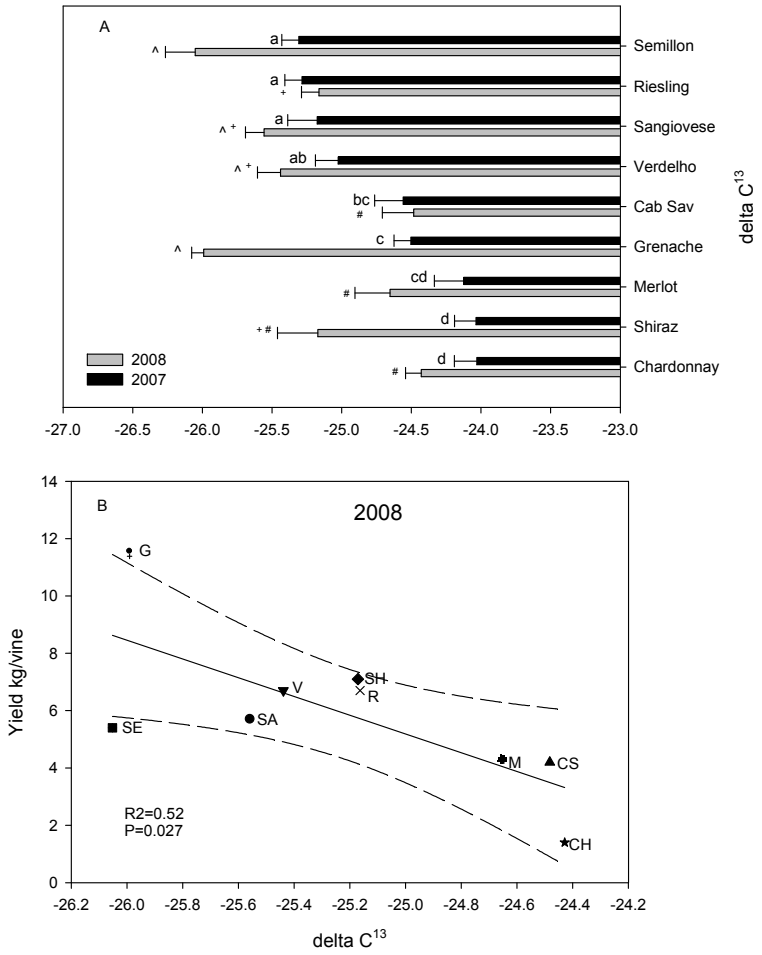


Figure 15. A. mean foliar isotope ratios measured in a range of varieties at harvest. B. relationship between yield at harvest and foliar isotope ratios.

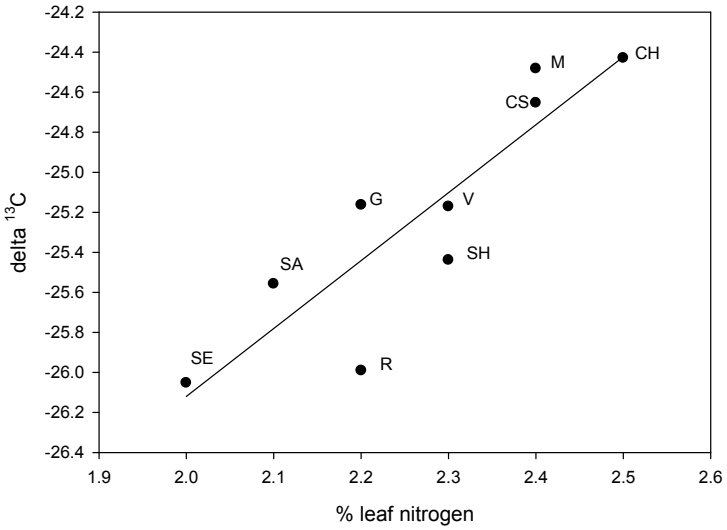


Figure 16. Relationship between lamina nitrogen at harvest and carbon isotope discrimination (delta ^{13}C) season 2008.

Clearly, many factors are acting to influence carbon isotope partitioning, not all of which are accounted for in the simple model used here and a more comprehensive model which takes into account some of these influencing factors may be necessary to enable stable isotope ratios to be used to differentiate the varieties on the basis of their response to environment.

Hydraulic conductivity

In order to effectively exploit soil water reserves hydraulic contact between the roots and the leaves must be maintained. Movement of water through this pathway is driven by gradients in water potential. For example, soil at field capacity may have a water potential close to zero, say -10kPa, and the transpiring leaves of a vine in this soil may have a midday water potential of -1.0MPa. The air surrounding these transpiring leaves will have a much lower water potential. At 20^{o}C and 96% relative humidity air has a water potential of -5.5MPa and on a hot dry day of 30^{o}C and 10% relative humidity air water potential will reach -320MPa. Clearly, the integrity of the leaf cuticle and the stomata play the primary role in regulating water movement through the vine because the largest water potential gradients exist at the leaf/air interface. However, an effective hydraulic pathway between root and leaf must exist to maintain leaf hydration. Schultz (2003), showed that Grenache and Shiraz, for example, differed strongly in the hydraulic properties of their petioles and internodes and he suggested that this was the key reason for their isohydric or anisohydric behaviour. We have therefore investigated some of the hydraulic properties of water conducting tissues in a range of vine varieties.

There were significant differences in internode hydraulic conductivity among varieties with Grenache being significantly higher than Merlot, Riesling, Sangiovese and Cabernet Sauvignon (Figure 17). When conductivity was regressed against a number of variables such as leaf area, canopy area, stomatal conductance, pruning weights in both the 2007 and 2008 seasons, no strong relationships were evident (data not shown). However, hydraulic architecture is an important aspect of plant response to water deficit and probably deserves further investigation in seeking an explanation for grapevine varietal differences.

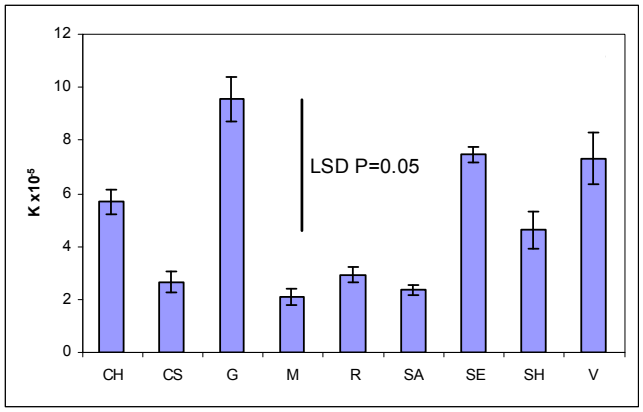


Figure 17. Internode hydraulic conductivity ($m^3.MP^{-1}.s^{-1}$) of nine grape varieties. Error bars are SE , n=12.

For example, differences in vessel size would cause the cultivars with larger vessels to have more cavitation events under moderate water stress and prolonged cavitation events under more severe stress (Sperry and Saliendra 1994; LoGullo *et al.* 1995) and plants with larger vessels develop cavitation at higher water potentials than plants with lower ones (Tyree and Dixon 1986), which the leads to stomatal closure. This probably explains the tendency for Grenache and other more isohydric varieties to exhibit stomatal closure at relatively high water potentials. Changes in xylem vessel size may also be an adaptive response to long term water deficit since it has been shown that modifications of vessel size and of shoot hydraulic conductivity are induced in grapevines by different water availability levels. Xylem sap flow and leaf transpiration was lower in water-stressed plants and the vessels of these plants had lower transectional areas (Lovisolo and Schubert 1998). Shoot hydraulic conductivity, shoot specific conductivity and leaf specific conductivity were lower in water-stressed plants and at higher stress levels shoot conductivity was further reduced by vessel embolisms (Lovisolo and Schubert 1998).

Conclusions

The performance of vines in the field is driven by a range of morphological and physiological characteristics which are determined by the genetic background of individual varieties. In this chapter we have assessed a number of grape varieties for their response to changes in their environment. Most tests were carried out on nine varieties growing at one location under uniform conditions and these varieties included many of those more commonly grown in Australia. While the variety mix

could not be exhaustive, it did represent over 77% of the major wine grape varieties currently grown in Australia (Table 7). Grenache was a strong performer in most of the tests but it represents less that 2% of the vines grown in Australia, although world-wide it is one of the most commonly planted varieties.

	ha	%
Red		
Shiraz	44082	46
Cabernet Sauvignon	27537	28
Merlot	10990	11
Pinot Noir	4770	5
Other (including Grenache)	8639	9
White		
Chardonnay	29831	49
Semillon	6481	11
Sauvignon Blanc	6135	10
Riesling	4516	7
Colombard	2660	4
Muscat Gordo Blanco	2134	4
Other	9463	15

Table 7. Area planted to grape varieties in Australia 2009.
Source: Australian Bureau of Statistics publication 1329.0.55.002

Some of the measurements made, pruning weights for example, simply reflect the season-long performance of the vine under prevailing conditions while others such as canopy temperature provide an instantaneous measure of performance at the time of measurement whereas measurement of stable isotope ratios can give information about vine response integrated over a whole season. If carried out over a range of conditions, high and low VPD for example, short term tests can give information about performance of different varieties under both mild and adverse conditions. While we can understand the changes in canopy temperature in terms of current transpiration which is regulated by stomatal conductance, factors which drive the rate of water loss from excised leaves, for example, may be more complex. This simple test may be influenced by many factors such as the permeability of the leaf cuticle, amount of stored water in the leaf, the hydraulic conductivity of the leaf vasculature, stomatal density, stomatal structure, leaf characteristics such as surface topography and hairiness, petiole hydraulic conductivity, susceptibility of the vasculature to

cavitation, and probably more. Some of these characteristics may be relevant to the control of water loss in the intact vine while some may not.

Some of the tests provide us with clues about the mechanisms responsible for their control. For example, the sensitivity analysis shown in Figure 12 and Table 6 allows the ranking of varieties according to the sensitivity of their stomata in responding to changes in VPD and these figures are highly positively correlated (r^2=0.56 P=0.015) with xylem sap ABA (Figure 14). The conclusion here is that leaves that are better able to raise the concentration of ABA available to the stomata will respond more sensitively to VPD change. We have reported elsewhere (Speirs, Loveys and Binney, GWRDC report CSP06/03) that the ABA concentration in sap sampled from leaf petioles with a leaf pressure bomb (the technique used here) probably reflects both root and leaf activity and so it is tempting to speculate that the isohydric character of a scion variety could be amplified by grafting to a rootstock better able to supply ABA and Speirs *et al.* (2010) also report that rootstocks do differ in this regard. We make the assumption here that a variety showing enhanced isohydric character would be better able to deal with more stressful environmental conditions imposed by climate change and/or the application of deficit irrigation and therefore that the manipulation of ABA physiology may be beneficial.

Given the diverse range of tests applied and responses reported in this chapter it would not be surprising if there was little consistency among the varieties. This was not the case, however and the ranking of varieties was surprisingly consistent among the tests. This meant that a simple analysis of variance test allowed a comparison and ranking of the varieties (Figure 18). Grenache clearly stood out as the best performer and Cabernet Sauvignon, Merlot and Chardonnay the worst. The remaining varieties could not be statistically differentiated. One of the project objectives relevant to this chapter was to develop a simple test to differentiate the varieties on the basis of the isohydric or anisohydric character. While we would like to unequivocally specify one particular test to describe and predict the isohydric/anisohydric character of a grapevine genotype, the ability of vines to make both short and long term adaptive changes in their morphology, anatomy and physiology make this very difficult and this is well illustrated in chapter 6 of this report where we show that by making long term changes in irrigation management Cabernet Sauvignon, a variety that would generally be classified as anisohydric, can be made to exhibit characters more commonly associated with isohydric varieties.

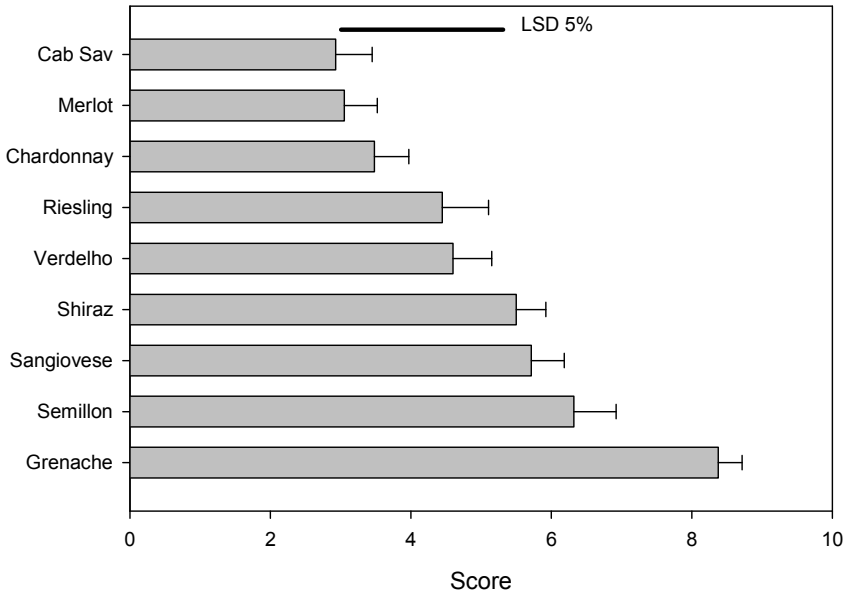


Figure 18. Mean ranking of 9 grape varieties across all the tests reported above. Error bars are SE, n=17.

B. Influence of climatic conditions on the performance of isohydric and anisohydric cultivars

The work that contributed to this chapter was the result of integration of project CSP 05/02 with SAR 05/01 and SAR 05/02. This collaboration resulted in a publication in the Australian Journal of Grape and Wine Research (Australian Journal of Grape and Wine Research 14, 250-259, 2008) and this publication is reproduced in full here with permission.

Modelling variety-dependent dynamics of soluble solids and water in berries of *Vitis vinifera*

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Abstract

Background and Aims: We modelled the dynamics of soluble solids, largely sugars, and water in 12 *Vitis vinifera* varieties. Emphasis was placed on maximum concentration of soluble solids (S_{max}) and time of maturity for their viticultural importance.

Methods and Results: We measured the concentration of soluble solids and water at weekly intervals during berry ripening. The dynamics of concentration of soluble solids was characterised with a sigmoid model, whereas water concentration was characterised with a concentration–response type curve. Scaling exponents for soluble solids (α_s) and water (α_w) were calculated as the slope of the log–log regression between amount of soluble solids or water per berry and berry fresh mass. S_{max} ranged from 27.1% in Shiraz to 21.2% in Riesling, was associated with both α_w and α_s, and was largely unrelated to source size (leaf area, pruning weight, light interception), source activity (stomatal conductance), sink size (yield components) and source : sink ratios. The time of maturity ranged from 26 January in Verdelho to 27 February in Crimson Seedless, and was an inverse function of the rate of change in concentration of soluble solids, which was in turn a direct function of stomatal conductance.

Conclusions: Traits related to carbon assimilation influenced time of maturity, but their link with maximum concentration of soluble solids in berries was not evident.

Significance of the Study: Quantitative models of accumulation of soluble solids are presented that provide a baseline for comparisons among varieties.

Keywords: *genotype, maturity, phenology, phenotypic plasticity, sink, source, stomatal conductance, sugar*

Introduction

Sugar concentration of grapevine berries is a major criterion for crop management and winemaking decisions, including harvesting time. Variety, environmental conditions and their interaction influence the timing of harvest maturity, as determined by concentration of both sugar and quality-related flavour and colour compounds (e.g. Vian et al. 2006, Gomez-Miguez et al. 2007). Recent warming trends have advanced the time of maturity of Shiraz, Cabernet Sauvignon and Chardonnay in Australia, and indirect evidence indicates some decoupling between accumulation of sugars and secondary compounds in ripening berries (Godden and Gishen 2005, Petrie and Sadras 2008). Consistent with these maturity trends, trends for high alcohol content in Australian wines during the last two decades are evident for red but not for white varieties (Godden and Gishen 2005). These trends have multiple drivers, including variety-dependent differences in sugar accumulation, which are only partially known.

In grain crops, physiological maturity (i.e. maximum grain dry weight) is closely linked to the dynamics of water (Rondanini et al. 2007, and literature cited therein). In comparison with grain crops, the links between maturity, soluble solids and water dynamics in berries of grapevine have received less attention (e.g. Ristic and Iland 2005). The allometric condition for increasing concentration of soluble solids is that the scaling exponent for soluble solids α_s, i.e. the slope of the regression between amount of soluble solids per berry and berry fresh mass in a log–log scale, is greater than 1 (Sadras and McCarthy 2007). The condition for decreasing water concentration is that the scaling exponent for water α_w, i.e. the slope of the regression between amount of water per berry and berry fresh mass in a log–log scale, is lower than 1. Whereas the allometric conditions for increasing concentration of soluble solids and decreasing water concentration follow from the definitions, i.e. the scaling exponent is the ratio of the relative rate of accumulation of soluble solids or water and the relative rate of berry growth (Pearsall 1927, Niklas 1994), the magnitude of the scaling exponents depends on environmental factors (Sadras and McCarthy 2007, Sadras et al. 2007) and may also differ with variety-dependent traits.

doi: 10.1111/j.1755-0238.2008.00025.x
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Table 1. Varieties compared at Nuriootpa (Barossa Valley, South Australia).

Variety	Clone	Type	Vine age (years)	Measurements
Cabernet Sauvignon	LC84	R	18	S, W^2, f, LA, Pw, Yc, gs
Chardonnay	I10V1	W^1	32	S, W^2, f, LA, Pw, Yc, gs
Crimson Seedless	USDA 1992	T	13	S, W^2, f, Yc
Dawn Seedless	88–05 Olmo G4-36/VX/ UCD	T	16	S, W^2, f, Yc
Fantasy Seedless	USDA 1992	T	13	S, W^2, f, Yc
Grenache	139HT 401-1	R	15	S, W^2, f, LA, Pw, Yc, gs
Merlot	8R	R	9	S, W^2, f, LA, Pw, Yc, gs
Red Globe	88–07 Olmo 10–23 D	T	17	S, W^2, f, LA, Pw, Yc, gs
Riesling	I10V14	W^1	17	S, W^2, f, LA, Pw, Yc, gs
Semillon	D10V12	W^1	13	S, W^2, f, LA, Pw, Yc, gs
Shiraz	BVRC12	R	11	S, W^2, f, LA, Pw, Yc, gs
Verdelho	SA168	W^1	11	S, W^2, f, LA, Pw, Yc, gs

f, fractional PAR interception; gs, stomatal conductance; LA, leaf area; Pw, pruning weight; R, red wine; S, total soluble solids in berries; T, table grape variety; W^1, white wine; W^2, water concentration in berries; Yc, yield components.

This study presents a quantitative comparison of the dynamics of soluble solids and water in berries of wine and table grape varieties. The primary focus is maximum concentration of soluble solids and the timing of maturity for their implications for vineyard management and winemaking. An allometric approach is used to account for size-dependent changes in soluble solids and water in berries.

Materials and methods

Site and crops

The vines were established on a red brown earth (Northcote 1979) at the South Australian Research and Development Institute, Nuriootpa (34°S, 139°E) in the Barossa Valley of South Australia. Climate, soils and viticultural practices of the region have been described elsewhere (Gladstones 1992, Dry and Coombe 2004). Varieties were at least 9 years old at the time of the experiment (Table 1). Unlike management in commercial vineyards that accounts for specific varietal requirements, the experimental blocks under study were managed similarly. Vines are own-rooted and cordon-trained to a single-wire trellis and have been consistently spur pruned since establishment. Each variety comprises a single row with at least 37 vines. The vines are arranged with a row-by-vine spacing of 3.50 m × 2.25 m and have been managed with supplementary irrigation (single 4-L/h drippers per vine) since establishment.

Berry sampling and analysis

Berries were sampled weekly, between 8:00 and 11:00 a.m., by cutting with scissors through the pedicel as close as possible to their point of attachment. Samples were taken from a minimum of 10 consecutive vines within each varietal row. Three berries were sampled per bunch selecting one each from the bottom, middle and top of each bunch. No more than five bunches were sampled per vine. Depending on berry size, each sample comprised 50 (Grenache and table varieties) or 70 (other varieties) berries. Berries were collected into polyethylene bags, placed immediately into a container cooled with ice in the field, and stored at −20°C in the laboratory prior to analysis.

Water concentration was derived from fresh weight and dry weight determined in subsamples dried at 60°C to constant weight. A second subsample was thawed in the same plastic bag used for sampling, and crushed between the palm of the hand and a bench top using consistent light pressure. The free-run juice was decanted into a 50-mL centrifuge tube and spun at 2000 × *g* for 10 min. Total soluble solids, largely sugars (Coombe and Iland 2004), were measured on the clarified juice sample using an RFM710 digital refractometer (Bellingham and Stanley Ltd, Kent, UK).

Modelling the dynamics of soluble solids and water

The dynamics of concentration of soluble solids *(S)* was characterised with a sigmoid model, i.e. a transition function with symmetry about a transition centre in both the x and y dimensions:

$$S = \frac{S_{max}}{1 + e^{\left[-\frac{x - x_0}{b}\right]}} \quad (1)$$

where x is time, S_{max} is the transition height, representing maximum concentration, x_0 is the transition centre, i.e. the time when concentration is half the maximum, and b = transition width × 2.197^{-1} (SYSTAT 2002). The transition width is the time (days) it takes for concentration of soluble solids to raise from 0.25 S_{max} to 0.75 S_{max} (SYSTAT 2002). The ratio S_{max} : transition width was taken as a measure of the rate of change in *S*. From the fitted curves, we derived two measures of timing of maturity. A physiological measure of berry maturity, accounting for both

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the intra-specific variation in maximum concentration of soluble solids and the shape of the fitted curve, was the time when S reached 0.95 S_{max} (Rawson and Turner 1982). An oenological estimate of maturity was the time when berries reached a concentration of soluble solids of 20% (Pearce and Coombe 2004).

The dynamics of water concentration was characterised with a concentration–response type curve (Saxena et al. 1997):

$$W = W_{min} + \frac{W_{max} - W_{min}}{1 + (x/x_{50})^n} \quad (2)$$

where W is water concentration, and subscripts indicate maximum and minimum, x_{50} is the time when water concentration is halfway between maximum and minimum, and n is the Hill slope, a scalar that controls the slope of the curve (Saxena et al. 1997).

Allometric analysis

The scaling exponent for soluble solids α_s was calculated as the slope of the regression between amount of soluble solids per berry and berry fresh mass in a log–log scale, and the scaling exponent for water α_w, as the slope of the regression between amount of water per berry and berry fresh mass in a log–log scale (Sadras and McCarthy 2007, Sadras et al. 2007). Given the tight associations ($r \geq 0.97$), model I and model II regressions yielded similar scaling exponents (Niklas 1994); model I regressions were thus used for consistency with previous studies (Sadras and McCarthy 2007, Sadras et al. 2007).

Sources and sinks

To investigate the physiological drivers of S_{max} and timing of maturity, we measured source size and activity, and sink size in the varieties indicated in Table 1. Three measures of source size were obtained: fractional interception of photosynthetically active radiation (PAR), canopy leaf area and pruning weight. Fractional PAR interception was calculated as the ratio of PAR measured at ground level (in five positions parallel to the row at 0.3 m intervals, and three replicates per variety) and incident PAR (average: 2065 μmol/m^2/s) measured with a ceptometer on 7 February 2007. Total canopy leaf area was calculated by measuring the leaf area of leaves randomly sampled from the fourth node of shoots and multiplying this by the average number of nodes per shoot and shoots per vine. Ten leaves were sampled on 10 replicate vines, giving 100 leaves in total for each variety. Leaves were scanned (Mirascan, BenQ Taipai, Taiwan) and leaf area was calculated using SigmaScan Pro 4.0 (SYSTAT Software Inc., California, USA). For each variety, shoots per vine were counted on 10 vines early in the season prior to flowering (November 2006). Nodes per shoot were counted on the same 10 vines at the end of the season when leaf senescence was occurring (May 2007). Pruning weights of the same 10 vines were measured after spur pruning (June 2007). Yield was estimated from bunch numbers counted in five vines (coefficient of variation = 0.26), and bunch fresh weight from a sample of 10 bunches (coefficient of variation = 0.37).

Stomatal conductance was measured as a surrogate of source activity. Mid-morning (9.00–11.00) stomatal conductance was measured with an AP4 porometer (Delta T Devices, Cambridge, UK) on 6 days during the berry ripening stage. All measurements were taken on the western side of the vine on cloudless days. Before commencing measurements each day, the porometer was calibrated using ambient temperature and humidity measured with the porometer. Fifteen leaves over five replicate vines were measured for each variety, and values logged after readings became stable (<10–15 s).

Phenological development to maturity

To investigate variety-dependent time to maturity further, we used published data to account for phenological variation before and after veraison (Pearce and Coombe 2004). Regression was used to analyse the contribution of flowering-to-veraison and veraison-to-20°Brix in the variation in time to maturity for a collection of 23 varieties established at Nuriootpa, in the same location where the current study was established.

Results

Seasonal conditions, yield and source : sink ratio

The experimental season was similar to the typical crop-growing season at Nuriootpa except for higher maximum temperatures (Table 2). A climatic water deficit, i.e. reference evapotranspiration – (rain + irrigation) in the order of 800 mm, was also typical of the viticultural practices in the region.

Yield range was 25-fold, and the source : sink ratio calculated as fractional PAR interception : yield varied by a factor of 29 (Figure 1). Source : sink ratio based on PAR interception was similar to source : sink ratios based on leaf area : yield ($r^2 = 0.88$, $P = 0.0002$) and pruning weight : yield ($r^2 = 0.96$, $P < 0.0001$).

Dynamics of soluble solids and water concentration in berries

Figure 2 and Table 3 summarise the dynamics of concentration of soluble solids in berries. A sigmoidal model with three parameters (Eqn 1) applied to all varieties ($0.85 \leq$ adjusted $R^2 \leq 0.98$), except for Crimson Seedless, which required a 4-parameter model to account for the pronounced initial tail. Crimson Seedless was thus excluded from analysis of curve-derived parameters.

The maximum concentration of soluble solids ranged from 21.2% in Riesling to 27.1% in Shiraz. The time (day of year) of physiological maturity ranged from 37 (6 February) in Riesling to 69 (10 March) in Merlot, and the time of oenological maturity ranged from 26 (26 January) in Verdelho to 58 (27 February) in Crimson Seedless.

Figure 2 and Table 4 summarise the dynamics of water concentration in berries. A dose–response curve described the dynamics of water concentration in berries of all varieties (Figure 2; $0.86 \leq$ adjusted $R^2 \leq 0.99$). The maximum concentration of water in berries was above 90% for table varieties, and below 88% for wine varieties.

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Table 2. Growing conditions during the 2006–2007 season at Nuriootpa (Barossa Valley, South Australia) and long-term (LT) comparison.

Month	Max. temp. (°C)		Min. temp. (°C)		Radiation (MJ/m²)		ETo† (mm)		Rain + irrigation (mm)	
	2006–2007	LT	2006–2007	LT	2006–2007	LT	2006–2007	LT	2006–2007	LT‡
September	20.2	16.9	6.1	6.0	17.4	15.7	88	72	20	61
October	23.9	20.3	7.1	8.0	22.7	19.8	135	111	3	47
November	27	24.1	10.5	10.1	24.3	23.0	157	141	25	31
December	28	26.5	12.3	12.0	25.6	24.7	175	167	39	24
January	29.4	28.9	14.6	13.7	25.2	25.5	175	180	63	18
February	32.4	28.7	15.6	14.0	24.4	23.4	167	152	12	20
March	25.9	25.7	12.9	11.8	17.9	19.3	122	127	33	23
Total							1019	950	195	234

†Reference evapotranspiration calculated with FAO56 model described in Allen et al. (1998).
‡Rainfall only.

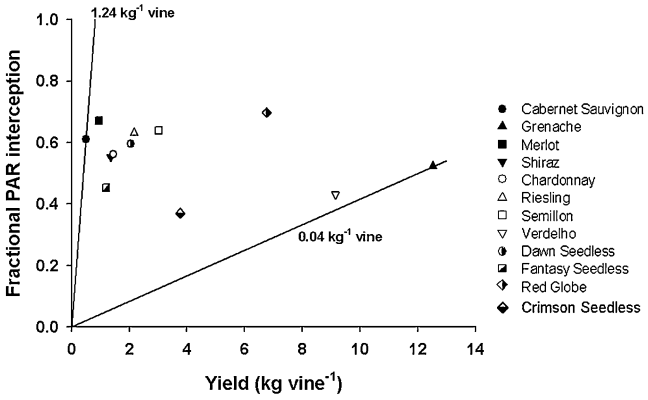


Figure 1. Canopy size, characterised as the fraction of photosynthetically active radiation (PAR) intercepted by the canopy, and fruit yield of 12 grapevine varieties at Nuriootpa, 2006–2007. The lines are the boundary source : sink ratios.

The minimum water concentration in berries ranged between 79% in Crimson Seedless and 65% in Shiraz, and averaged 76% in table varieties, 72% in white wine varieties and 68% in red wine varieties. The concentration of water in berries at physiological maturity (0.95 S_{max}) ranged from 69.7 to 80.0%, and averaged 78.7% in table varieties, 74.7% in white wine varieties and 71.8% in red wine varieties.

Allometric relationships

As expected for post-veraison berries actively increasing their sugar concentration (Coombe and Iland 2004), the scaling exponent for soluble solids was consistently greater than 1 (range: 1.92 to 2.65), whereas the scaling exponent for water was consistently lower than 1 (range: 0.78 to 0.89). Maximum concentration of soluble solids in berries was related to both scaling exponents, but more strongly with the scaling exponent for water (Figure 3). Shiraz, the variety with the greatest concentration of soluble solids, had a scaling exponent for water of 0.78 ± 0.051 compared with 0.86 ± 0.015 for Riesling, the variety with the lowest S_{max}. The allometric coefficients for soluble solids and water were unrelated to yield, canopy size or source : sink ratios (not shown).

Relationships between maximum concentration of soluble solids, sources and sinks

High S_{max}, as in Shiraz (Figure 2, Table 3), could be related to high source : sink ratio. To test this hypothesis, we explored the associations between S_{max} and several measures of source size and activity, sink size and source : sink ratios. Owing to the imperfection of each of these individual variables, we analysed them collectively (Table 5). Except for a marginal positive association with pruning weight, maximum concentration of soluble solids in berries was largely unrelated to source size and activity, sink size and activity, and source : sink ratios (Table 5).

Relationship between timing of maturity, sources and sinks

The timing of maturity was closely associated with the time required for berries to increase concentration of soluble solids from 25 to 75% of maximum ($r^2 = 0.37$, $P < 0.05$ for oenological maturity, and $r^2 = 0.81$, $P = 0.0002$ for physiological maturity). These associations reinforce the conclusion from independent measurements at Nuriootpa that post-veraison development is the main driver of intra-specific variation in time to maturity in this environment, whereas the contribution of variation of pre-veraison development is negligible (Figure 4).

The date of maturity was an inverse function of the rate of change in concentration of soluble solids, which was in turn a direct function of stomatal conductance (Figure 5). Associations between rate of accumulation of soluble solids and stomatal conductance were always positive and statistically significant in 5 out of 6 measurement days ($P < 0.02$). Stomatal conductance was an inverse function of source : sink ratio (Figure 6).

Discussion

We monitored and modelled the dynamics of soluble solids and water in berries of a diverse collection of grapevine varieties under a narrow set of environmental con-

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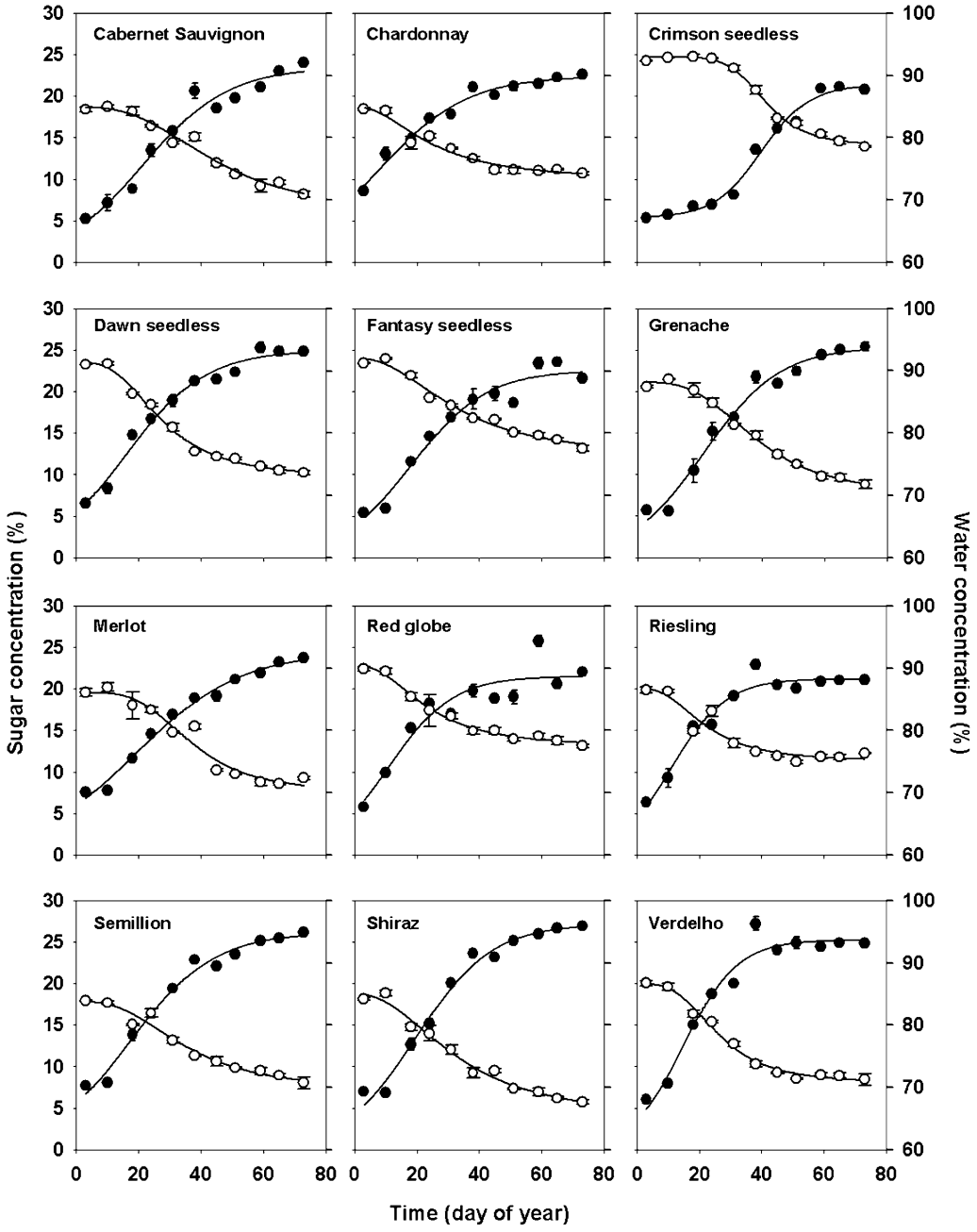


Figure 2. Dynamics of concentrations of soluble solids (closed symbols) and water (open symbols) in berries of *Vitis vinifera* varieties at Nuriootpa, 2006–2007. Error bars are two standard errors of the means. Parameters of the fitted curves are in Table 3 (soluble solids) and Table 4 (water).

ditions viz. single season, single location and uniform crop management. The plasticity (sensu Bradshaw 1965) of the varietal patterns of accumulation of water and soluble solids in berries in relation to management and environmental factors thus requires specific testing. Sadras and McCarthy (2007) found some degree of plasticity in the accumulation of sugar and dehydration of Shiraz berries.

Minimum water concentration

Implicit in the comparison among the varieties in this study was the expectation of distinct patterns of soluble solids and water dynamics in table grape varieties, putatively selected for large berry size and higher water content at maturity, in contrast to wine varieties where turgor maintenance was possibly less relevant. Liu et al. (2006) reported generally lower content of sugar in table varieties compared with their wine counterparts; implicit in this finding is a greater water content in table varieties. Consistently, here, we found that the ranking of minimum water concentration in berries, i.e. 76% in table varieties, 72% in white wine varieties and 68% in red wine varieties, supports the notion of distinct patterns of water dynamics, possible related to differential selective pressures between table and wine varieties. This contrasts with the small intra-specific variation for this trait

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Table 3. Parameters of a sigmoidal model (Eqn 1) accounting for the dynamics of soluble solids in berries.

Variety	S_{max} (%)		x_0 (d)		b (d)		Transition width (d)	Timing of maturity (day of year)	
	estimate	SE	estimate	SE	estimate	SE		Physiological	Oenological
Cabernet Sauvignon	23.5	1.18	21.3	2.31	13.7	2.30	30	62	45
Chardonnay	22.4	0.54	7.9	1.29	14.1	1.82	31	49	38
Crimson Seedless†								60	58
Dawn Seedless	24.9	0.68	16.0	1.29	12.6	1.45	28	53	34
Fantasy Seedless	22.5	0.96	18.5	1.96	11.9	2.04	26	53	43
Grenache	25.3	0.85	21.4	1.51	12.1	1.47	27	57	37
Merlot	24.4	0.81	19.0	1.60	16.9	1.75	37	69	44
Red Globe	21.5	1.11	11.2	2.62	10.1	2.98	22	41	37
Riesling	21.2	0.58	11.3	1.35	8.7	1.44	19	37	36
Semillon	26.1	0.73	17.2	1.31	13.3	1.45	29	56	33
Shiraz	27.1	0.74	19.8	1.25	12.0	1.26	26	55	32
Verdelho	25.2	0.77	15.1	1.41	8.5	1.39	19	40	26

S_{max} is the maximum concentration, x_0 is the time when concentration is half the maximum, and b is related to the transition width, i.e. the time it takes concentration of soluble solids to increase from 0.25 S_{max} to 0.75 S_{max}. From the fitted curves, two measures of timing of maturity were derived: physiological, calculated as the time when berries reached 0.95 S_{max}, and oenological, calculated as the time when berries reached 20% concentration of soluble solids.
†A different model was used for Crimson Seedless, hence the lack of comparable parameters, except for the timing of maturity derived from fitted curves.

Table 4. Parameters of a dose–response model (Eqn 2) accounting for the time dynamics of water concentration of berries.

Variety	W_{min} (%)		W_{max} (%)		x_{50} (d)		n		Water concentration at maturity (%)	
	estimate	SE	estimate	SE	estimate	SE	estimate	SE	Physiological	Oenological
Cabernet Sauvignon	67.5	4.14	84.9	0.78	44.9	8.53	2.83	0.943	72.5	76.1
Chardonnay	73.1	1.86	85.0	1.12	24.6	4.59	2.13	0.892	75.3	76.5
Crimson Seedless	78.7	0.56	92.9	0.30	41.3	0.97	6.34	0.873	79.9	80.2
Dawn Seedless	72.7	0.88	91.4	0.58	27.5	1.38	2.93	0.427	75.1	79.4
Fantasy Seedless	76.1	2.09	92.0	0.76	32.6	4.34	2.31	0.597	79.9	81.6
Grenache	69.9	1.12	88.1	0.39	38.1	1.84	3.27	0.423	73.7	79.4
Merlot	70.0	2.46	86.1	1.10	36.9	4.31	3.78	1.470	71.4	75.4
Red Globe	77.3	0.81	90.3	0.61	23.3	1.80	2.41	0.479	80.0	80.5
Riesling	74.9	1.73	86.7	1.62	22.0	4.36	2.71	1.532	77.3	77.5
Semillon	69.4	2.39	83.8	0.87	35.0	5.24	2.75	0.939	72.5	77.2
Shiraz	64.9	2.74	84.9	0.96	32.9	4.57	2.27	0.596	69.7	75.2
Verdelho	70.6	0.78	86.6	0.64	26.0	1.45	3.24	0.581	73.8	78.5

W is water concentration, and subscripts indicate maximum and minimum, x_{50} is the time when water concentration is halfway between maximum and minimum, and n is the Hill slope, a scalar that controls the slope of the curve. Water concentration at physiological (0.95 S_{max}) and oenological maturity (20% soluble solids) was derived from fitted curves.

in grain crops (Calderini et al. 2000, Rondanini et al. 2006, Gambin et al. 2007).

Maximum concentration of soluble solids

The maximum concentration of soluble solids in berries is physiologically interesting and relevant for winemaking. We found substantial varietal differences (Figure 2, Table 3) and no association between S_{max} and variables related to the carbon economy of the crop (Table 5). Maximum concentration of soluble solids was related to both allometric coefficients for soluble solids and water, but the relationship was slightly stronger for water (Figure 3). Many studies indicated that water dynamics in berries is (i) cultivar-dependent; and (ii) linked to sugar accumulation (Greenspan et al. 1996, Tyerman et al. 2004, Keller et al. 2006). For instance, Tyerman et al. (2004) showed that single berry hydraulic conductance was two- to fivefold higher in Shiraz than in Chardonnay;

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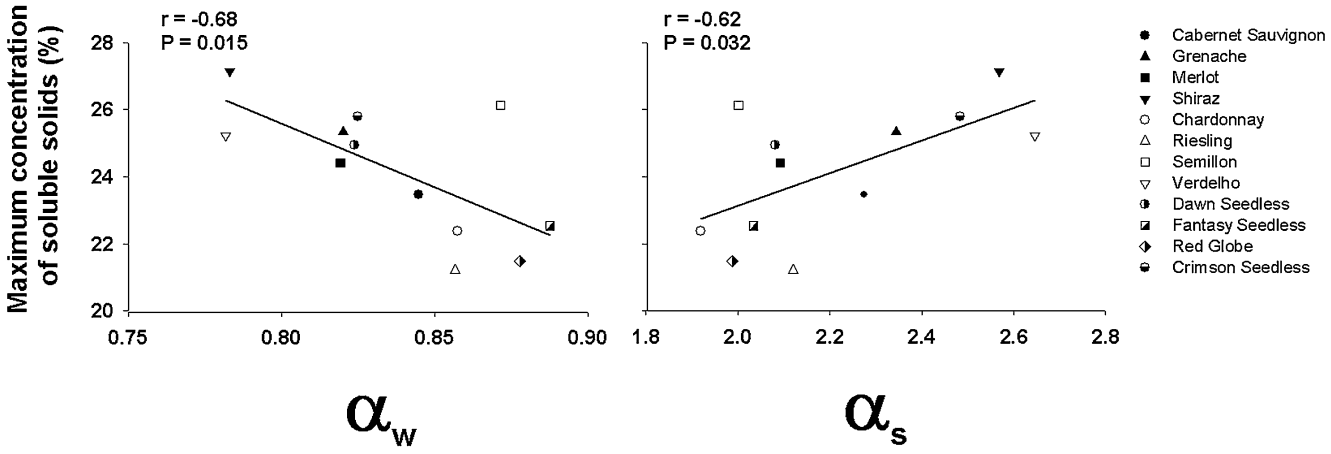


Figure 3. Relationships between maximum concentration of soluble solids in berries and scaling exponents for water (α_w) and soluble solids (α_s). The solid lines are linear regressions. Scaling exponents are dimensionless.

Table 5. Variation in maximum concentration of soluble solids in berries among varieties was unrelated to source size, source activity, sink size, and source : sink ratio.

Type of variable	Variable		*P*>
Source size	Leaf area		0.53
	Pruning weight		0.06
	Fractional light interception		0.27
Source activity	Stomatal conductance	25 January	0.60
		08 February	0.66
		09 February	0.93
		14 February	0.67
		15 February	0.73
Sink size	Yield		0.62
	Bunch number		0.59
	Bunch weight		0.96
Source : sink	Leaf area : yield		0.97
	Pruning weight : yield		0.93
	Fractional PAR Interception : yield		0.64

P indicates significance of linear regressions.

this trait could, therefore, play a significant role in the varietal-dependent dynamics of sugars. More work is needed to develop conceptual models linking sugar and water dynamics of berries accounting for both cultivar and environmental conditions (Genard et al. 2007).

The viticultural importance of S_{max} relates to the need to conciliate a target sugar concentration with the concentration of other compounds related to berry colour and flavour. Thus, the trade-offs between sugar and other components resulting from delayed or anticipated harvest would be quite different for a variety such as Shiraz, where concentration of soluble solids could reach high values, up to 27% under our experimental conditions, in comparison with varieties such as Riesling where concentration levels-off at typically lower values, 21% under our conditions. Godden and Gishen (2005) demonstrated substantial, variety-dependent changes in the profiles of Australian wines between 1984 and 2004, including a marked increase in alcohol in Cabernet Sauvignon, Merlot, Grenache and Shiraz. In comparison, alcohol remained stable in Chardonnay, Riesling and Semillon. Differential responses of sugar accumulation to recent warming trends (Petrie and Sadras 2008), and variety-dependent coupling of sugar and other compounds, are some of the potential factors underlying the contrasting time trends in the profile of Australian wine reported by Godden and Gishen (2005). A dynamic view of the processes leading to the final berry composition, rather than a snapshot at the end of the season, will contribute to better understanding and open opportunities for the variety-specific management of crop and winemaking decisions.

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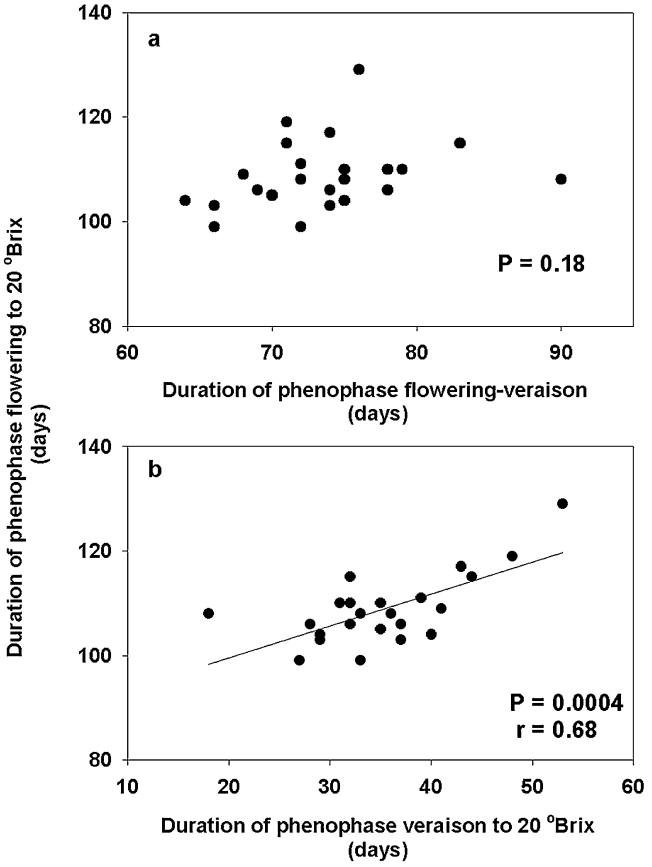


Figure 4. In the Barossa Valley, intra-specific variation in time from flowering to 20°Brix is (a) unrelated to the duration of pre-veraison phase, and (b) closely related to the duration of the post-veraison phase. Data are medians from four seasons and 23 varieties at SARDI experimental station at Nuriootpa (Pearce and Coombe 2004).

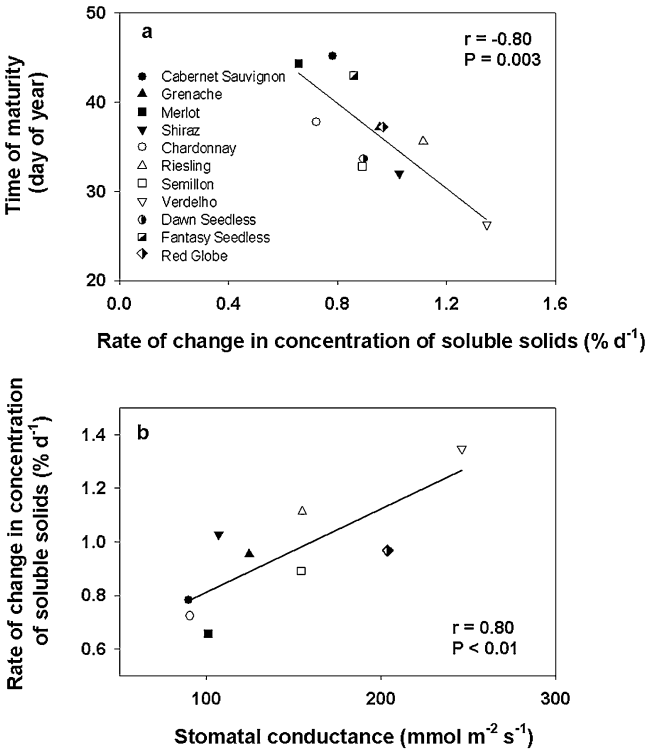


Figure 5. Relationships between (a) time of maturity (20% soluble solids in berries) and rate of change in concentration of soluble solids, and (b) rate of change in concentration of soluble solids and stomatal conductance. Stomatal conductance is the average of six measurement dates between 25 January and 15 February; error bars are two standard errors. Different number of points in the graphs are related to the exclusion of Crimson Seedless, for which rate of accumulation of soluble solids was not calculated due to its unique dynamics (Figure 2), and the different intensity of measurements as summarised in Table 1.

Timing of maturity

Variety-dependent variation in timing of maturity is a primary function of post-veraison development (Figure 4). Consistent with this, the timing of maturity was an inverse function of the rate of accumulation of soluble solids in berries after veraison. It could be expected therefore that source : sink ratios would influence the rate of accumulation of soluble solids and therefore time of maturity (Bindi et al. 2001). Source-to-sink relationships in turn involve both size, e.g. canopy leaf area, and activity per unit size, e.g. photosynthetic rate per unit leaf area. In general, variation in the relative size of sources and sinks is more relevant than variation in activities per unit source or sink (Monteith 1977). Here, we found that the rate of accumulation of soluble solids in berries, the key driver of timing of maturity, was a direct function of stomatal conductance (Figure 5). Leaf photosynthetic rate depends on stomatal and non-stomatal controls (Grassi and Magnani 2005, Chen et al. 2006, Jiang et al. 2006, Massonnet et al. 2007).

The variation in stomatal conductance with source : sink ratio (Figure 6) can be interpreted in terms of photosynthetic responses to end-product utilisation. In annual crops, low grain set or removal of grain often leads to reduced photosynthetic rate, and reciprocally, stronger sinks could increase photosynthesis (Evans 1993, Fischer 2008). In perennials, or even in annuals where vegetative organs could act as strong sinks, stems and roots could buffer the effects of high source : sink ratio resulting, for instance, from reproductive thinning (Sadras et al. 2000). This could account for the lack of photosynthetic response to source : sink manipulation in some studies with grapevine (Chaumont et al. 1994), in contrast to studies where a 3.5-fold range in source : sink ratio generated through bunch thinning and shoot removal lead to changes in single leaf photosynthesis ranging from negligible to twofold (Edson et al. 1993). The variation in response with leaf position in the study of Edson et al. (1993) probably reflects the modularity of the plant (Hardwick 1986, Kaitaniemi and Honkanen 1996). Based on experimental and modelling studies with Cabarnet Sauvignon, Quereix et al. (2001) proposed that the decrease in stomatal conductance associated with feedback inhibition of photosynthesis could be related to a phloem-based feedback signal. We, therefore, propose that the negative association between stomatal conductance and source : sink ratio in our experiments is possibly associated with feedback/feedforward sink effects on leaf photosynthesis. The influence of source : sink on rate of accumulation of soluble solids in berries reinforce the

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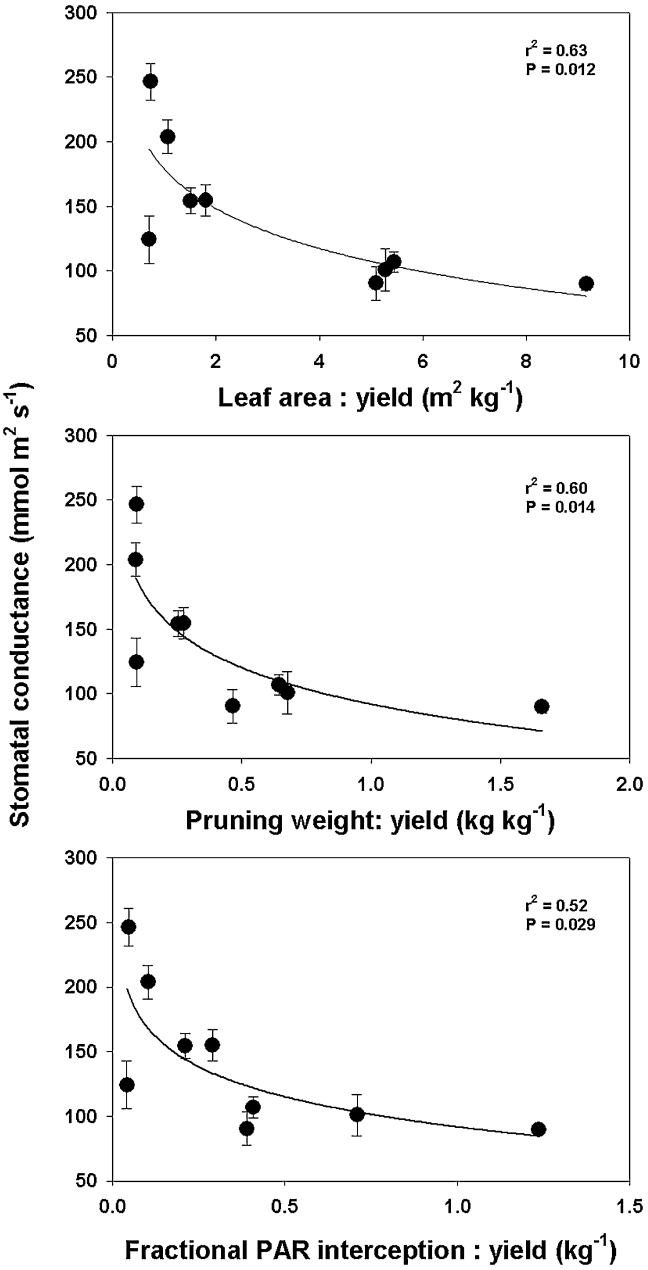


Figure 6. Relationship between stomatal conductance and three measures of source : sink ratio. Stomatal conductance is the average of six measurement dates between 25 January and 15 February. Error bars are two standard errors.

importance of management practices, chiefly water and nutrient supply, on time of maturity.

In conclusion, we have modelled distinct patterns of accumulation of soluble solids and water in berries of grapevine varieties, and advanced on the putative links between berry and leaf physiology. We showed that the maximum concentration of soluble solids in berries was unrelated to carbon-related traits, and speculate that water dynamics may play a role. The timing of maturity was clearly associated with the rate of accumulation of soluble solids, which was in turn related to stomatal conductance. Varietal differences in stomatal conductance may involve an important component of phenotypic plasticity in terms of photosynthesis and stomatal responsiveness to source : sink ratio.

Acknowledgements

We thank Fernando Iniesta Hornero for his assistance with berry sampling and laboratory analyses, and the Australian Grape and Wine Research and Development Corporation (Grants SAR 05-01; CSP 05-02) and the River Murray Improvement Program for funding.

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Manuscript received: 14 April 2008

Revised manuscript received: 17 June 2008

Accepted: 14 August 2008

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C. Response of vines to elevated temperature

The work that contributed to this chapter was the result of integration of project CSP 05/02 with SAR 05/01 and SAR 05/02. This collaboration resulted in a publication in Functional Plant Biology: Soar, C.J., Collins, M.J. and Sadras, V. (2009) Irrigated Shiraz vines (*Vitis Vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. Functional Plant Biology 36, 801-814. It is reproduced here in full with permission.

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

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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.

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10.1071/FP09101 1445-4408/09/090801

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 depends 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

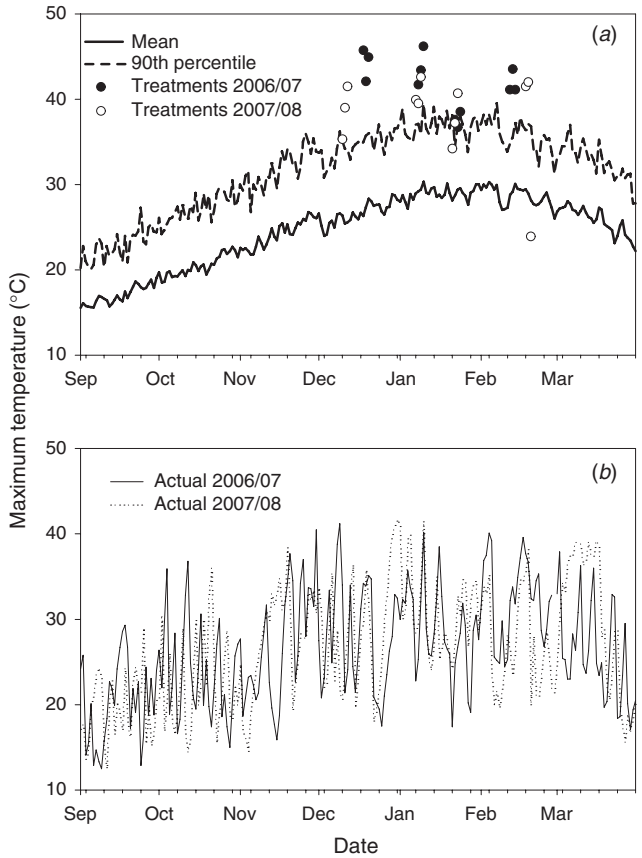


Fig. 2. (*a*) Maximum temperature in heating treatments (circles) compared with long-term average (solid line) and 90th percentile (dashed line) and (*b*) actual maximum temperature during the experiments. Long-term (50 years) records at Nuriootpa, South Australia, are from the Australian Bureau of Meteorology.

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

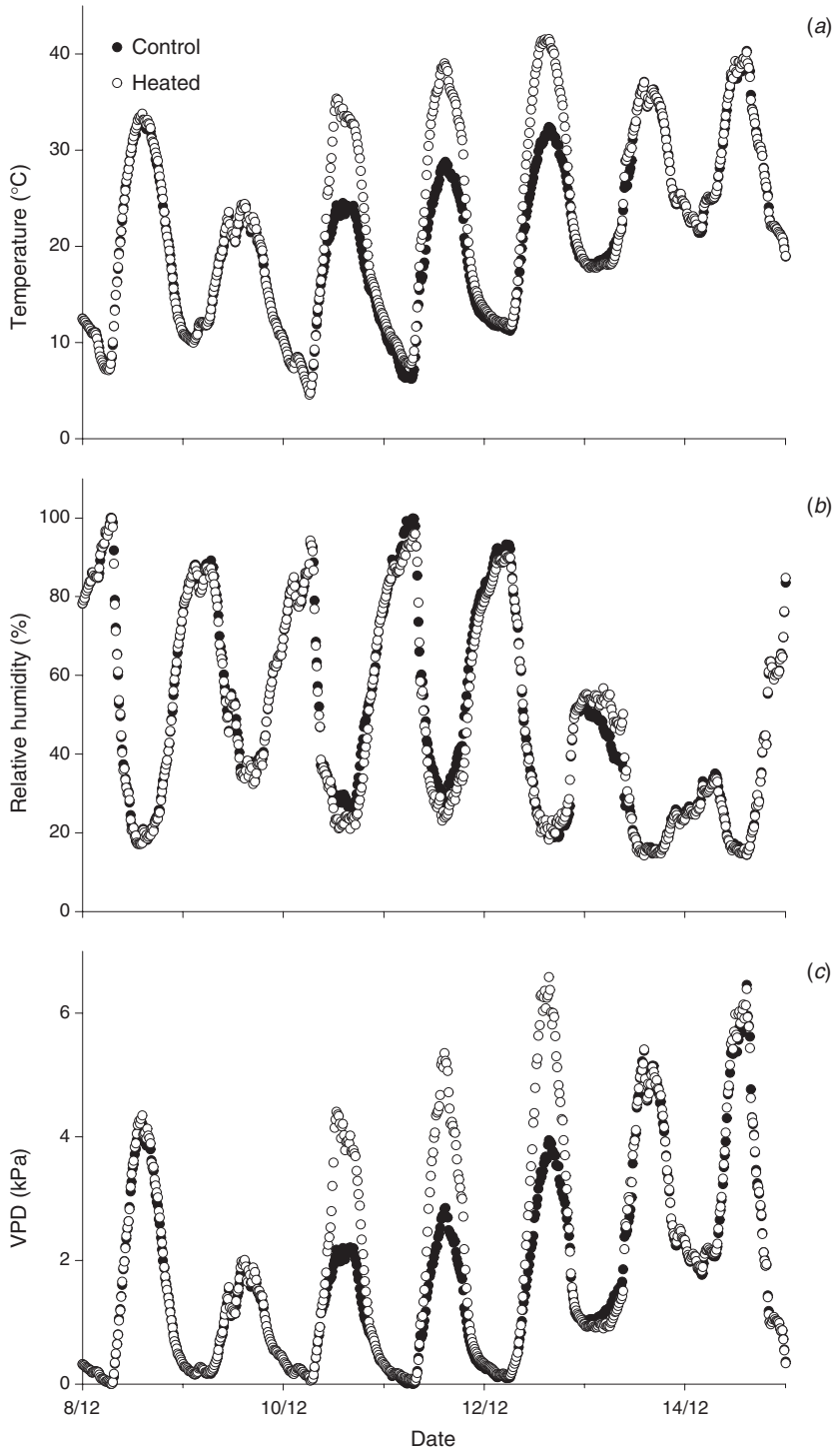


Fig. 3. Temperature, relative humidity and vapour pressure deficit inside (open symbols) and outside (closed symbols) the heating chambers during the post-set treatment in 2007. The treatment was applied from the 10th to the 12th of December inclusive. As background, trajectories are expanded by two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

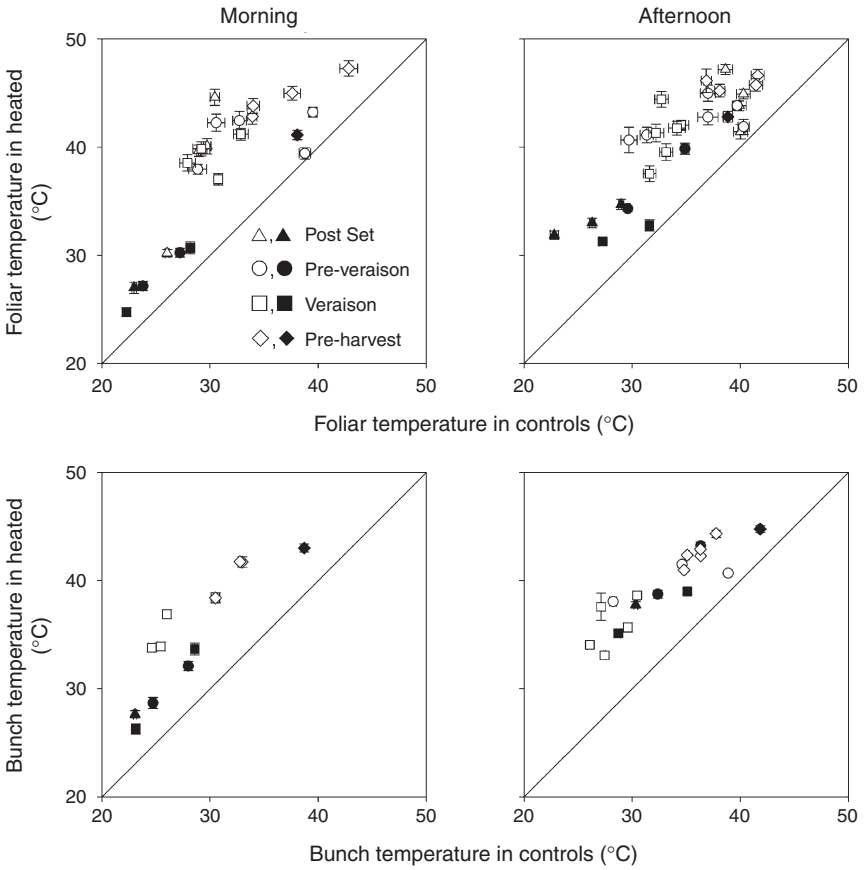


Fig. 4. Comparison of foliar temperature and bunch temperature in heated and controls vines in 2006–07 (open symbols) and 2007–08 (closed symbols). Foliar temperature is single leaf (2006–07) or canopy (2007–08). The solid line is $y = x$ and error bars are s.e.m.

($P > 0.05$) 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

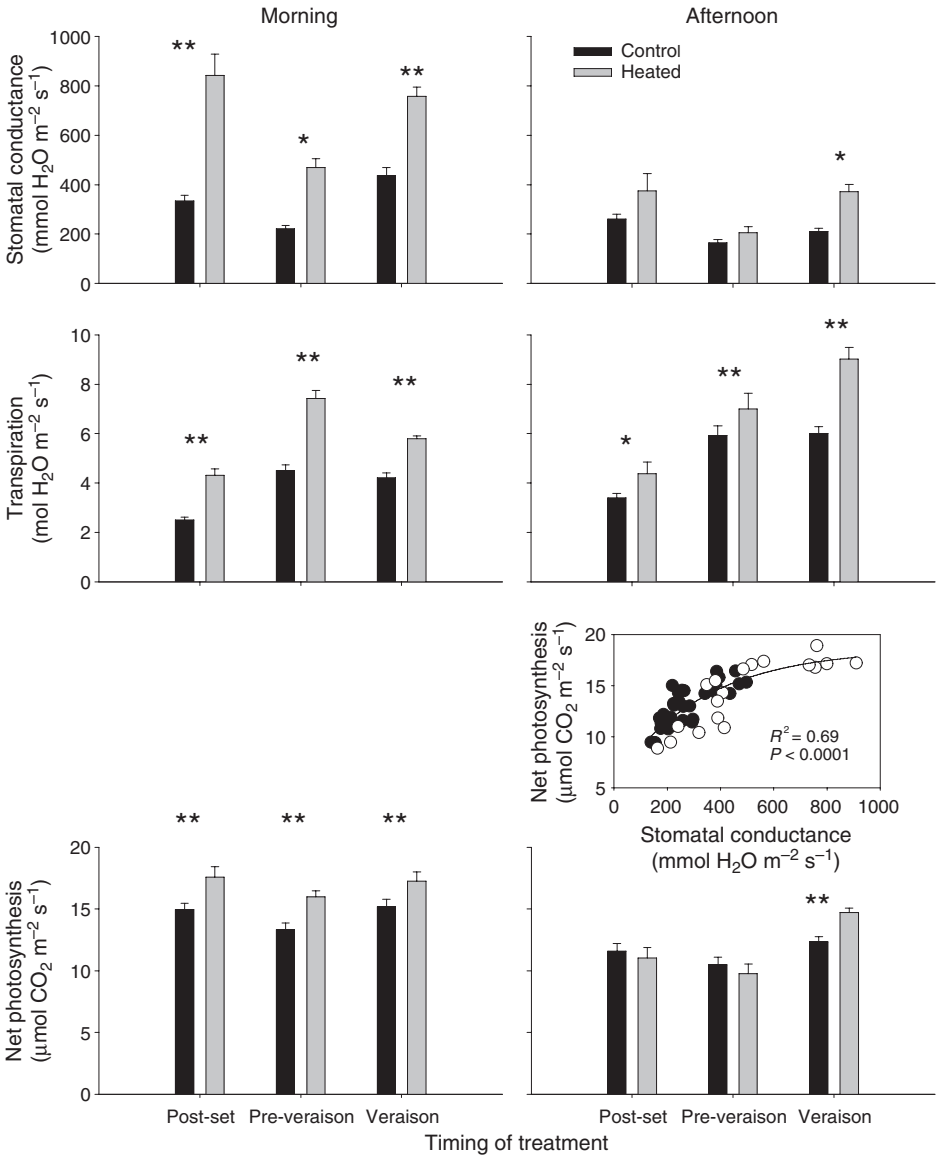


Fig. 5. Stomatal conductance, leaf transpiration and net photosynthesis of heated and control vines in the morning and afternoon of the second day of heating for the post-set, pre-veraison and veraison treatments. Error bars are standard errors of the means and asterisks indicate significant differences 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.

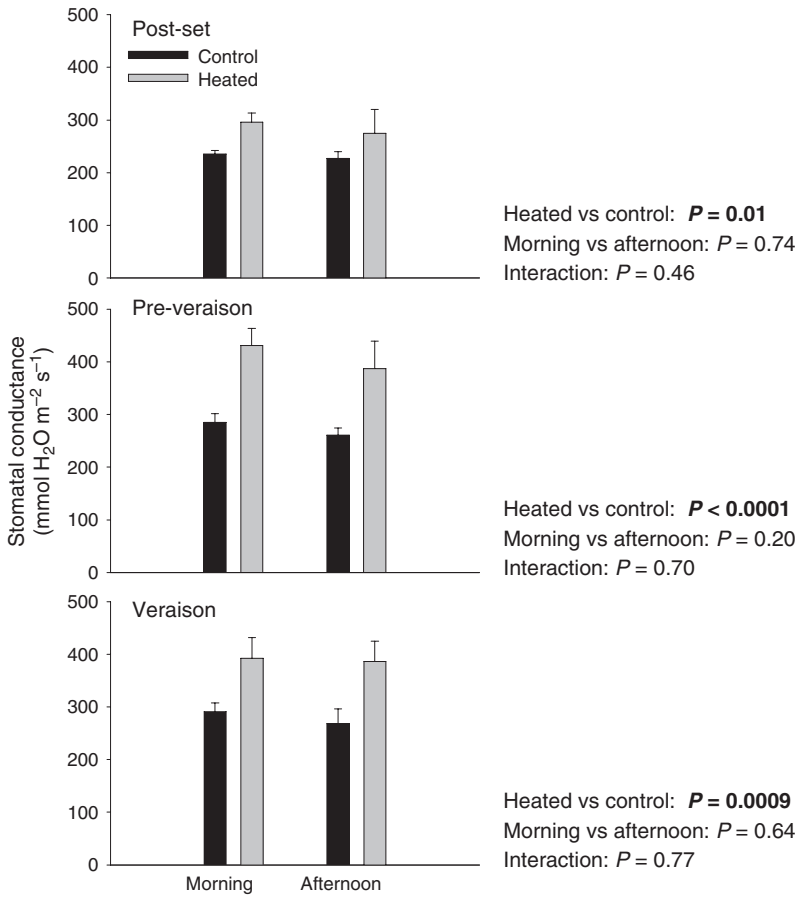


Fig. 6. Stomatal conductance measured with diffusion porometer in heated and control vines in the morning and afternoon of the second and/or third day of heating for the post-set, pre-veraison and veraison treatments. Error bars are s.e.m. *P*-values are from ANOVA. Data from 2007–08.

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

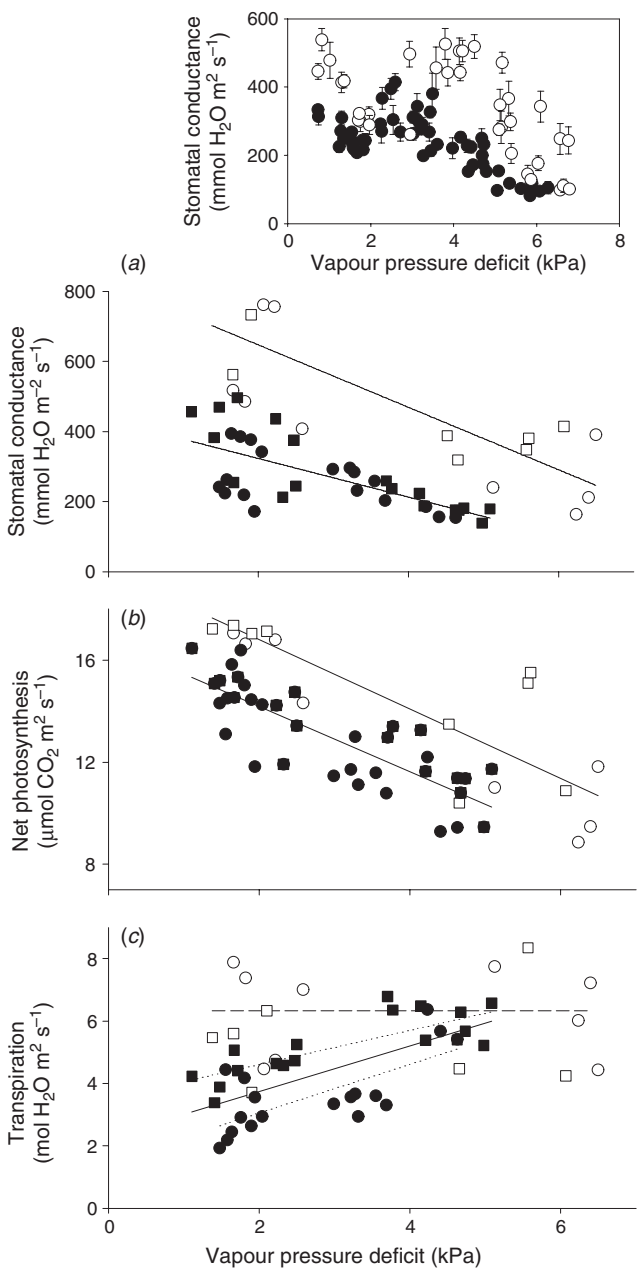


Fig. 7. Relationships between (*a*) stomatal conductance, (*b*) transpiration, and (*c*) net photosynthesis and vapour pressure deficit for heated (open symbols) and control (closed symbols) vines. Measurements were taken in the morning (circles) and afternoon (squares) of the second day of heating applied 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

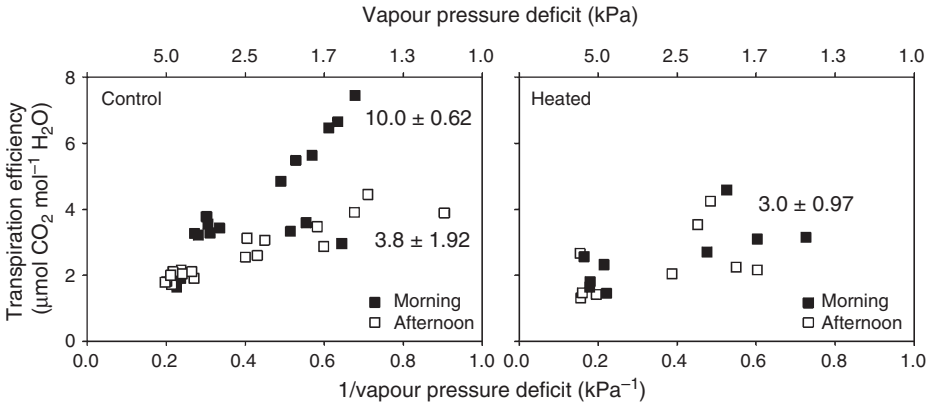


Fig. 8. Transpiration efficiency as a function of the inverse of vapour pressure deficit in leaves from control and heated vines. Measurements were taken in the morning and afternoon of the second day of heating applied at either post-set, pre-veraison and veraison; each point is the average of nine 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.

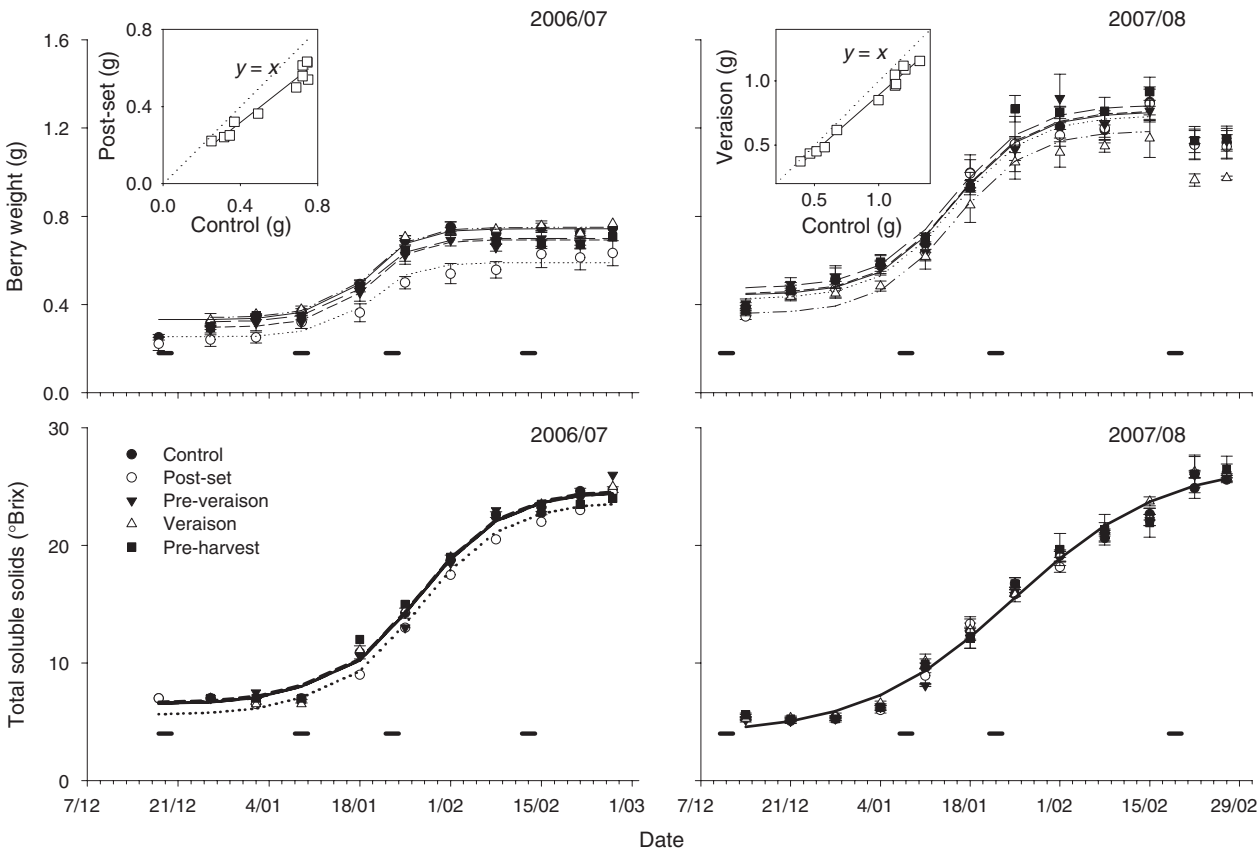


Fig. 9. Dynamics of berry growth and total soluble solids in berries from controls, and from vines heated during 3 days at one of four phenological stages (horizontal bars). Error bars represent s.e.m. Fitted curves are Eqn 1. In 2007–08, measurements of berry weight during the shrinkage period (Sadras and McCarthy 2007) were not used to fit the curves (two last dates). Insets compare berry weight of controls and post-set (2006/07) and controls and veraison (2007/08) treatments.

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.

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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).

D. Effect of variety on response to elevated temperature

Introduction

In the previous chapter we showed that in Shiraz vines elevated temperature, applied at a number of phenological stages, did not have a major effect on the dynamics of berry growth and sugar accumulation apart from a relatively small reduction in berry size and sugar accumulation when heat was applied shortly after fruit set. It was also shown that recently irrigated Shiraz vines were able to up-regulate stomatal conductance during the heat treatment thereby to some extent mitigating the effect of the high ambient temperature through leaf cooling brought about by the high rates of transpiration. However, it was recognised that the experimental procedure adopted in these experiments allowed only for the manipulation of day temperature and night temperature was the same in both control and heated vines. During heat wave conditions it is common for both day and night temperatures to be elevated. As noted in the previous chapter, night temperature can affect plant processes with consequences for crop yield and quality. We have therefore modified our experimental protocol to enable us to test the effects of elevated day and night temperatures on vine response.

Results and Discussion

The heat tents successfully modified both day and night temperature. An example for the budburst phenological stage is shown in Fig 1.

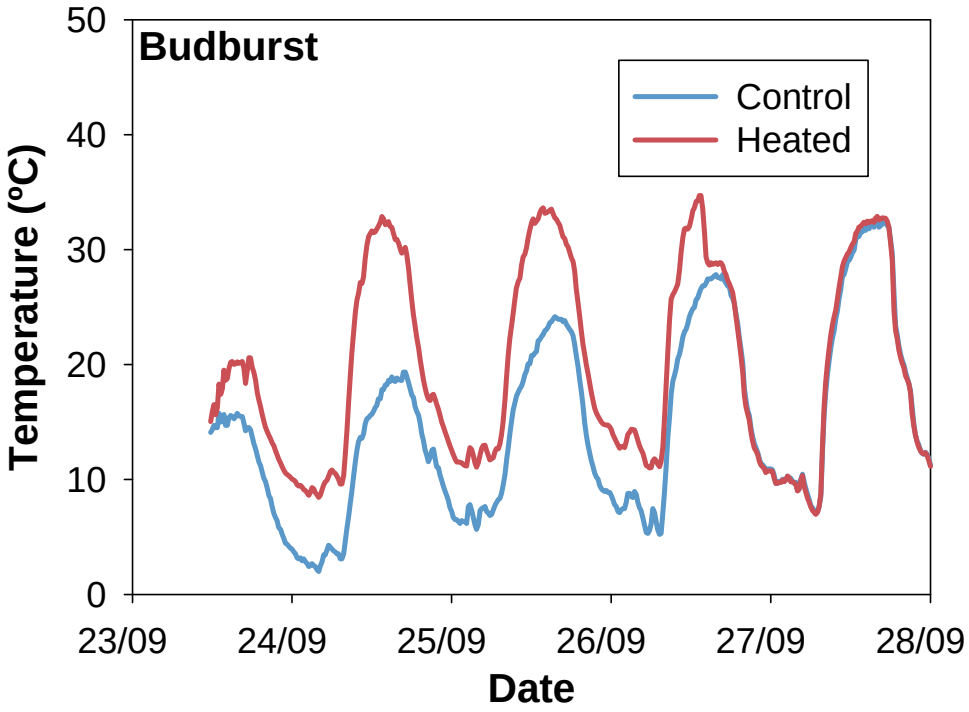


Figure 1. Air temperatures in heated and control canopies.

Both day and night temperatures were significantly elevated compared with ambient conditions (Fig 2). The average difference in temperature over the 3 experimental days and the maximum temperature difference, which occurred at around solar noon, are shown in Table 1. Transpiration of the heated vines was significantly higher than in control vines during the morning at veraison and pre-harvest.

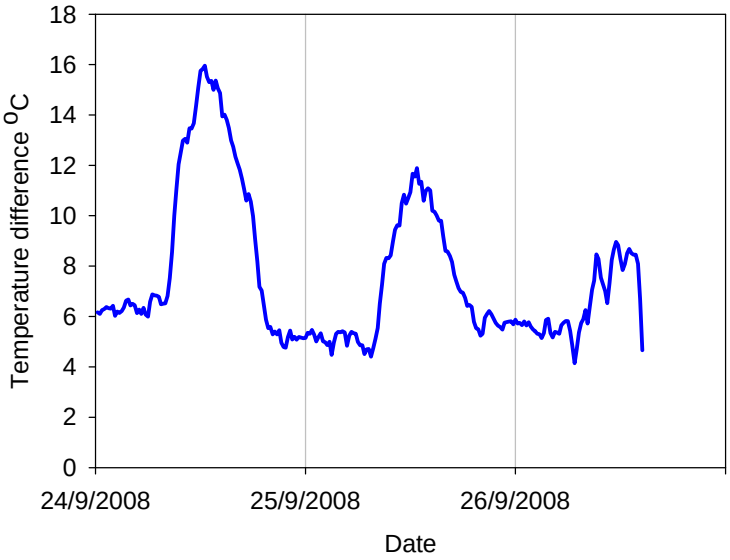


Figure 2. The difference in temperature between the heated and control canopies during the 3 treatments days at budburst

Phenology	Dates	Average temp diff	Max temp diff
Budburst	Sept 24-27	6.61	15.9
Flowering	Nov 10-13	3.09	9.8
Post-set	Dec 9-12	4.16	9.7
Pre-veraison	Jan 6-9	4.49	9.0
Veraison	Jan 20-23	3.62	7.86
Pre-harvest	Feb 17-20	3.12	7.5

Table 1. Average and maximum temperature difference (°C) between the canopies of control and heat tent vines during the 3 treatment days at each phenological stage

In the afternoon the difference was reduced and was only significant in the measurements made at veraison (Figure 3).

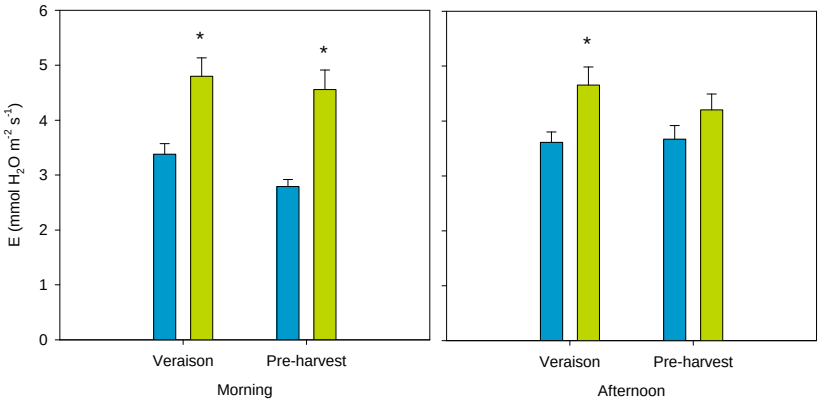


Figure 3. Mean transpiration of Shiraz vines at 2 phenological stages in heat tents and control vines at ambient temperature. Readings during the morning (8.00 to 13.00) and afternoon (13.00 to 18.00) are presented. Bars are SE (n=9) and * denotes a significant difference (P<0.05).

Assimilation during the morning was not significantly different between treatments and was significantly reduced during the afternoon at both sampling periods (Fig 4). Measurements made during the night showed significantly higher respiration in the heated vines (data not shown) as might be expected because of the higher air temperatures.

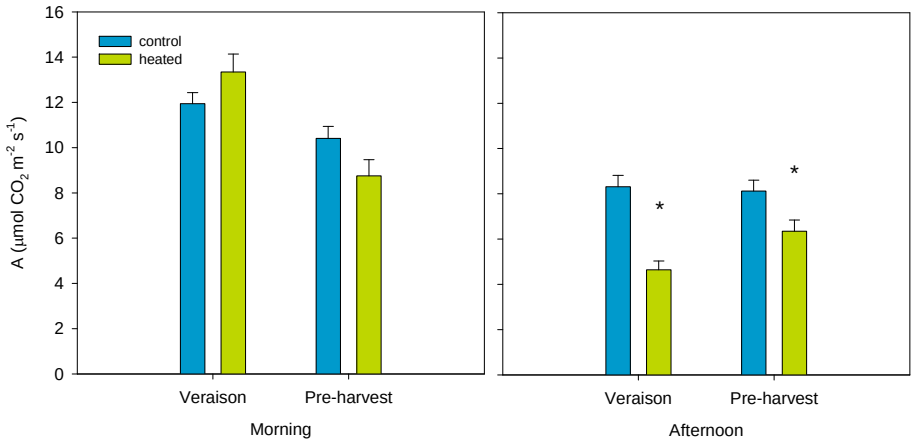


Figure 4. Mean assimilation of Shiraz vines at 2 phenological stages in heat tents and control vines at ambient temperature. Readings during the morning (8.00 to 13.00) and afternoon (13.00 to 18.00) are presented. Bars are SE (n=9) and * denotes a significant difference (P<0.05).

The increased transpiration in the heated vines was driven by a significantly higher stomatal conductance during the morning period and higher evaporative demand (VPD). In the afternoon stomatal conductance tended to be lower than in control vines, although not significantly so (Fig 5).

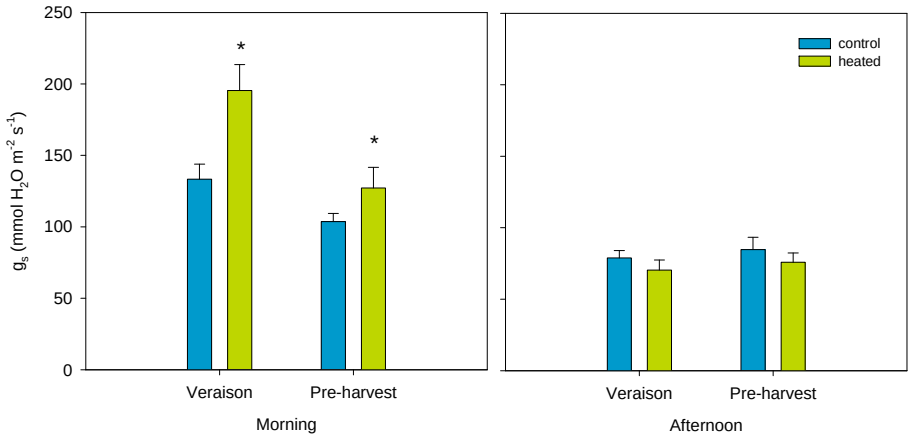


Figure 5. Mean stomatal conductance of Shiraz vines at 2 phenological stages in heat tents and control vines at ambient temperature. Readings during the morning (8.00 to 13.00) and afternoon (13.00 to 18.00) are presented. Bars are SE (n=9) and * denotes a significant difference (P<0.05).

Leaf water potential tended to be lower in the heated vines and was generally lower in the later pre-harvest sample period (Fig 6).

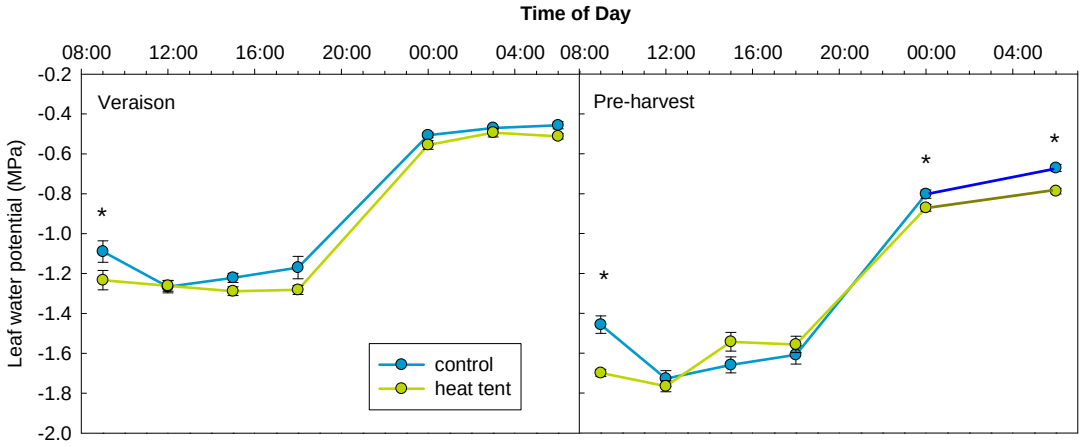


Figure 6. mean leaf water potential of Shiraz vines at 2 phenological stages in heat tents and in control vines at ambient temperature. Bars are SE (n=9) and * denotes a significant difference ($P<0.05$).

These data are in line with previous findings and the transpiration data suggest that there will be more rapid depletion of soil water reserves in the heated vines. Measurements made over a 24h period showed that stomatal conductance and transpiration were not significantly different between treatments during hours of darkness (data not shown) so that higher night temperature in the heated vines were not the cause of the lower leaf water potentials measured during the day. These results generally confirm those obtained in the previous year and show that, give adequate water, Shiraz is able to up-regulate stomatal conductance during high temperature events and perhaps gain some benefit from additional leaf cooling but at the expense of more rapid depletion of soil water reserves. The addition of heating during the night appeared to accentuate the effects of daytime heating and increased respiration rates during the night may have consequences for the amount of assimilate available for fruit development during the critical post veraison period by changing the source-sink relationship. It has been noted previously that high temperature events can result in arrested fruit sugar accumulation despite recovery in leaf gas exchange in the post-heat period (Speirs *et al.* 2010).

These experiments suggest that the effect of short periods of high ambient temperature may be reduced through up-regulation of stomatal conductance leading to enhanced leaf cooling. This is dependent on the availability of sufficient soil water to meet the vine's enhanced requirements during the high temperature event. However, soil water may not always be sufficient, especially where deficit irrigation is being practised and/or there is insufficient warning of heat wave conditions. We have therefore investigated the combined effect of high temperature and insufficient water. These experiments were carried out on Semillon vines in the South Australian Riverland district. In many of the tests reported here in chapter 1 Semillon behaved similarly to Shiraz and the anisohydric tendency of this variety have been described by Rogiers *et al*. (2009). Rogiers *et al.* (2009) also note that Semillon shows significant noctural transpiration which contributes to low pre-dawn leaf water potentials and would lead to low transpiration efficiency (ratio of carbon fixed to water transpired).

Average maximum air temperature in the heat tents during the 3 day heating period was 44^{o}C compared to the average maximum of 33^{o}C outside the tents. Average minimum night temperature in the tents was 22^{o}C which was 8^{o}C higher than ambient. These high temperatures resulted in an average maximum VPD over the 3 day heating period of 8.1 kPa compared with average maximum ambient VPD of 4.7 kPa during the same period. Average minimum VPD was 5-fold higher in the heat chambers than outside (0.26 kPa compared to 1.29kPa, Figure 7).

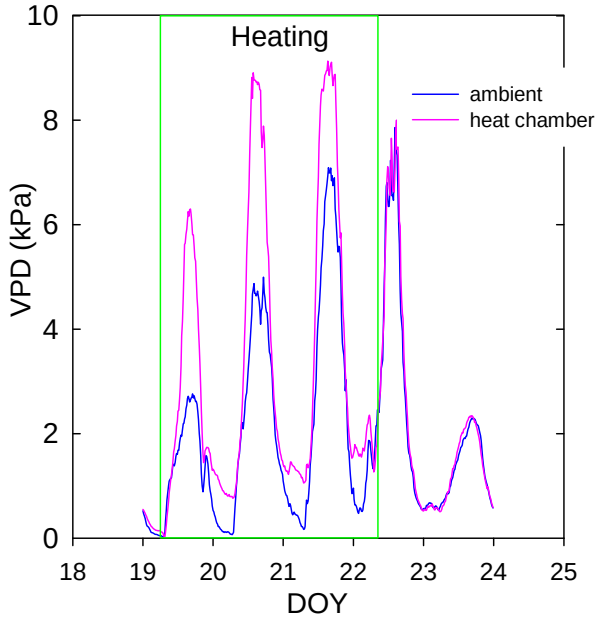


Figure7. Vapour pressure deficit (VPD kPa) during the period of heat treatment

There was no significant difference between stomatal conductances of

the selected vines when measured on Jan 5th before treatments were applied (Figure 8).

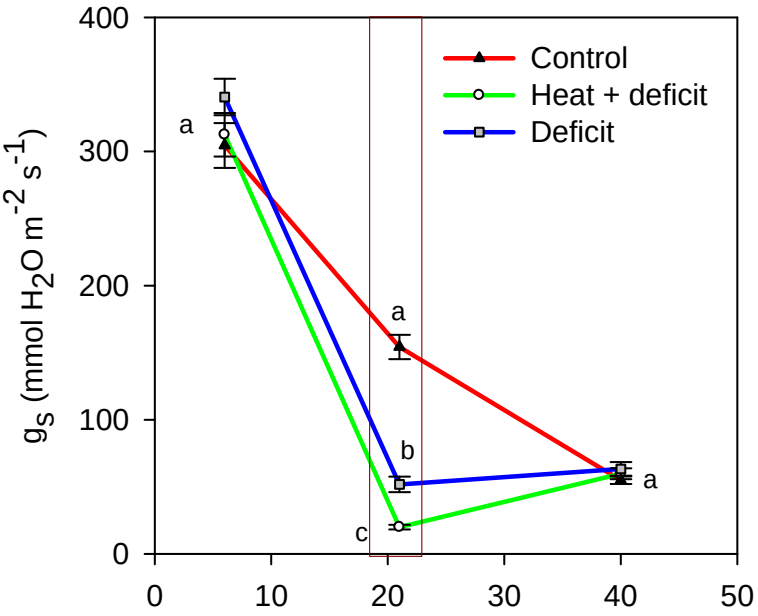


Figure 8. Stomatal conductance of Semillon vines measured before water was turned off (Jan 5th), on 2nd day of heating (Jan 21st) and after irrigation restored (Feb 9th). Different letter at any sample day indicate significant difference ($P<0.05$). Bars are SE (n=5). X axis = day of year and heating period is shown in the brown box.

During the 35 day experimental period there was a decline in stomatal conductance of the control vines which was consistent with seasonal trends noted before at this vineyard (Speirs *et al*., 2010). By the time the heat treatment was applied (days 19 to 22) there had been a significant reduction in stomatal conductance in the vines not receiving water since Jan 5th (the onset of stress symptoms was delayed by a 14mm rain event on Jan 12th) and conductance was further depressed in the heated vines (Fig 8). However, recovery in both the deficit alone and deficit + heated vines was complete as there was no difference in conductance compared to irrigated control vines at the last sample time 17 days after heating ceased. Midday leaf water potential was significantly lower in the heat + deficit vines than in control and was significantly lower again in the deficit alone. This was probably due to the higher stomatal conductance shown by these vines. Pre-dawn leaf water potential was also lower in the deficit vines compared to the controls although the heated vines were significantly higher than deficit alone (Fig 9).

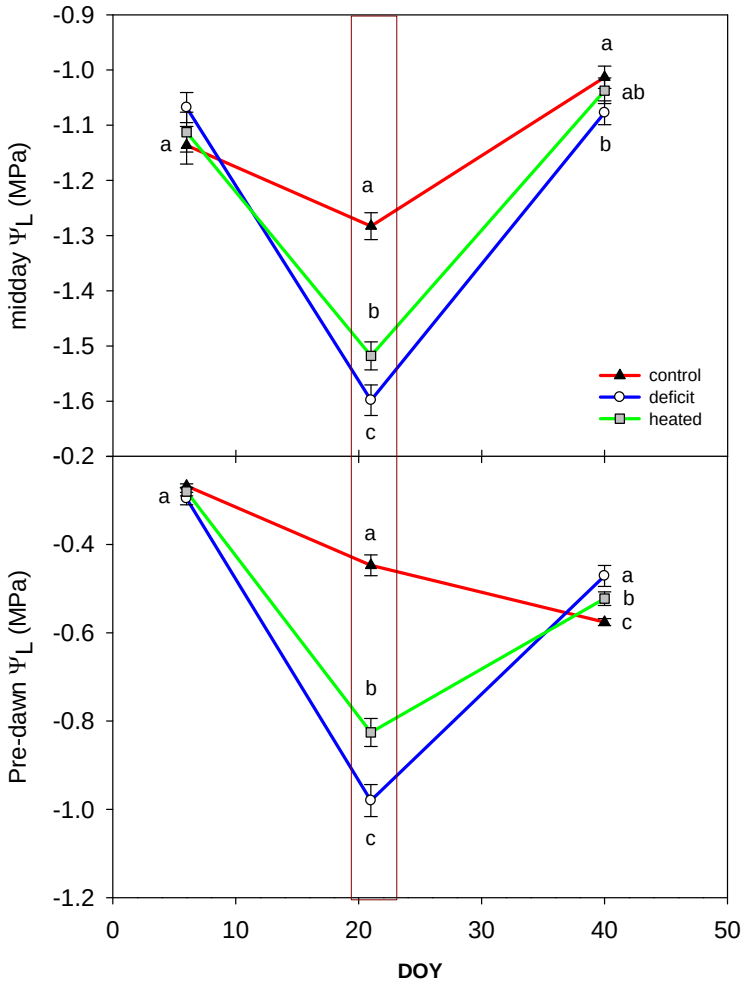


Figure 9. Midday and pre-dawn leaf water potential of Semillon vines measure before water was turned off (Jan 5th), on 2nd day of heating (Jan 21st) and after irrigation was restored (Feb 9th). Different letter at any sample day indicates significant difference (P<0.05). Bars are SE (n=5).

In line with the changes in stomatal conductance, assimilation rates were significantly reduced by the water deficit treatment. The high temperatures in the heat tents further depressed assimilation which, on average, was close to zero during the 3 day heat period (Fig 10A). Recovery was complete, however, and there was no significant difference between treatments 18 days after irrigation was restored. Transpiration of the vines, as assessed from the gas exchange data, showed similar trends (Fig 10B).

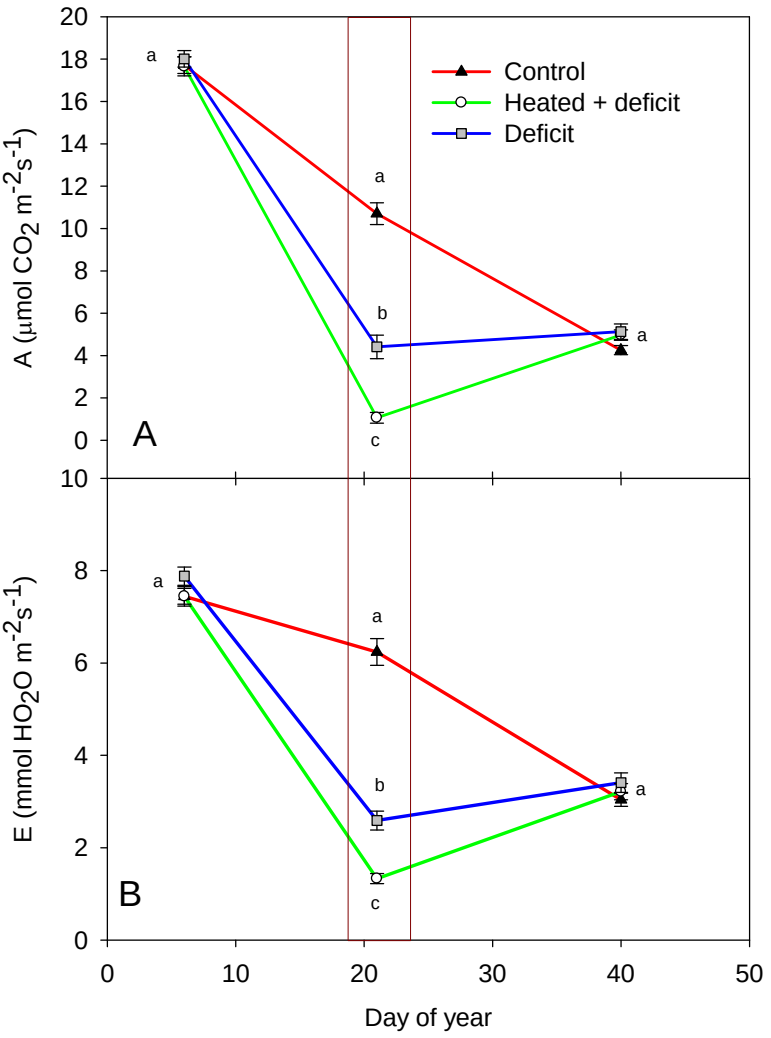


Figure 10. A. Assimilation and B. transpiration of Semillon vines measure before water was turned off (Jan 5th), on 2nd day of heating (Jan 21st) and after irrigation was restored (Feb 9th). Different letter at any sample day indicates significant difference (P<0.05). Bars are SE (n=5).

Sap flow sensors were installed in the vines at the beginning of the experiment. Figure 11 shows daily integral water use for the period corresponding from the rain event to the end of the experiment. Water use in the deficit vines was reduced compared with the controls. Due to high vine to vine variability the average water use of the vines selected for the heat treatment was similar to that of controls. However, during the 3 day heat event water use of these vines increased and then fell to approximate the pre-heat rates after irrigation was restored. The effect of the heat exposure on water use is better illustrated by considering the 3 days prior to the heat event, during the heat event and in the 3 days post the heat event.

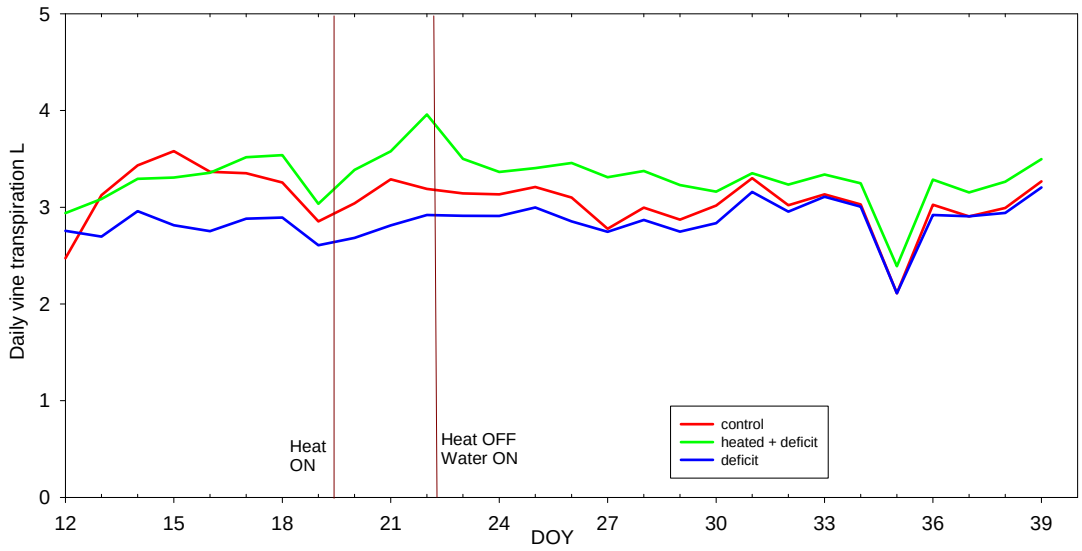


Figure 11. Daily vine transpiration as assessed from sap flow sensors.

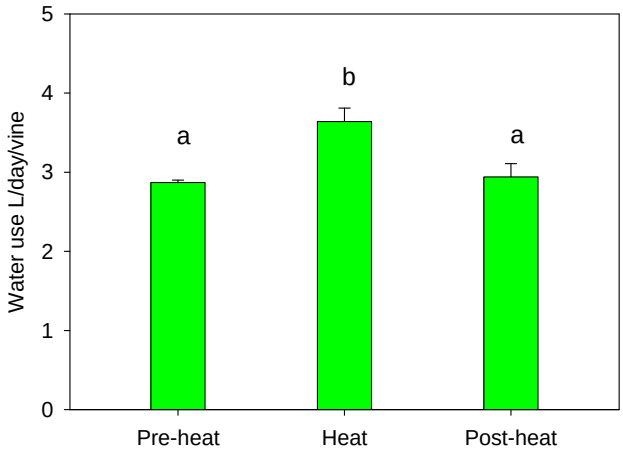


Figure 12. Average daily water use of the vines selected for heat treatment assessed from sap flow sensors for 3 days prior to the heat treatment, during the heat treatment and for 3 days post-treatment. Different letters over the columns indicate significant difference ($P<0.05$). bars are SE n=3.

These data are shown in Figure 12. Exposure to the high chamber temperature significantly increased water use compared with both pre and post heat periods. During the day water use of the heated vines actually declined, relative to the pre-heat period, probably due to the extremely low stomatal conductance during this period. The increase in daily water use was primarily due to a large increase in nocturnal sap flow (Figure 13). When the heat chambers were removed the diurnal pattern of sap flow returned to that of the deficit alone vines with lower nocturnal flow.

The Semillon vines used in this experiment behaved as expected and responded to the lack of irrigation with reduced stomatal conductance, reduced assimilation, reduced

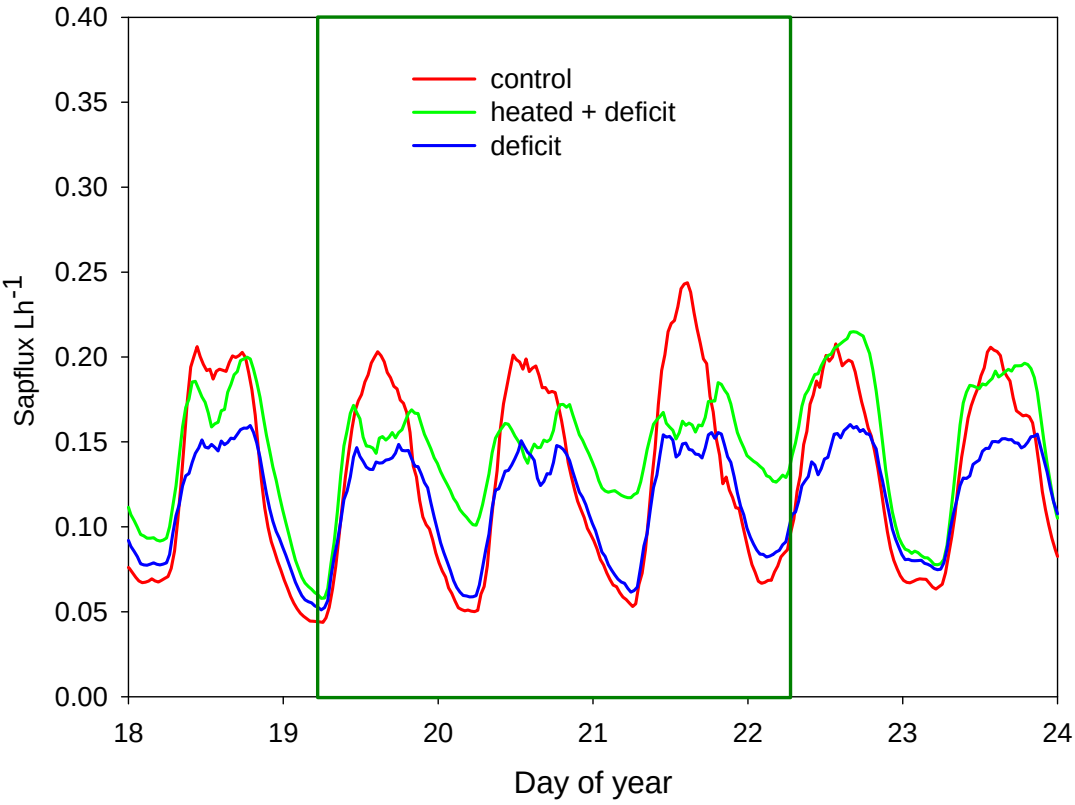


Figure 13. Sap flux (L h^{-1}) of control, deficit and deficit+heated vines before, during and after the heat treatment.

pre-dawn and midday water potential and reduced transpiration. Superimposing elevated day and night temperatures on the water deficit treatment caused a further reduction in stomatal conductance and daytime transpiration. Daily water use measured by sap flow was higher during the 3 day heat event than in the previous 3 day period and this was primarily due to increased nocturnal transpiration. There are a number of possibilities to explain this observation. The sap flow sensors measure flow through the vine trunks and do not differentiate between water that is actually transpired and lost to the atmosphere and that which replaces water lost from the vine tissues during daytime transpiration. Rogiers *et al.* (2009) also noted high nocturnal transpiration in Semillon vines during nights of high VPD. These measurements were made with gas exchange equipment which would only have measured the transpiration component and would not have detected the component replacing daytime water loss. The pre-dawn water potentials of our deficit only vines were lower than those of the deficit + heat treatment vines suggesting that recharge of tissue water had been more effective at the higher night temperatures, which may account for the higher nocturnal flow. Although the VPD in the heat chambers was

extremely high, the low stomatal conductance contributed to low daytime transpiration compared with the deficit only vines. This, and higher midday leaf water potential, suggests that daytime tissue water depletion in the heated vines was not excessive, again arguing against recharge being only the reason for the high nocturnal sap flow. It is not possible from our data to correctly apportion relative figures for transpiration and recharge but the data suggest that a high proportion will be transpiration. During the heat event over 40% of daily sap flow occurred during the night and this is a significant component of the vines daily water budget. This trait may further differentiate isohydric from anioshydric grape varieties as Rogiers *et al.* (2009) showed that nocturnal transpiration of Semillon was about 4 times higher than that of Grenache.

E. Influence of irrigation pre-history on isohydric / anisohydric responses

Introduction

In the preceding chapters we have studied a range of grape varieties to assess their responses to a number of tests designed to differentiate them on the basis of their isohydric/anisohydric response to environment and water deficit. We have also measured the responses of a limited number of varieties on exposure to heat stress. All of this work was carried out at two sites, one in the Barossa Valley and one in the South Australian Riverland. We thought it appropriate to extend this range of sites to include another important viticultural region so that we could assess the performance of varieties exhibiting a range of isohydric/anisohydric characteristics under conditions typical for the region and also under a prolonged water deficit regime. Accordingly we have identified a vineyard in the McLaren Vale region where Shiraz, Grenache and Cabernet Sauvignon vines were growing in close proximity and over two seasons we have studied the physiology and productivity of these vines.

Results and Discussion

1) 2007-2008 season

Soil moisture in the Shiraz block is shown in Fig 1. In both the control and deficit vines there appeared to be little response to the irrigation events at soil depths other than at 20cm. While we attempted to place the moisture sensors below drippers it is possible that due to irrigation line movement this relationship was disturbed.

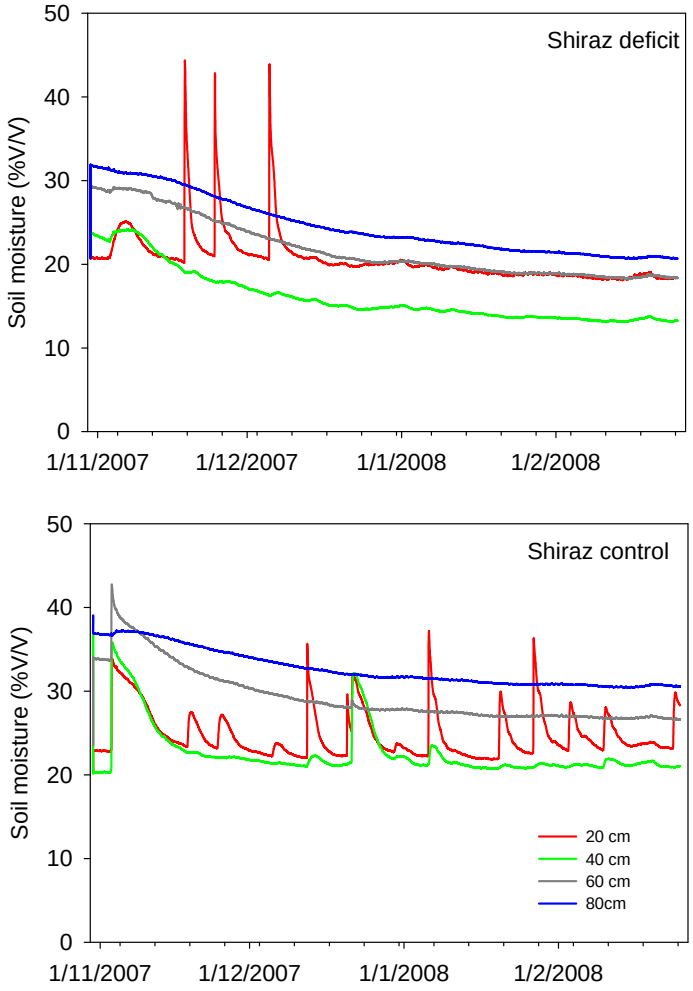


Figure 1. Volumetric soil water as determined by capacitive probes at 4 depths

Nevertheless, the data suggest that after the irrigation to the deficit treatment was shut off in early December there was a gradual decline in soil moisture at all depths. There was no consistent effect of the water deficit in any variety on stomatal conductance, with a general decline as the season progressed (Fig 2). There was some recovery in both control and deficit Cabernet Sauvignon and Shiraz towards the end of the season which was not evident in the Grenache deficit vines.

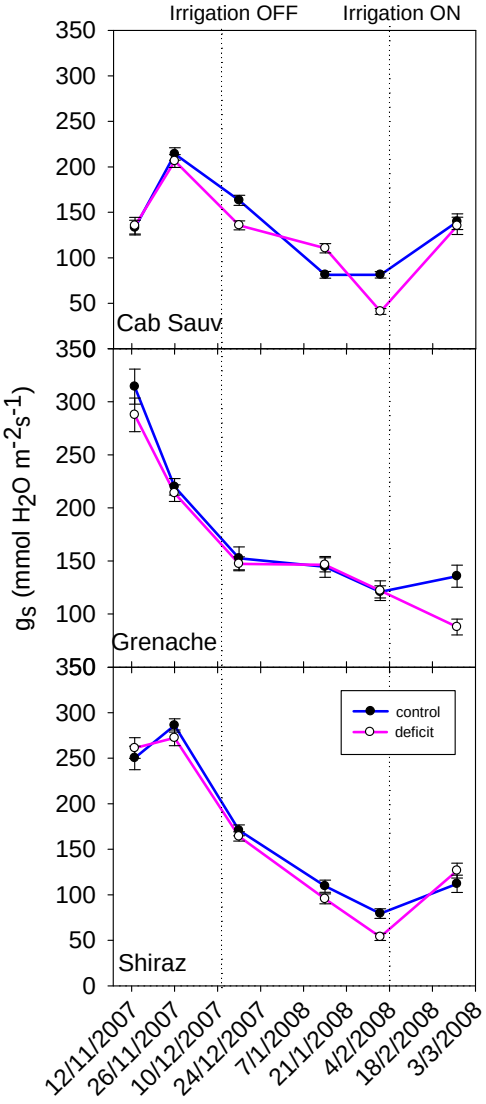


Figure 2. Stomatal conductance of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

Leaf water potential was similarly unresponsive to treatment with a general decline through the season evident (Fig 3). There was a linear accumulation of sugar during the sampling period (mid January to harvest at the end of February) with no difference evident between treatments (Fig 4). Titratable acidity and juice pH at harvest were similarly unresponsive to treatment although Cabernet Sauvignon had a higher juice pH than the other two varieties (Fig 5). Fruit yield was significantly lower in Cabernet Sauvignon than the other varieties (Fig 6) and this was due to lower bunch number and lower average berry weight (Figs 7). There were no significant treatment effects for any of these yield components.

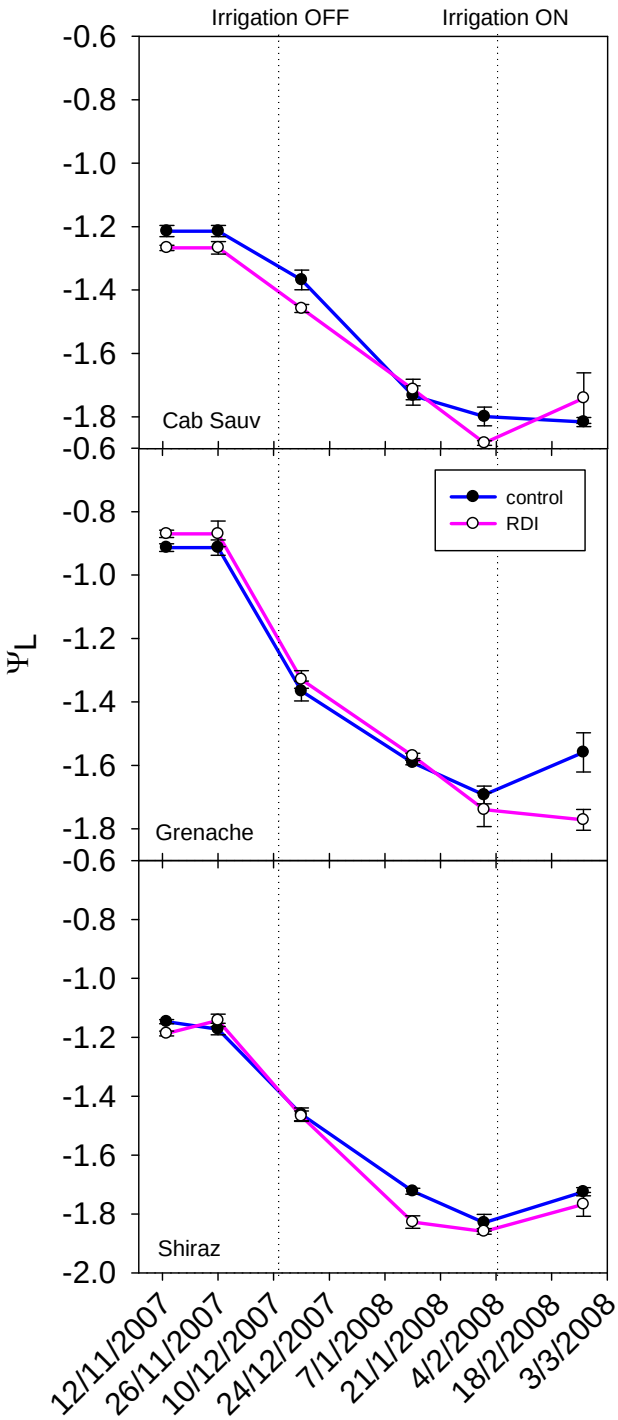


Figure 3. Midday leaf water potential of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

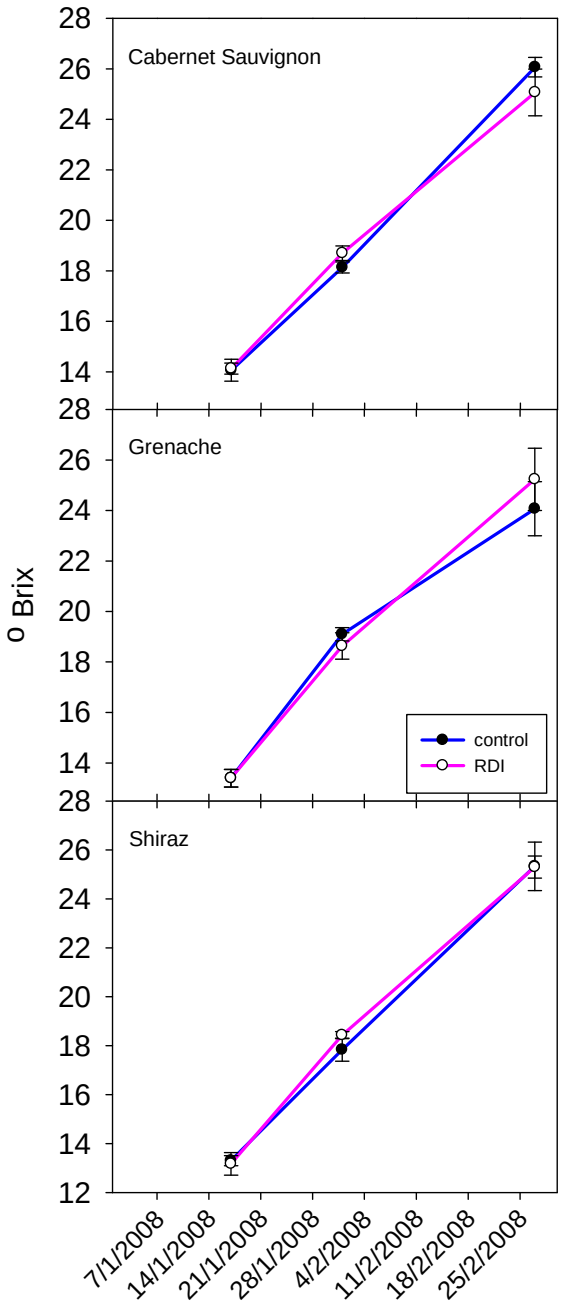


Figure 4. Fruit soluble solids of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

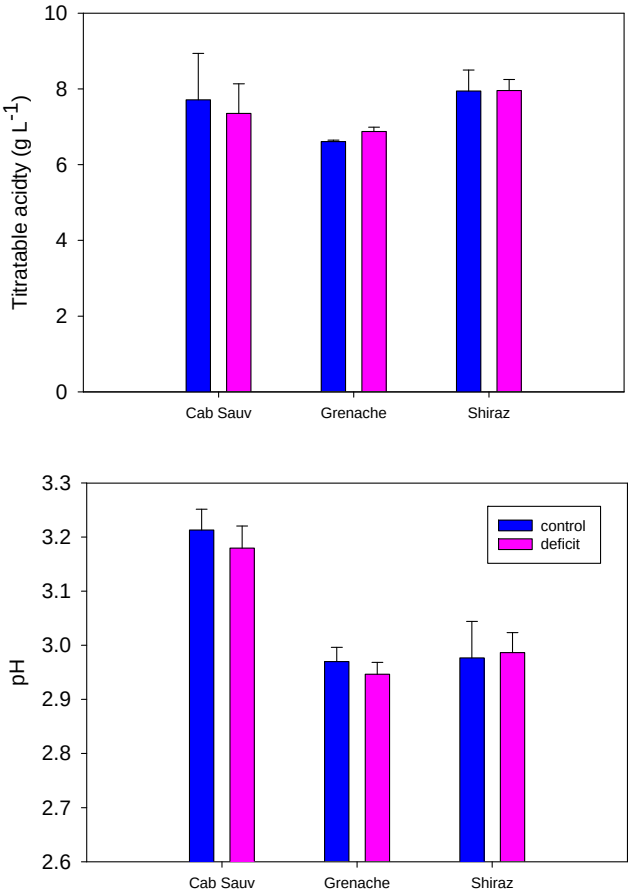


Figure 5. Titratable acidity and juice pH of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

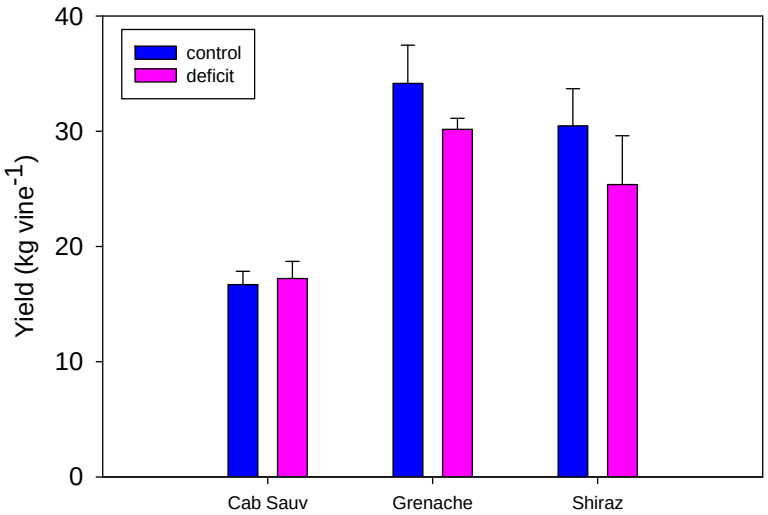


Figure 6. Yield per vine of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

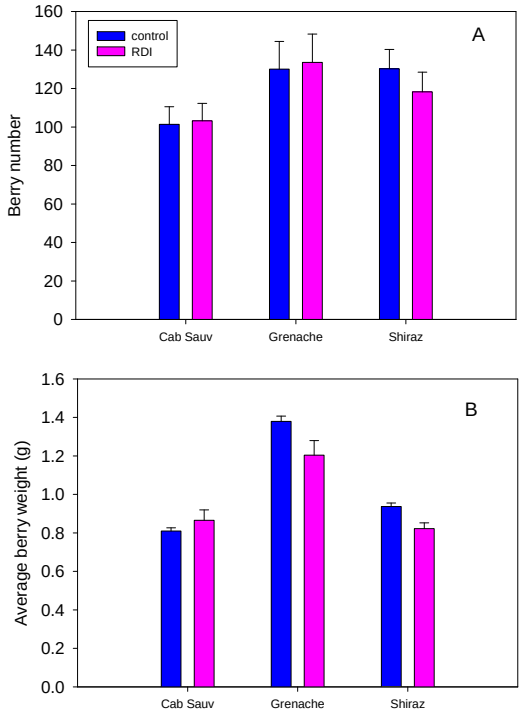


Figure 7. Berry number per bunch and average berry weight of Cabernet Sauvignon, Grenache and Shiraz vines in the 2007/8 season

2) 2008-2009 season

In contrast to the 2007/8 season the water deficit treatment resulted in a significant reduction in stomatal conductance compared with the irrigated control vines in all three varieties. Both control and deficit vines showed some recovery in conductance later in the season but only the Grenache vines recovered completely, relative to the controls, after irrigation was restored (Fig 8).

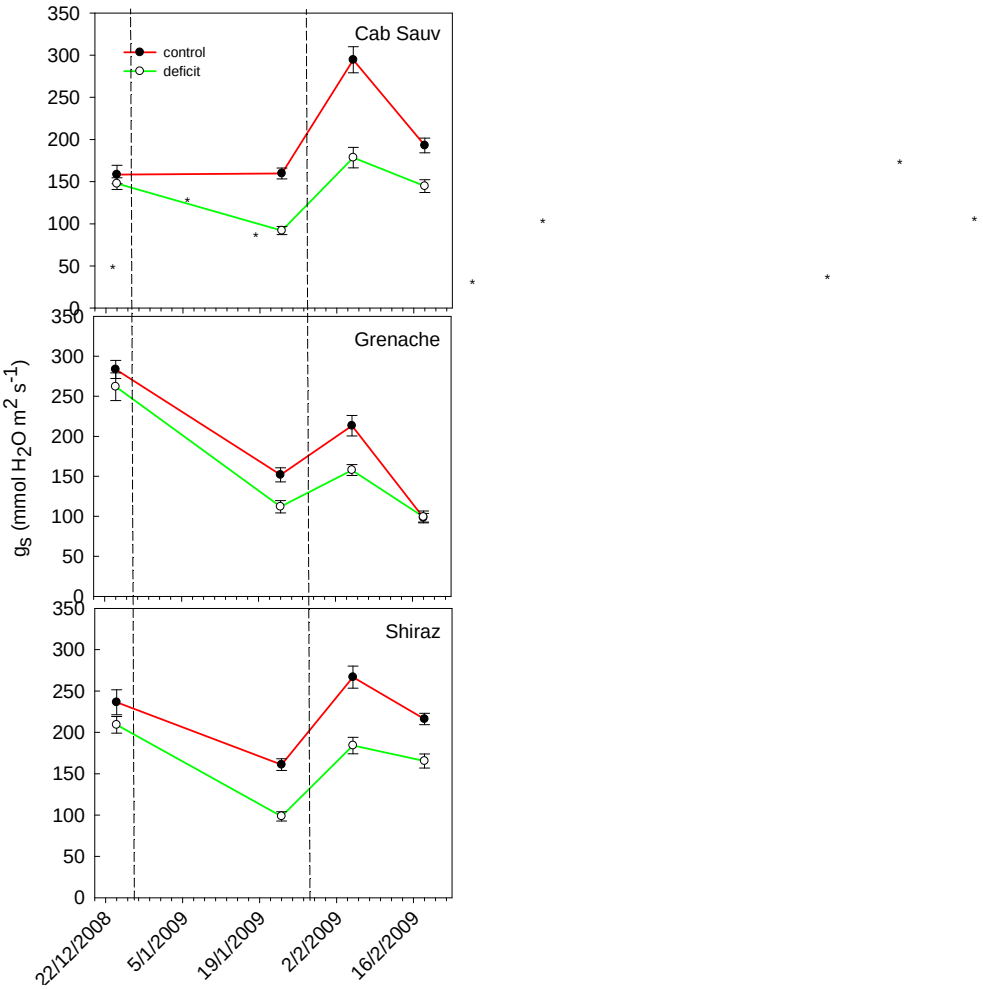


Figure 8. Stomatal conductance of Cabernet Sauvignon, Grenache and Shiraz vines in the 2008/9 season

Yield was considerably lower in all varieties than in 2007/8. All varieties showed a reduction in yield compared with their irrigated controls, although this was significant only for Cabernet Sauvignon. Grenache yield was significantly higher than either of the other varieties. (Fig 9).

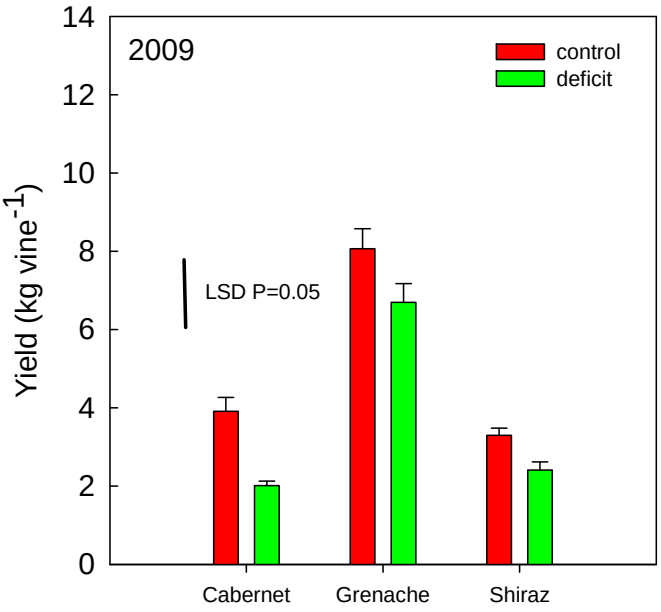


Figure 9. Yield per vine of Cabernet Sauvignon, Grenache and Shiraz vines in the 2008/9 season

The 2007/8 season was remarkable for the similarity of performance of the three vine varieties and the apparent lack of effect of the reduced irrigation during the December to March period. The soil at this site has a high clay content and high water holding capacity and it can only be concluded that winter and current rainfall at this site was sufficient to sustain the vines and allow them to produce a significant crop. Measures of vine performance such as stomatal conductance and leaf water potential were not influenced by the lack of irrigation and were not significantly different between varieties. By contrast, the 2008/9 season did produce differences between the control and deficit vines although there was little effect of variety on stomatal conductance. Yield of both control and deficit vines was greatly reduced compared with the previous season. These seasonal differences were most likely driven by rainfall. The 2008/9 season had particularly low rainfall during the spring in October/November and was virtually zero during January and February (Fig 10). Irrigation at this site was controlled by the site manager and was similar for the two seasons, suggesting again that rainfall was a significant driver of vine performance at this site. In the face of the greater pressures on the vines in the 2008/9 season Grenache performed better, in terms of yield, than Shiraz and Cabernet Sauvignon.

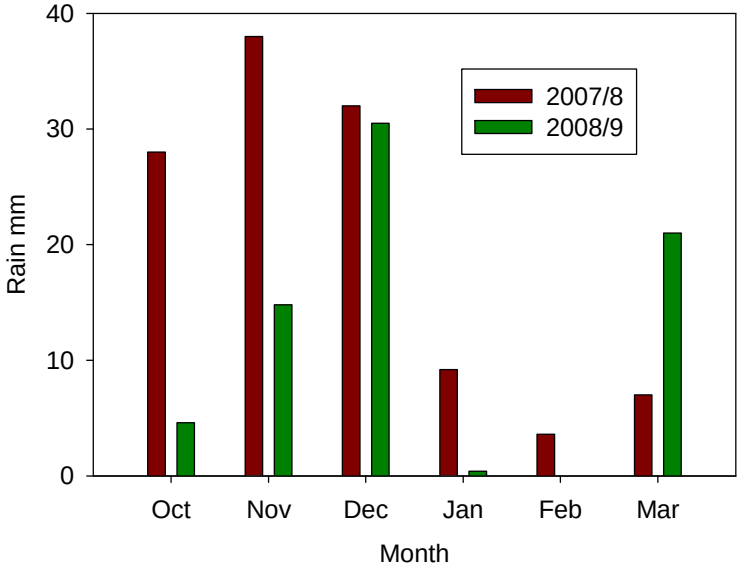


Figure 10. Monthly rainfall at Piramimma Winery McLaren Vale during the periods October to March for 2007/8 and 2008/9. Figures provided by the Bureau of Meteorology.

F. Effects of irrigation prehistory on the response of Cabernet Sauvignon vines to environmental change.

Introduction

In previous chapters we have shown that there are reasonably consistent differences between varieties in their responses to changes in environmental variables such as soil water and the transpiration pressure imposed on the vines by changes in temperature and humidity. This has allowed for a broad classification of vines into those demonstrating more isohydric tendencies and those showing more anisohydric characters. Of the vines tested, Grenache consistently showed isohydric characters and generally performed well in terms of its ability to produce a crop under deficit irrigation. Amongst the other varieties tested there was a high level of variability in their performance, suggesting that their performance may have been influenced by recent history of exposure to environmental variables such as soil water or atmospheric VPD. To test this idea we have studied the gas exchange characteristics of Cabernet Sauvignon, a variety that generally displayed more anisohydric characters, when challenged with long term soil water deficits and short term changes in evaporative demand.

Results and Discussion

The irrigation treatments resulted in consistent differences in stomatal conductance throughout the season (Figure 1).

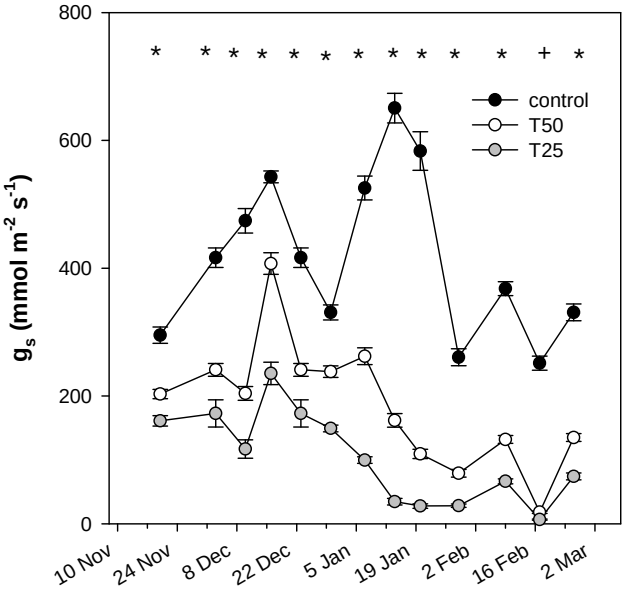


Figure 1. Leaf stomatal conductance in control and deficit irrigation vines throughout season 2008/2009. Significant differences are indicated by symbols, with * indicating all treatments are significantly different and + indicating control vines are different from T50 and T25 vines. Bars are SE (n=20).

Control vines were always significantly ($P<0.05$) higher than T50 and T25 and at most sample days T25 was significantly lower than T50. There was a downward trend through the season in T50 and T25. Midday leaf water potential largely reflected stomatal conductance with control values consistently significantly higher than T50 and T25 (Figure 2). As with stomatal conductance there was a seasonal downward trend in midday leaf water potential. Pre-dawn leaf water potential, measured during the period of leaf gas exchange measurements, was significantly lower in the water deficit treatments (Figure 3). These consistent differences in the key indicators of vine performance resulted in significant differences in canopy size measured in January (Figure 4).

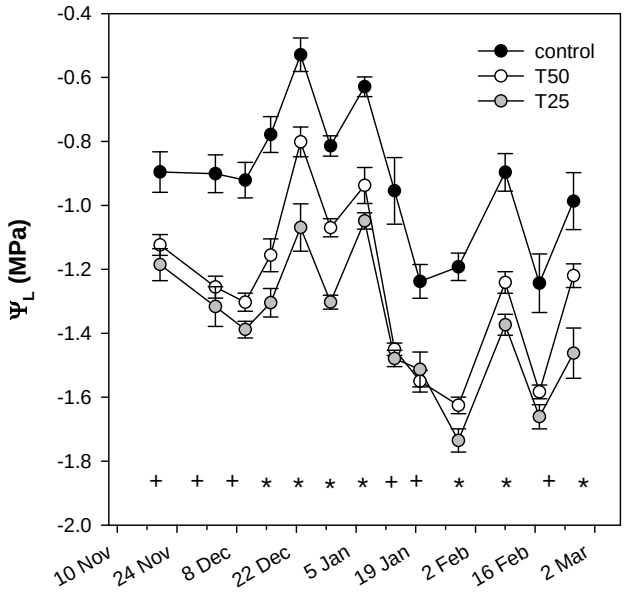


Figure 2. Midday leaf water potential (Ψ_L) of control and deficit irrigation vines throughout season 2008/2009. Significant differences are indicated by symbols, with * indicating all treatments are significantly different and + indicating control vines are different from T50 and T25 vines. Bars are SE (n=5).

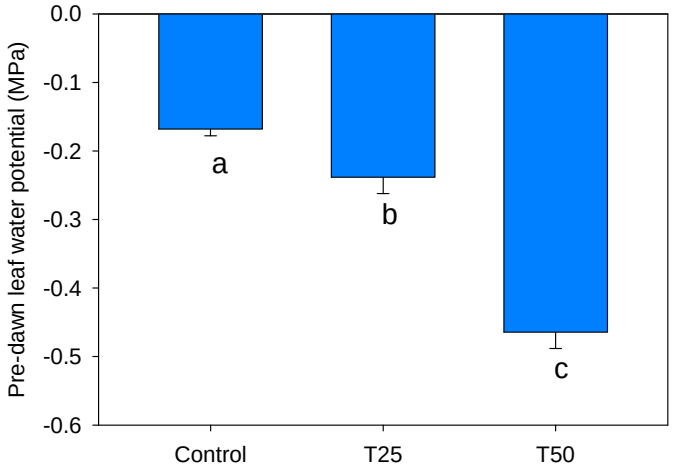


Figure 3. pre-dawn leaf water potential measured Jan 8th 2009. Different letters indicate significant difference (P<0.05). Bars are SE n=5.

One of the major indicators of the isohydric/anisohydric distinction between varieties seems to be the response to VPD. Accordingly, we assessed the response of these Cabernet Sauvignon vines to changes in VPD by manipulating the conditions in the gas exchange chamber.

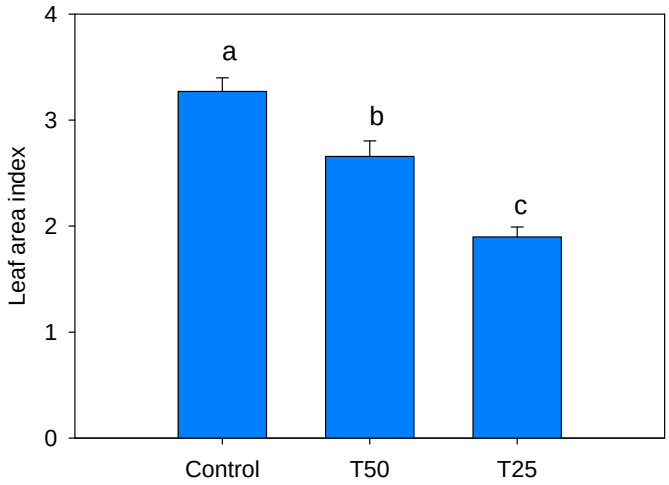


Figure 4. Leaf area index for control and deficit irrigation vines in season 2008/2009 measured at harvest.

Major treatment effects were evident. The control vines were relatively unresponsive to changes in VPD from about 1.5 kPa to 4 kPa. (Note: it was not possible to achieve VPD higher than this in the control because of the water added by the leaves to the stirred gas exchange chamber. At lower transpiration rates as shown for T50 and T25 higher VPD could be achieved), showing a more or less linear increase in transpiration as VPD was increased (Fig 5).

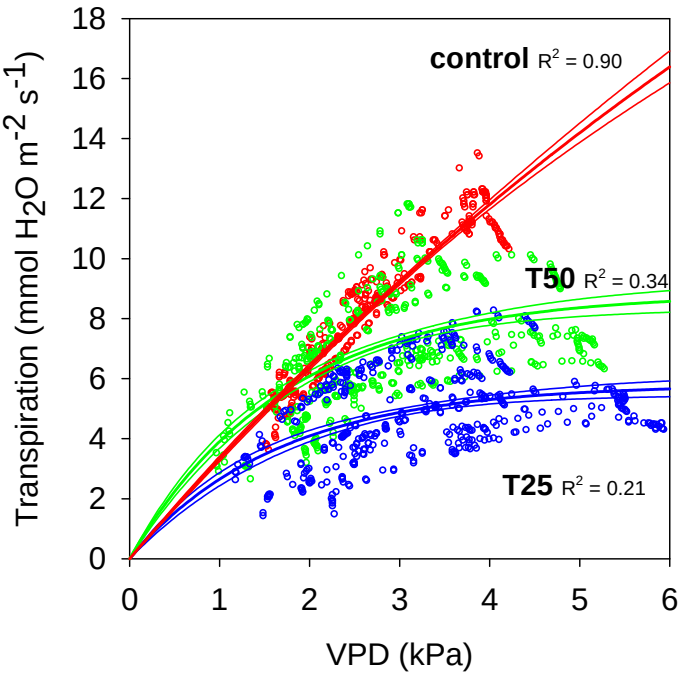


Figure 5. Leaf transpiration in control and deficit irrigation vines in response to increasing VPD within the LiCor chamber. Solid lines are regression relationships and 95% confidence interval bands for each irrigation treatment.

T50 and T25 became progressively more sensitive to VPD with transpiration in the T25 vines being reduced approximately 3-fold compared with the controls at high VPD. Tests of significance (Kruskal-Wallis) were performed and indicated a very large difference between the transpiration rates for each treatment ($p < 0.001$). The water deficit treatments were affecting transpiration through a sensitisation of stomata to VPD (Fig 6).

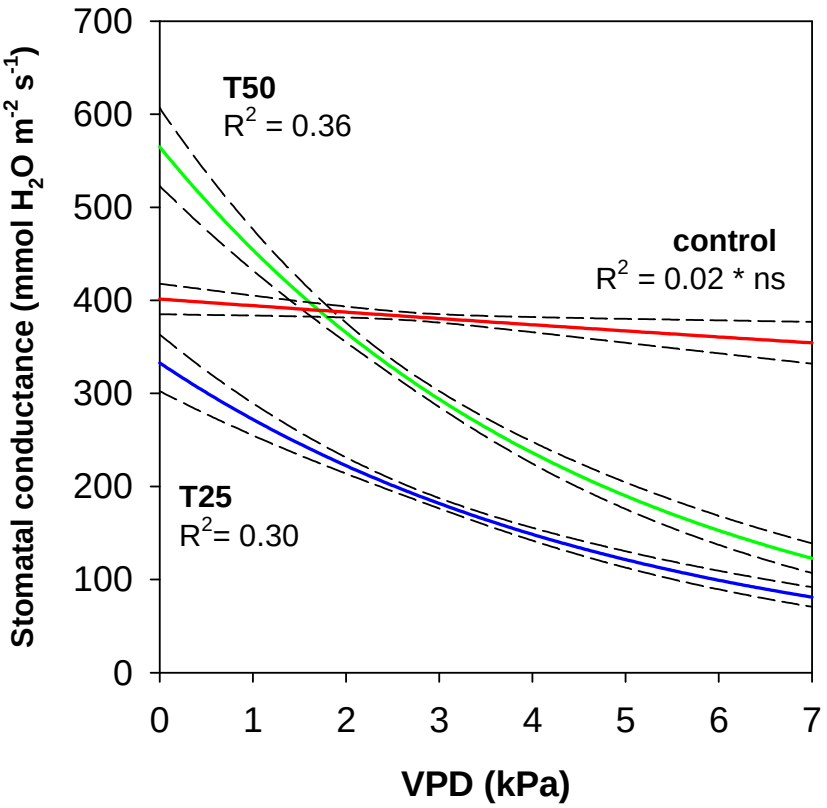


Figure 6. Stomatal conductance in control and deficit irrigation vines in response to increasing VPD within the LiCor chamber. Solid lines are regression relationships and 95% confidence interval bands for each irrigation treatment.

Stomatal conductance in the control vines was unaffected by increased VPD whereas stomata in the T50 and T25 vines progressively shut as VPD increased. The consequence of this type of response is frequently seen in field vines experiencing a degree of water deficit and adapts them successfully to optimize their gas exchange during the daily course of VPD change. Stomata of vines experiencing mild water deficit typically open widely in the early morning but then close as humidity falls and temperature rises, illustrated here in the T50 vines. Vines with abundant soil water are

unresponsive and are able to maximise gas exchange, and therefore carbon gain, by keeping their stomata open throughout the day despite increased evaporative demand (a behaviour illustrated by our control vines). As water deficit intensifies (as in our T25 vines) gas exchange is limited even under conditions of relatively low evaporative demand and becomes even more tightly controlled as VPD increases. Despite these major differences in stomatal response among the treatments intrinsic water use efficiency (A/gs) appeared to be similar in all treatments as the assimilation/stomatal conductance relationships all fitted approximately the same regression line (Fig 7).

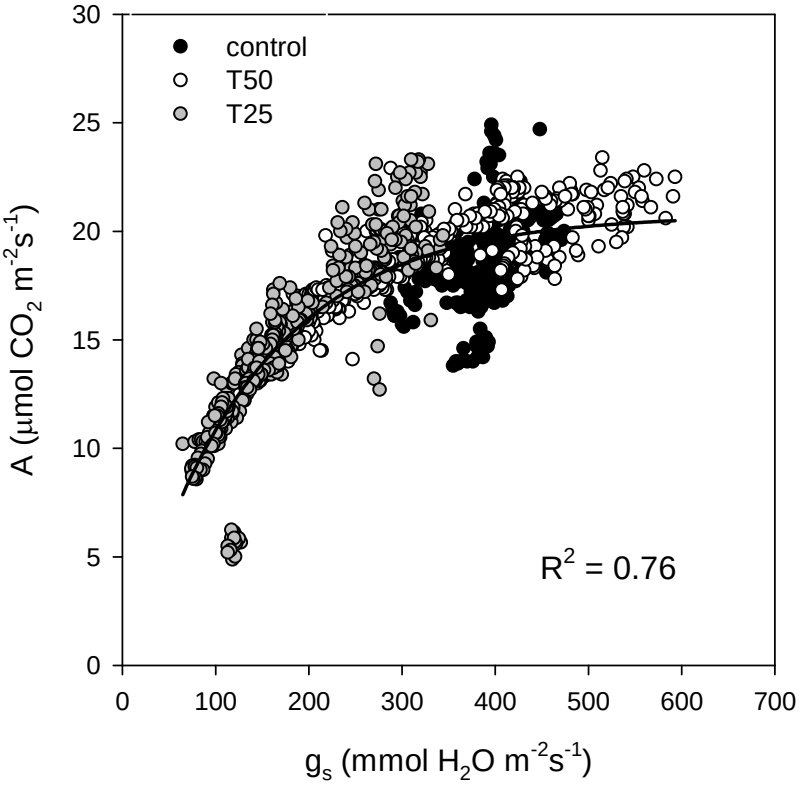


Figure 7. Intrinsic water-use efficiency (A/gs) relationships for control, T50 and T25 vines.

However, A/Ci curves did suggest some treatment effects (Fig 8). These data allowed the calculation of Vc max (maximum rate of carboxylation), J max (whole chain electron transport) and Rd (mitochondrial respiration in the light) according to Farquhar *et al.* (1980) (Table 1). Vc max and J max were not affected in the T50 treatment but was significantly reduced in T25 when compared with the control. Rd may have been increased in the deficit treatments but high variability meant that the differences were not significant.

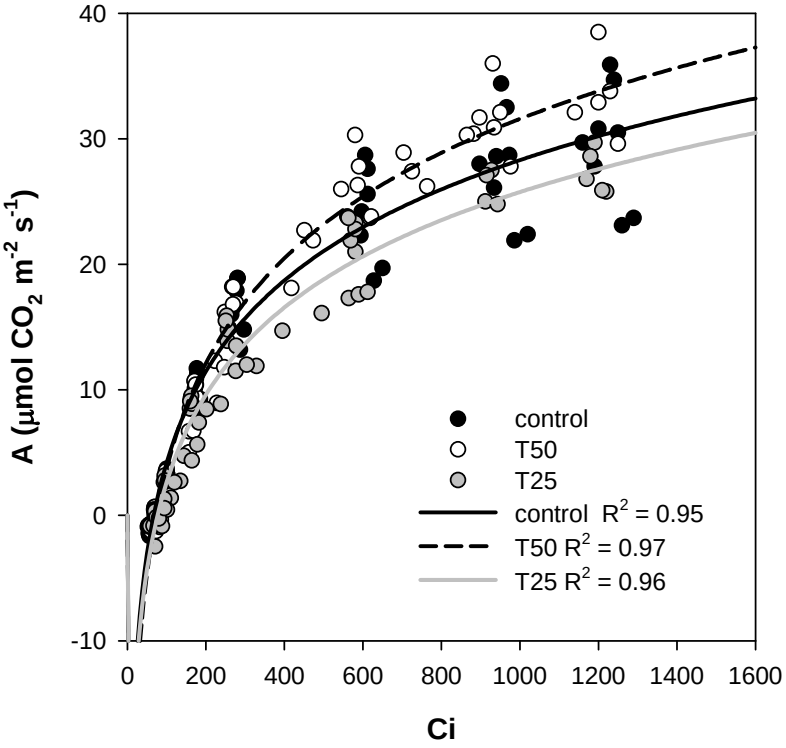


Figure 8. A-Ci curves for control, T50 and T25 vines at Oxford Landing, Waikerie.

Table 1. Vc max, J max and Rd calculated from A-Ci curves for irrigation treatments. Different letters in any column show significant difference (P<0.05)

Treatment	Vc max	J max	Rd
control	132[a]	132[a]	1.2[a]
T50	135[a]	139[a]	1.4[a]
T25	105[b]	111[b]	1.8[a]

These data show that the Cabernet Sauvignon vines have adapted to their irrigation regime through changes in stomatal response to evaporative demand and the question arises whether this is being driven by chemical signals, probably arising in the roots, or by hydraulic properties. In the control vines stomatal conductance was unaffected by changes in leaf water potential whereas both T25 and T50 showed a strong positive relationship between conductance and leaf water potential (Figure 9). There was no significant difference between the relationships T50 and T25, with the slope of the relationship being almost identical for the two treatments. The control vines appear able to sustain high levels of stomatal conductance even at quite low values of leaf water potential, suggesting that there is no intrinsic hydraulic limitation at water potential values as low as -1.4MPa whereas at the same water potential T50 and T25 vines had much lower stomatal conductance. This suggests that some factor other than low leaf water potential is driving stomatal response in the water deficit vines.

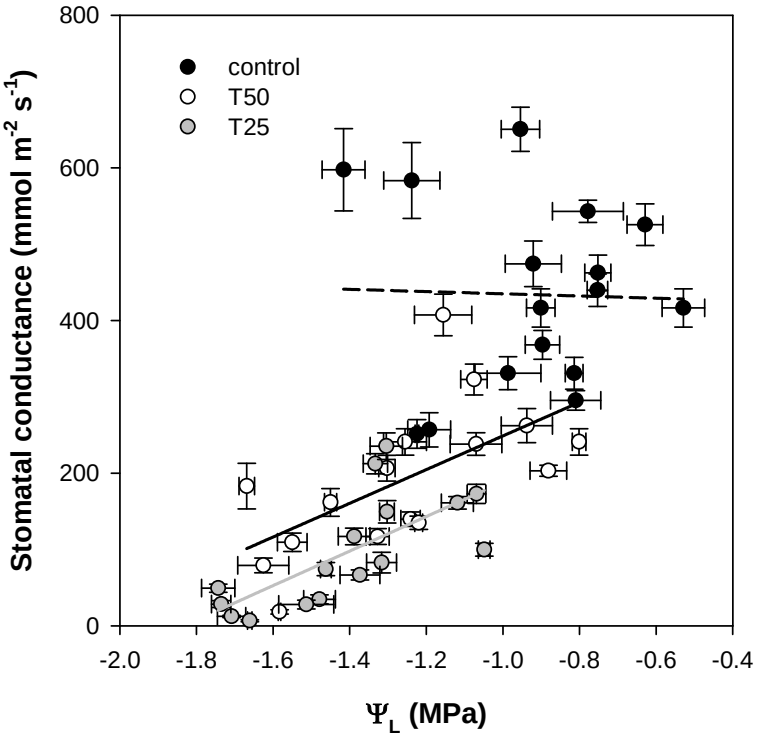


Figure 9. Relationship between stomatal conductance and leaf water potential for control and deficit irrigation treatments. T50 gs = 221x + 470, R^2 = 0.38, P<0.05) and T25 g_s = 226x + 415, R^2 = 0.50, P<0.05)

We have previously provided much evidence that the synthesis in roots of abscisic acid (ABA) and its transport to the shoot is the main driver of stomatal response to soil water deficit (Speirs *et al.* 2010) and so we investigated changes in ABA in the three treatments.

Plotting the relationship between stomatal conductance and ABA on a logarithmic scale reveals an interesting pattern between the different treatments. Low levels of ABA (up to about 120 ng/mL) did not affect stomatal conductance. This is the concentration found in the control vines. At higher levels of ABA there was a linear negative relationship with conductance. ABA does not affect stomatal conductance in control vines but does play a role in stomatal closure in T50 and T25 vines. An estimating equation approach was used to fit the bent stick model (Fig 10). That result gave a slope comparable to that observed using traditional least squares. The similarity was in part due to the moderately high R^2 of 0.72 and significantly, higher than an exponential decay model which gave a R^2 of 0.55.

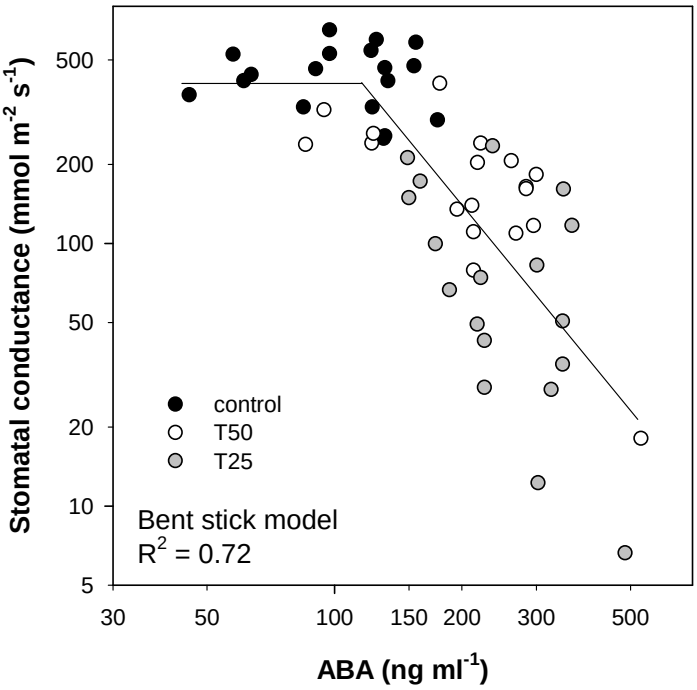


Figure 10. Relationship between stomatal conductance and xylem sap ABA concentration (Log scales)

This relationship between xylem sap ABA and conductance is remarkable similar to that reported by Speirs *et al.* (2010) for a number of grape varieties growing at several locations, with an inflection point at an ABA concentration of about 100 ng/mL. Speirs *et al.* also noted that this relationship between xylem sap ABA and stomatal conductance may be highly conserved among species since a very similar relationship was evident for sunflower (Tardieu *et al.* 1996) even though the concentration of bulk leaf ABA was some 80-fold lower in sunflower that is normally encountered in grapevine. When the concentration of ABA in xylem sap is below this critical concentration it is likely that arrival of new ABA to the leaf apoplast is balanced by metabolism and stomata are able to open widely. When the xylem concentration increases due to increased root synthetic activity stimulated by soil water deficit, rate of arrival of ABA outstrips breakdown leading to a net increase in apoplast ABA and stomatal closure. This mechanism may be further fine-tuned by changes in the activity of the ABA 8'-hydroxylase enzyme, the activity of which is governed by a gene sensitive to ambient humidity (Okamoto *et al.* 2009; Speirs *et al.* 2010). At high humidity the rate of breakdown of apoplast ABA is enhanced, allowing greater stomatal opening.

Prediction of stomatal conductance from LWP and ABA

Both LWP and ABA were considered potential predictors of stomatal conductance. In each case there was no predictive ability of either ABA or LWP for unstressed (control) vines as shown in Table 2.

Table 2. Adjusted R^2 for three models for predicting stomatal conductance

Treatment	**ABA+LWP**	**ABA**	**LWP**
control	0.000	0.000	0.000
T50	0.439	0.440	0.329
T25	0.489	0.169	0.466

There was predictive power for vines in treatments T50 and T25. When considering regression models that take into account both ABA and LWP (bivariate regression) there was no relationship for control vines for either variable (Table 3).

Table 3. Estimation of stomatal conductance from ABA and LWP for each of three treatments.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
control				
(Intercept)	433	138	3.12	0.008
ABA	-0.469	1.029	-0.455	0.656
LWP	-57	168	-0.339	0.740
T50				
(Intercept)	413	93	4.42	0.001
ABA	-0.461	0.238	-1.94	0.075
LWP	93.6	95.0	0.986	0.340
T25				
(Intercept)	425	85	5.01	0.000
ABA	-0.190	0.149	-1.275	0.228
LWP	198	63	3.12	**0.008**

In T50 vines ABA has a marginal effect ($P < 0.1$) above that of LWP and in T25 vines significant indication ($P < 0.01$) that LWP had a significant effect above that of ABA (Table 3). The effect of ABA alone is shown in Table 4 and shows no effect for control vines, a very significant effect ($P < 0.01$) for T50 but only a marginal effect (

0.05 < P < 0.10) in T25. By contrast LWP alone (Table 5) was significant (P < 0.05) in T50 and very significant (P < 0.01) in T25 vines.

Table 4. Estimation of stomatal conductance from ABA for each of three treatments

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
control				
(Intercept)	465	97	4.77	0.000
ABA	-0.282	0.841	-0.34	0.742
T50				
(Intercept)	331	43	7.70	0.000
ABA	-0.622	0.174	-3.58	**0.003**
T25				
(Intercept)	189	49	3.83	0.002
ABA	-0.358	0.178	-2.01	**0.063**

Table 5. Estimation of stomatal conductance from leaf water potential for each of three treatments

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
control				
(Intercept)	419	131	3.19	0.007
LWP	-16	138	-0.17	0.910
T50				
(Intercept)	468	97.6	4.79	0.000
LWP	219	76.0	2.89	**0.012**
T25				
(Intercept)	416	86.0	4.82	0.000
LWP	227	60.0	3.76	**0.002**

A comparison of the effects of ABA and LWP in T50 indicted that ABA was a marginally better predictor but in T25 LWP was the clearly the better predictor. Overall measurements of ABA were more variable than measurements of LWP and this additional source of error would have contributed to the poorer prediction of stomatal conductance by ABA. These analyses show that when there is abundant soil water (control) ABA does not reach a threshold value and has no influence on conductance. Conductance was similarly unresponsive to leaf water potential under these conditions. At moderate water deficit (T50) stomata are sensitively responding to xylem ABA but when water availability falls further (T25) hydraulic effects begin to have a greater influence on leaf performance. A leaf water potential threshold of -1.4MPa to -1.5MPa is suggested from data in Figure 9. If this hydraulic component is

related to susceptibility to xylem cavitation it might be expected that more isohydric varieties would have a higher threshold than anisohydric varieties (Schultz 2003).

7. Outcomes and conclusions

When subject to a range of simple tests designed to differentiate vines based on their isohydric/anisohydric characters there was a surprising consistency over the nine grape varieties tested. Grenache clearly stood out as the best performer and was the most isohydric-like in behaviour, whilst Cabernet Sauvignon, Merlot and Chardonnay the worst. The remaining varieties could not be statistically differentiated. While we would like to unequivocally specify one particular test to describe and predict the isohydric/anisohydric character of a grapevine genotype, the ability of vines to make both short and long term adaptive changes in their morphology, anatomy, and physiology in response to environmental pressures such as increased atmospheric evaporative demand or soil water deficit makes this very difficult. A statistical analysis of the relative influence of root-sourced chemical signals (ABA) and hydraulic factors in controlling stomatal conductance (Results section F) clearly showed that under severe water deficit hydraulic factors dominate whereas under conditions of mild water deficit stomata are predominately responsive to chemical control. This is clearly illustrated by the effect of seasonal differences (Results section A) on stomatal response to increasing evaporative demand. Varieties such as Merlot and Cabernet Sauvignon with low stomatal sensitivity to VPD in 2007, a drought year, were capable of significantly increasing their stomatal sensitivity to VPD in 2008 when conditions were less stressful, irrespective of larger canopies and higher yields in that year. In addition to this, from a vineyard management perspective, long term changes in irrigation management can equally have an affect on isohydric/anisohydric responses. We show that by making long term changes in irrigation management Cabernet Sauvignon, a variety that would generally be classified as anisohydric, can be made to exhibit characters more commonly associated with isohydric varieties (Results section F).

This project has enhanced our knowledge of vine response to high temperature events and has shown why it is that application of extra irrigation water before the arrival of heatwave conditions can be beneficial. The ability of anisohydric vines such as Shiraz to allow increased transpiration in response to elevated temperatures can help alleviate the impact of high day temperatures in a heat wave, however the positive

benefit of this increased transpiration can be quickly outweighed by rapidly depleting soil moisture and subsequent development of water stress if extra water is not regularly applied. Equally, vines subject to short and long term severe water deficit before heatwave conditions may not benefit to the same extent from extra water because their physiology has been changed to favour stomatal closure. Results with both Semillon and Shiraz also suggest that if night temperatures are elevated night-time transpiration rapidly becomes a significant part of daily water use, limiting the vines ability to recharge and cope with subsequent periods of elevated temperatures.

8. Recommendations

We have been able to demonstrate major differences in the ways that grapevine varieties respond to challenges they face in the form of increasing evaporative demand which may be combined with varying levels of soil water deficit. When these challenges are minimised, for example during exposure to low evaporative demand and with access to abundant soil water, all varieties will perform well in terms of vigour and the ability to ripen a crop. However, as the environment becomes more stressful through increased evaporative demand which may be combined with soil water deficit, grapevine varieties show differential responses. While it was obviously not possible to assess all vine varieties for their responses we selected representatives of both isohydric and anisohydric classes and the variety mix selected did represent nearly 80% (on an area basis) of the vines grown in Australia. Grenache, an example of an isohydric variety, performed well under most conditions when compared with varieties such as Merlot and Cabernet Sauvignon which showed more anisohydric characters. However, exposure to long term soil water deficit caused Cabernet Sauvignon vines to express characteristics such as stomatal sensitivity to increasing evaporative demand more like an isohydric vine. This suggests that strategic irrigation management can be used to alter vine response to potentially damaging environmental conditions. These experiments with Cabernet Sauvignon were carried out with vines grafted to Ramsey rootstock and since we have highlighted the importance of the root's ability to supply chemical signals in modifying canopy responses, future work may benefit from a better understanding of this aspect of root/shoot interactions with a view to defining the properties of rootstocks able to induce isohydric or anisohydric responses in scions. The

observation that well watered Shiraz vines were able to up-regulate stomatal conductance when faced with heatwave conditions is an important addition to our knowledge which may help in designing management strategies to deal with these events which are predicted to become more frequent as our climate changes. Whether other varieties behave in a similar way and whether the expression of the trait is determined by the vines isohydric/anisohydric character requires further investigation. Results with Semillon vines suggests that when combined with water deficit stomata are not able to respond by opening when exposed to high temperature. During exposure to high temperature water use of the Semillon vines during the day was actually less than vines not experiencing the high temperature despite a significant increase in VPD but overall daily water use was increased due to a large (40%) increase in nocturnal transpiration. This water use is unproductive and represents a major component of the water budget of these vines. This deserves further investigation as it may represent another property which differentiates isohydric from anisohydric varieties.

The knowledge gained in this project should assist in the selection of vine scion and rootstock genotypes which will perform better under conditions on increased evaporative demand and soil water deficit. It also provides information which may be used to shape management practices designed to reduce the impact of heatwave conditions.

Appendix 1. Communication

Some of the work carried out during this project has been presented to industry and to peers as follows:

- Seminar presentations to a number of industry groups during delivery of Module 4 of the GWRDC/DAFF *Water and Vine – managing the challenge* initiative.
- Presentations to the GWRDC *Soil and Water Initiative* advisory group
- Seminar presentations at University of Lancaster, UK and Geisenheim Research Centre, Germany
- In-house seminars
- A publication in Functional Plant Biology (section B of this report)
- A publication in Australian Journal of Grape and Wine Research (section C of this report).
- Seminar presentation at the 8th International Symposium on Grapevine Physiology & Biotechnology in Adelaide 2008

Report on visit to Dr Manfred Stoll and Professor Hans Schultz, Geisenheim Research Centre, Section of viticulture, D-65366 Geisenheim, Germany and Dr Ian Dodd and Professor Bill Davies, Lancaster Environment Centre, Lancaster, Lancashire LA1 4YQ, United Kingdon.

These visits were undertaken during August and September 2008 and were part of Dr Marisa Collins' career development plan. In Geisenheim Dr Collins undertook some collaborative work to assess the effects of water deficit on gas exchange characteristics of a number of grape varieties common to the area as well as Grenache and Shiraz for comparison with work carried out on these varieties in Australia. In addition she contributed to some on-going work which is currently being prepared for publication (see abstract below).

Automatic image analyses to reveal the effects of vessel element anatomy on leaf specific conductivity of field-grown grapevines

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Abstract
Anatomical measurements (diameter and frequency) of xylem vessels were determined at two different positions of petioles under water stress and non-water stress conditions for two different varieties (*Vitis vinifera* L. cvs. Syrah and Silvaner). Based on anatomical measurements using an automated image analysis software, the theoretical xylem conductance of the different positions and the resulting leaf specific conductivity was calculated. These characteristics resulted in different estimated leaf specific conductivities LSC among the two varieties. Syrah showed a larger number of smaller vessels contributing to the LSC making this variety better adapted to drought conditions than Silvaner.

Appendix 2. Intellectual Property

The information generated by this project will enter the public domain and will be accessible for future reference.

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Appendix 5. Acknowledgements

The authors would like to acknowledge:

Technical support

- Ms. Sue Maffei, Ms. Kerryn O'Brien and Mr. Allan Binney

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Industry collaborators

- Yalumba and all of their staff at their Oxford Landing (Waikerie) vineyard, particularly the vineyard manager Robert Strachan (site of deficit irrigation and VPD work on Cabernet Sauvignon and Semillon)

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- Rino Zafarelli (site of deficit irrigation x variety work in McLaren Vale region)

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