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Improving water use efficiency, canopy structure and grape quality by better matching rootstock and scion physiology to irrigation practice

FINAL REPORT TO

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CRV 99/8**

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Cover picture: Root Systems of a range of grapevine rootstock varieties

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The Cooperative Research Centre for Viticulture is a joint-venture between Australia's viticulture industry and leading research and education organisations. It promotes cooperative scientific research to accelerate quality viticultural management from vine to palate. Australian grapegrowers and winemakers are key stakeholders in the CRCV, contributing levies matched by the Commonwealth Government and invested by the Grape and Wine Research and Development Corporation in the Centre.

Abbreviations

g_s	stomatal conductance
E	transpiration
A	CO_2 assimilation
Ψ_{leaf}	leaf water potential
ABA	Abscisic acid
PRD	Partial Rootzone Drying
WUE	Water Use Efficiency
VPD	Vapour Pressure Deficit
PAR	Photosynthetically Active Radiation
Ψ_{stem}	stem water potential
TSS	Total Soluable Solids
TA	Titratable acidity
A/E	Transpiration efficiency

Definitions:

Water use efficiency; tonnes of grapes produced per ML of water from rainfall and irrigation expressed in tonnes (t) of grapes per MegaLitre of water (ML).

Transpiration Efficiency; component of WUE, being the ratio of carbon dioxide assimilated through photosynthesis to water vapour lost through transpiration expressed as μmol CO_2/mol H_2O.

Drought Tolerance; refers to the ability of the grapevine to maintain normal physiological processes such as transpiration and photosynthesis when subjected to water stress

Table of Contents

Cover picture: Root architecture of 5 common rootstock varieties grown in the glasshouse and watered daily to field capacity. Vines were 2 years old at harvest.

1. Abstract

Research on Partial Rootzone Drying (PRD) has highlighted the importance of root sourced chemical messages such as abscisic acid (ABA) in controlling water use in grapevine. The main objectives of CRV 99/8 were to determine whether changing root genotype by grafting to commercial rootstocks would provide a further means to manipulate these signals and thus modify water use efficiency, and to determine how grapevine varieties differ in response to atmospheric and soil moisture deficits. This work was carried out at vineyards at the University of Adelaide and the SARDI Barossa Valley Field Station. The results show that rootstocks have potential to influence vine water use and that these differences are reflected in xylem sap ABA concentration when vines are under stress. Variation was also found in the response of different commercial scion varieties to water deficits, which should be considered when deciding upon management of water resources in the vineyard.

2. Executive Summary

Prior to the commencement of CRV 99/8 published information on how rootstocks could be expected to perform under water limiting conditions was based largely on survey data and anecdotal information. The aims of this project were to provide scientific evidence for the potential of rootstocks to alter vine water use and to establish a better understanding of the physiological mechanisms driving this potential. Research on partial rootzone drying (PRD) had previously demonstrated the importance of root derived chemical signals, in controlling water use efficiency (WUE). A major objective of CRV 99/8 was to determine if rootstocks might be used to further manipulate these root-derived signals to improve grapevine WUE.

The original proposal for CRV 99/8 also recognised the importance that scion genotype might play in determining grapevine WUE. There was some evidence in the literature for a difference between the water use strategy of Shiraz versus Grenache and their response to water deficits (Schultz, 1997). Thus another major objective was to characterise the physiological response of some common *Vitis vinifera* varieties to water deficit, again focusing on the role of ABA.

Comparisons of 7 commercially available rootstocks grafted to Shiraz clone BVRC12 were performed over three seasons in the Coombe vineyard of the University of Adelaide. The irrigation was designed such that all vines received an identical amount of water in a given season but irrigation amount was varied between seasons such that a comparison of all rootstocks was made under different seasonal and irrigation conditions. Most physiological measurements were made on a semi-diurnal basis such that there were 5 to 6 measurements taken per day per rootstock. The diurnal change in stomatal conductance (g_s), transpiration (E), CO_2 assimilation (A) and leaf water potential (Ψ_{leaf}) of the clonal Shiraz varied significantly in each year as a function of rootstock. The best comparison between irrigation levels was achieved in the 01/02 and 02/03 growing seasons where the total water (irrigation and rainfall) in 01/02 was approximately half that received in 02/03. The relative performances of the rootstocks were similar in the two years with only a few exceptions. Rootstocks such as Ramsey and Teleki 5C consistently endowed higher assimilation and transpiration rates per

unit leaf area compared to the ungrafted Shiraz control, whereas other rootstocks such as K51-40 and 420a were consistently lower than the own rooted controls for the same parameters. The concentration of abscisic acid in the petiolar xylem sap (ABA_{sap}) extracted using a root pressure chamber was strongly correlated with stomatal conductance for all combinations when vines were subjected to stress under the low water inputs of 02/03, but not under the high water inputs of 01/02. The relative differences in g_s between rootstocks correlated strongly with the relative differences in ABA_{sap} in both years. Thus there is potential, with some precautions, for ABA_{sap} to be used to distinguish between high water use and low water use rootstocks in field conditions.

It is a reasonable assumption that if two vines have equal water supply, one with higher leaf transpiration rates should have lower Ψ_{leaf}. This assumption did not hold for most of the rootstocks in this trial which suggests that water supply was not equal across all vines which is suggestive of differences in the capacity of the different rootstock root systems to supply water to the scion. Differences in root architecture may account for this variation in water supply capacity and indeed some evidence was obtained in this project to show that such variation exists.

Three scion varieties, Shiraz, Grenache and Chardonnay, were assessed for their response to changes in evaporative demand. In terms of stomatal conductance, Grenache was far more sensitive to changes in Vapour Pressure Deficit (VPD) than the other two varieties. The consequence of this was that on cool days there was little difference between the three varieties in terms of leaf gas exchange and vine water status, however on hot dry days, Grenache leaves closed their stomata (lower g_s) to conserve water and maintain higher leaf water potential. We have postulated that this higher sensitivity of varieties such as Grenache to environment may make these varieties more responsive to irrigation management, a concept that has, and will continue to, receive attention in related CRCV/GWRDC projects (CRCV 2.1.8). Furthermore the response of these varieties to grafting to different rootstocks is not expected to be the same.

Throughout this project xylem sap ABA has been measured such that a link could be made between ABA signals and vine gas exchange arising from different rootstocks or varieties. An assumption is often made that ABA detected in the xylem sap originates from the roots. We set up experiments in collaboration with another project (CRV 01/05) managed by Dr Jim Speirs from CSIRO to begin to address the question as to the original source of ABA detected in the sap and leaves of grapevine. Gradients in xylem sap and leaf ABA were detected in both glasshouse- and field-grown Shiraz increasing in concentration towards the apex. These gradients correlated with gradients in stomatal conductance decreasing towards the apex. A combined approach coupling the physiology of the gradients with a molecular biological approach, looking at the activity of the key genes in the biosynthetic pathway for ABA (9-*cis* epoxycarotenoid dioxygenase (NCED) and zeaxanthine epoxidase (Zep)), demonstrated that the ABA gradients were in part generated by local synthesis in the shoots. This work has highlighted the fact that ABA measured in the shoots of grapevine is a consequence of both root and shoot derived ABA, albeit that ABA produced in the shoots may be in response to hydraulic and/or chemical signals arising from the roots.

We acknowledge the support of the University of Adelaide for providing access to the Coombe vineyard and vineyard management staff. Similarly we acknowledge the South Australian Research and Development Institute for providing access to the "Block 1" vineyard and vineyard management staff for the varietal comparison work. Thanks to Dr Michael M^{c}Carthy from SARDI and Professor Peter Dry for their assistance throughout the course of the project.

3. Background

At one time it was generally thought that the ability of leaves to control their gas exchange, that is the amount of carbon dioxide entering the leaf and the amount of water vapour exiting the leaf, was controlled by physiological events initiated in the leaves themselves. This thinking arose because of observations which linked leaf water relations, the synthesis of hormones in leaf tissue and the ability of these hormones to control the leaf stomatal pores through which all gas exchange occurs. The idea was that a water stress event would lead to a loss of leaf turgor, which switched on the synthesis of a plant hormone called abscisic acid (ABA) and the ABA would cause stomatal closure, thus reducing or eliminating further water loss. This theory was able to explain some known responses to water stress. However, careful observations showed that it was often possible to see a reduction in gas exchange in the absence of any changes in leaf water relations and alternative explanations were sought. Attention was turned to the roots, since these organs are in direct contact with the drying soil, and it was found that roots responded quickly to changes in soil moisture and that enough ABA could be transported from the roots to the leaves to act as a very effective stomatal control mechanism in grapevine (Loveys, 1984). Soon after this, experimental methods were developed, using plants with their roots divided between two containers, so that it was possible to differentiate between water supply effects and hormonal effects on leaf gas exchange (Gowing *et al*. 1990). There is now a very strong body of evidence to show that the roots are sensitive detectors of soil water status and they are able to transmit this information to the leaves in the form of chemical signals (Davies *et al*. 1987). The way that the leaves respond to these signals is also important, suggesting that the whole plant response will depend on the rootstock/scion combination. It was against this background of plant physiology research that the partial rootzone drying (PRD) method of grapevine irrigation was developed with support from the GWRDC (Dry, 1997, Loveys *et al.*, 1998, 1999).

Research conducted during the development of PRD showed that simple changes to current irrigation practice could bring about significant and beneficial changes to water use efficiency and canopy structure and that these changes were driven by chemical signals produced in the roots and transported to the leaves where they bring about fundamental changes in stomatal function. Since transpiration is affected to a

greater degree than photosyntheseis as a result of this partial stomatal closure (Düring et al., 1997, Iacono, 1998), water use efficiency is increased. For example, Loveys *et al*. (1998) showed that by using PRD, own-rooted Cabernet Sauvignon and Shiraz, growing near Adelaide, could produce a crop of 15-22t/ha with an irrigation input of less than 1ML/ha (rainfall will be additional to this), and that Riesling on Ramsey rootstock could produce up to 30t/ha with an irrigation input less than half the average for the Riverland district. In these experiments, pruning weights (a measure of canopy vigour) were reduced by between 20 and 40%, compared with normally irrigated vines.

There is some considerable evidence that grapevine varieties differ in their drought tolerance. For example, Schultz, (1996) showed that Shiraz was intrinsically more drought tolerant than Grenache. When the vines were grown side by side in a French vineyard, unirrigated Grenache vines were unable to fully ripen a crop, whereas the Shiraz vines were able to. A number of physiological differences between the cultivars were identified. In particular, Shiraz maintained an ability to keep its stomata open at lower leaf relative water contents, thus allowing it to better exploit soil water reserves. In these experiments the Shiraz and Grenache were grafted to 110 Richter and 140 Ruggeri rootstocks respectively, and it was not clearly established whether the effects noted originated with the scion or the rootstock. More recently, Gibberd et al. (2001) (unpublished) have used measures of carbon isotope ratios in leaf tissues to demonstrate differences in the water use efficiency of a number of grape cultivars. Both scion and rootstock effects were evident. The effects of rootstock on drought tolerance of the scion were well demonstrated by experiments of Iacono et al. (1998). He grew the *Vitis vinifera* scion variety Müller Thurgau on a number of hybrid rootstocks and showed that some rootstocks were able to confer drought tolerance to the scion and some were not. Grapevine performance, especially in water-limiting conditions, is therefore determined by the genotype of both the leaves and the roots. There has been no concerted effort to explain these differences by gaining a better understanding of the physiological characteristics of both rootstocks and scions and their interaction. Such an understanding will yield improvement in WUE through optimisation of irrigation methods, especially those which rely on stimulation of stress responses such as RDI and PRD, by allowing irrigation input to be better matched to the true water requirement of own-rooted cultivars and rootstock/scion combinations.

Industry Context

It is recognised that the major constraint to the continued development of the winegrape industry in Australia is the availability of water (Scales et al., 1995). Vineyard expansion has created demand for an additional 70,000ML of water the bulk of which has come from water trading and efficiency gains in vineyards (Anon., 1996). A recent survey of Riverland and Sunraysia vineyards (Skewes and Meissner, 1997) showed that the efficiency of water use (WUE, t/ML) varied by an order of magnitude and depended on site, irrigation method and grape variety. Many growers were judged to be very inefficient in their use of water. Irrigation scheduling tools used by the industry also vary widely, with decisions based on the calendar, on some measure of soil moisture or on models of crop water use which rely on parameters such as pan evaporation. Ideally, irrigation scheduling would be based on some plant-based measure of water stress, such as pre-dawn water potential, stomatal conductance, midday leaf temperature, sap flow etc. but so far none of these has reached a stage of development where it can be widely adopted. Soil moisture may be a reasonable measure of plant stress since plant water status is closely linked to soil water status through the roots. Leaf water potential measured pre-dawn approximates soil water potential. Decisions about when to irrigate could therefore be taken, based on soil water content (eg. neutron probe). However, the work of Schultz (1997) graphically shows that this can be misleading. Unirrigated Grenache vines reached a pre-dawn water potential of –0.85MPa and that of Shiraz was much lower (apparently more water stressed) at –1.4MPa., yet the Shiraz vines were able to successfully ripen their crop but the Grenache vines did not. Irrigation scheduling based on soil water would therefore have applied far more water to the Shiraz vines than was required and may have withheld water from the Grenache vines when it was needed. The explanation for this is relatively simple: the Grenache vines were intrinsically more sensitive to water stress than the Shiraz, with stomata closing and photosynthesis declining at only moderate levels of soil water deficit, whereas the Shiraz vines were able to continue operating at lower levels of soil water. One component of the ability of Shiraz to sustain photosynthesis at lower values of soil moisture may be osmotic adjustment, that is the accumulation of solute molecules in leaves and/or roots which allow these organs to function at lower values of soil moisture. Although there are some reports of this phenomenon in grapevines (Godoy and Huitron, 1985; Schultz and Matthews, 1993) a systematic survey of the grape cultivars commonly grown in

Australia has not been undertaken. This may be particularly important in the case of rootstocks, and here we have very little knowledge.

While an irrigation scheduling tool based on plant performance may be desirable, constraints due to cost and a lack of appropriate guidelines have so far prevented any wide adoption. Soil moisture appears to be a reasonable alternative, but interpretation of soil moisture data should take into account crop genotype, for both own-rooted and grafted vines.

Rootstocks are used for a number of reasons. Non *vinifera* stocks confer resistance to nematodes and Phylloxera, and this may be the primary reason for their use. However, significant effects on canopy vigour and stress responses have been noted. If the physiological basis of these effects were understood, then better judgements of rootstock selection may be made. Cost and availability considerations often preclude the use of rootstocks in new plantings, but pressures from soil-borne organisms or water availability are likely to increase and rootstock use will then become more cost-effective.

The project outlined in this proposal will provide a sound background of knowledge on which to plan irrigation in own-rooted and grafted vines.

Relevance and priority assessment

The Australian winegrape industry aimed to improve water use efficiency by 30% by the year 2002 (Anon.1997). This project aims to provide more tools to reach this goal through provision of information which will describe the mechanisms controlling the intrinsic water use efficiency of the major winegrapes grown in Australia. It will identify biochemical markers which will allow an assessment of the stress responses and water use efficiency of both scion and rootstock varieties. This knowledge will allow irrigation input to be matched to crop requirement in a more meaningful way than at present and will be particularly relevant to PRD and RDI, where it will enlarge the scope and currency of these techniques. The knowledge gained will feed into programs of rootstock improvement and into models of grapevine performance which are currently under development.

4. Project Aims and Performance Targets

Objectives

- Characterise stress response mechanisms of major winegrape varieties and relate these to water use efficiency (WUE).
- Assess the influence of rootstock on expression of these mechanisms. Since the root is the primary organ sensing water stress and we have shown that root-derived signals are the driving force responsible for determining canopy performance, it is reasonable to expect rootstock selection to be a major determinant of WUE under different irrigation regimes.
- Develop biochemical markers, which will allow for rapid screening of grapevine varieties to assess their stress response properties.
- Produce recommendations for the most efficient irrigation strategy for specific varieties and scion/rootstock combinations.

The following list outlines the modified performance targets for the project.

Performance Targets

- Appoint Post Doctoral Fellow
- Propagate and establish potted vines
- Quantitative analysis of ABA production by different rootstocks
- Quantitative analysis of cytokinin production by different rootstocks
- Measure soil/plant water status, and effect on root derived hormones
- Assess responsiveness of leaves to root signals
- Assess role of sap pH in regulating hormonal control of stomatal aperture
- Measure pH of sap in stressed and control vines
- Survey vines for degree of osmotic adjustment
- Measure effect of rootstock on canopy growth
- Measure effect of increasing atmospheric CO_2 on stomatal response to VPD and ABA.
- Report outcomes to industry
- Collate data and prepare final report

5. Methods

A1 Methods: Physiology of rootstock influence over scion water use

Experiment design

In 2000/2001 all physiological measurements were taken between 10:00 am and 14:00 pm to approximate midday measurements.

Semi-diurnal measurements were taken across two seasons (2001/2002 and 2002/2003) at the Coombe vineyard of the University of Adelaide rootstock trial. In 1993 *Vitis vinifera* cv Shiraz (BVRC12 clone) grafted to 7 different rootstocks (420a, K51-40, Ramsey, 140 Ruggeri, Schwarzmann, SO4 and Teleki 5C) and an ungrafted control were planted into 11 rows (blocks) of 32 vines, such that each scion/rootstock combination was represented once per row as a 4 vine plot, where the two centre vines were used for measurements and the two outer vines were buffers. The distance between rows was 3.0 m and the distance between vines was 2.7 m. The rows within the trial run North-South such that in the morning the east face of the row is sun exposed and after 12:00 pm the west face becomes sun exposed.

Irrigation was applied by overhead drip lines 20 cm from the ground with two $2L.h^{-1}$ Agridrip® (Antelco, Bridgewater, South Australia) pressure compensating drippers per vine, one located 30 cm each side of the trunk, supplying Adelaide mains water. In 2002 water inputs into the block were maintained at a high level for the region, such that irrigation was applied at 1.3 $ML.ha^{-1}$. In 2003 watering was greatly reduced to just 0.4 $ML.ha^{-1}$.

Gas exchange measurements

Stomatal conductance (g_s), transpiration (E) and CO_2 assimilation (A) were measured using a Li6400 portable photosynthesis detection system equipped with a 6400-02B red/blue external light source and CO_2 mixer (Li-Cor Inc, Lincoln Nebraska). Measurements were taken on the western side of the rows on relatively cloudless days with similar VPD_{max} (~3.5kPa at 15:00pm Australian central daylight savings time) at six time points during the day in 2002, and at five times in 2003. At each time ambient temperature, relative humidity and light intensity (PAR) were measured with

the Li6400 and conditions within the sample chamber were set to closely mimic ambient conditions whilst clamped on a leaf. Sample CO_2 concentration was kept constant at 370ppm at all times using the mixer supplying industrial grade CO_2 (BOC gases, Australia). The Li6400 was clamped onto 3 leaves per vine and values were logged after all readings had become stable (ascertained as the point where the combined coefficient of variation for flow, CO_2 and H_2O in the sample chamber was less than 1%).

Leaf water potential and sap collection for ABA analysis

Leaf water potential (Ψ_{leaf}) was measured on each of the three leaves on which gas exchange was measured immediately after being removed from the chamber of the Li6400. The leaf was wrapped in a clear plastic bag, which was held closed by a 15mm fold-back clip, prior to excision using a single edged razor blade to slice through the petiole approximately 1cm from the point of attachment to the cane. The water potential was measured with a 3000 series Plant Water Status Console (Soilmoisture Equipment Corp, Santa Barbera, USA) using industrial grade nitrogen (BOC gases, Australia) to pressurise the chamber. After recording the water potential an overpressure of 100 kPa was applied and sap was collected using a 200 μL Gilson Pippetteman® and transferred to a pre-weighed and labelled microcentrifuge tube before being snap frozen in liquid nitrogen. Samples were stored at –40ºC until analysis.

Stem water potential and sap collection for ABA analysis

Measurement of stem water potential (Ψ_{stem}) was identical to that of leaf water potential with the addition of a bagging treatment for a minimum of 16 hours prior to measurement; and stem water potential was only measured at 7:00, 9:00. 13:00 and 17:00 in 01/02, and 9:00, 13:00 and 17:00 in 02/03. Plastic bags were made out of Pandafilm® (which were black on the interior and white on the exterior surfaces). These bags were placed over 3 leaves of each vine no later than 16:00 on the day preceding measurement, and were not removed until immediately before measurement of the water potential.

Sap pH and ABA analysis

The frozen sap samples were thawed in a darkened area of the laboratory (ABA is sensitive to light degradation) on the bench top and following centrifugation in a bench top microfuge (1min, 1500 g), the tubes were re-weighed to determine the weight of sap collected. The pH of the sample was measured with a MI-410 micro pH electrode (microelectrodes inc, Bedford, USA). 200 µL of MeOH containing 15.9 ng of deuterated ABA (3', 5', 5',7', 7', 7' – (d6) – ABA) was then added to each tube as an internal standard. The samples were then dried under vacuum using a Savant SC110a speed-vac plus (New York, U.S.A.). The dry residue was then redissolved in 50 µL dry acetone followed by the addition of 125 µL of the freshly prepared derivitising agent, ethereal diazomethane. The samples were covered for 20 minutes and then allowed to air dry after which the residue was redissolved in 80 µL of 100% MeOH, and following centrifugation in a bench top microfuge (3 min, 10000 g) the supernatant was transferred to a 200 µL GC vial dried and redissolved in 20 µL MeOH from which 1 µL was analysed by gas chromatography/mass spectrometry. The GC analysis was performed using a Hewlett Packard GC-MS system (HP 6890 series equipped with a HP 5973 series mass selective detector) with a DB-5MS column (J&W scientific). The GC-MS was operated in splitless mode with an initial oven temperature of 80ºC, followed by a ramp at 12ºC/min to 240ºC then held at this temperature for 10 min. The ion pairs diagnostic of ABA (190/194 and 162/166) were monitored using selective ion-monitoring mode (SIM).

Leaf area measurement

Relative leaf area of each of the Shiraz/rootstock combinations was determined by plucking every leaf from 6 measurement vines of each combination. A sub sample of 72 leaves per combination (12 from each vine) was taken and a leaf area to dry weight ratio was calculated as follows. The leaf area of all 72 leaves was measured using SigmaScan Pro version 4 (Jandel Scientific, California, USA) to analyse an image captured on a HP 2410 all-in-one scanner (Hewlett Packard, Australia). Pixel to length calibration was performed simply by scanning a piece of paper of known dimensions and doing a two-point calibration. The sub sample was then dried to constant weight at 60ºC to establish a dry weight to leaf area ratio. The total leaf area of each vine was then estimated by determining dry weight of each vine's leaves and converting this to area from the leaf area to dry weight ratio of the sub-sample.

Pruning weights

Pruning weights were taken in June 2002 and June 2003. Canes were removed to leave 2 node spurs, and the canes were bundled and weighted using field scales.

Root distribution studies on potted vines

Two year-old *V. vinifera* L. cv Shiraz grafted to Ramsey, 420a, Riparia Gloire and Own Roots were planted in tall cylindrical pots (D = 150 mm x H = 500 mm) containing potting mix consisting of 50% composted pinebark, 25% peat and 25% sharp white sand, 0.6 g/L $FeSO_4$, 5 g L^{-1} of the slow release fertilizer Osmocote® - long life plus trace elements and 2 g L^{-1} pH adjustment (2:1:1 gypsum:dolomite:lime). Plants were grown in controlled temperature glasshouses at 25ºC/20ºC day/night with night break lighting (to simulate longer day length) provided by 400 W metal halide lamps (Philips HPI400WGES) for 2 h from 23:00 to 01:00. Plants were trained to a single vertical shoot and were watered daily to field capacity.

After 2 years further growth the plants were destructively harvested and the roots gently washed free from soil with Adelaide tap water from a hand-held watering hose. After washing, the root systems were stored for no more than 48 hours in a 3ºC cold room to await sorting and analysis. All roots were cut from the crown with dissecting scissors and sorted into three diameter classes, >2 mm, 1-2 mm and <1 mm. The length of each root in the >2 mm class was measured, prior to being pooled, dried to constant weight at 60ºC and weighed. Roots in the other diameter classes were pooled immediately after excision, dried to constant weight at 60ºC and then weighed.

The effects of rootstock on root weight and length (>2 mm only) was then analysed for each root class using a single factor ANOVA, with pair wise comparisons being made using a Fisher's Protected LSD.

B1 Methods - Physiology of varietal responses to water deficit

Experiment design

Measurements were taken pre-veraison in the 2002/2003 season at the Nuriootpa field research station belonging to SARDI. The trial consisted of one row each of Shiraz (clone 1125), Grenache (clone 139HT) and Chardonnay (clone I10V1). Readings and

samples were taken across nine replicate vines in each row across three days of low VPD (mean max = 1.57 kPa) and again on three days of high VPD (mean max = 5.82 kPa). The block ran east to west such that most of the canopy was fully sun exposed throughout the measurement period.

Gas exchange measurements

Stomatal conductance (g_s), transpiration (E) and CO_2 assimilation (A) were measured as for the Coombe vineyard (see A1 above). Measurements were taken on the northern side of the rows on relatively cloudless days at five time points (9:00, 11:00, 13:00, 15:00 and 17:00). The Li6400 was clamped onto 2 leaves per vine and values were logged after all readings had become stable (ascertained as the point where the combined coefficient of variation for flow, CO_2 and H_2O in the sample chamber was less than 1%).

Leaf water potential and sap ABA analysis

Water potentials and sap ABA analysis were performed identically to that shown for the Coombe vineyard (see A above).

Leaf area measurement

Relative leaf area of the Shiraz, Grenache and Chardonnay vines were determined by plucking every leaf from 5 consecutive vines at the same row position in each row. A sub sample of 50 leaves per variety (10 from each vine) was taken and a leaf area to dry weight ratio was calculated as follows. The leaf area of all 50 leaves was measured using SigmaScan Pro version 4 (Jandel Scientific, California, USA) to analyse an image captured on a HP 2410 all-in-one scanner (Hewlett Packard, Australia). Pixel to length calibration was performed simply by scanning a piece of paper of known dimensions and doing a two-point calibration. The sub sample was then dried to constant weight at 60ºC to establish a dry weight to leaf area ratio. The total leaf area of each vine was then estimated by determining dry weight of each vines leaves and converting this to area from the leaf area to dry weight ratio of the sub-sample.

Ripening Measurements on Shiraz and Grenache

In 2003 total soluble solids (TSS), pH and titrateable acidity (TA) were measured on a single combined juice extract for Shiraz and Grenache respectively from a 50 berry sample taken across the same 12 vines used for gas exchange measurements in 2002. The two samples were collected into a screw cap vials and stored below 4ºC overnight to await analysis on the following day. The samples were weighed, and then squeezed with the juice collected into a 50 mL screw cap plastic centrifuge tube. The juice was then clarified by centrifugation at 2000 *g* for 3 min. The clarified juice was decanted into a fresh screw cap tube ready for analysis.

TSS was measured using refractometry and pH by direct measure using a calibrated pH meter. TA was determined using a pH meter method where 100 mL of deionised water was changed to pH 8.2 using 0.1N NaOH prior to the addition of a 10 mL aliquot of the clarified juice sample. Using a burette, the volume of 0.1N NaOH required to return the pH to 8.2 was determined. From this value the titratable acidity in g/L of tartaric acid equivalents was calculated as 0.75 times the titre value (Iland et al., 2000).

C1 Methods: Origins of ABA gradients in grapevine shoots

Often ABA concentrations are measured in the sap and leaves from grapevine shoots with the assumption that most of the ABA measured originates from the roots as part of a direct root to shoot signal of water deficit. The work in this section was designed to use natural gradients in ABA to study likely sources of ABA measured in the shoots of grapevine.

The experiments were done in collaboration with Dr Jim Speirs (CRV 01/05). The aim of the work was to investigate the source of gradients in xylem-sap [ABA] that lead to gradients in stomatal conductance in the shoots of grapevine. A combined molecular (CRV 01/05) and physiological (CRV 99/8) approach was used to compare the activity of the key genes in the ABA biosynthetic pathway (9-cis epoxycarotenoid dioxygenase (NCED) and zeaxanthine epoxidase (Zep)) with ABA concentration gradients to determine the likely source of the ABA measured in the shoots.

Detailed methods for this section of work and the results of the molecular component can be found in appendix 5. Results of this work will also be included in more detail in the final report for CRV 01/05.

6. Results/Discussion

A2 Results.

Gas exchange

Measurements were taken to compare the response of the different Shiraz/rootstock combinations in two seasons of contrasting water availability. The total water inputs (rainfall + irrigation) were vastly different between the two years and the response of the vines between the two years was indicative of different levels of stress between seasons. Whilst measurements were made on days of similar temperature and humidity the conditions over the entire growing season were considerably different between the two years.

The transpiration per unit leaf area of leaves for all combinations increased from the morning to the afternoon (Figure 1a and b) in both years as both irradiance and vapour pressure deficit increased. In general the transpiration rates measured in the 02/03 season were half of that observed under the lower water inputs of the 01/02 season. This was demonstrated by the comparison of stomatal conductance between the two seasons where g_s values in 02/03 were lower than in 01/02 (Fig 2a and b) despite the similar atmospheric conditions on measurement days. In the 01/02 season Shiraz on K51-40, SO4 and 140 Ruggeri had consistently lower transpiration rates than the other combinations with the largest differences between graft combinations at 15:00 h (Fig 1a) when the evaporative demand was greatest. Transpiration rates dropped again from 15:00 h to 17:00 h in all combinations except Shiraz on 140 Ruggeri where the transpiration did not change between 15:00 h and 17:00 h. Of the combinations with the highest transpiration in 01/02, Shiraz on Teleki 5C maintained the highest transpiration rate at all time points and Shiraz on Ramsey showed the largest drop in transpiration between the last two time points. Whilst some significant differences were found between the leaf areas of the different combinations the actual differences were quite small (data not shown). Conversion of transpiration per leaf

area to transpiration per vine on the basis of vine leaf area did not significantly change the ranking or the relative relationship between combinations in terms of transpiration (data not shown).

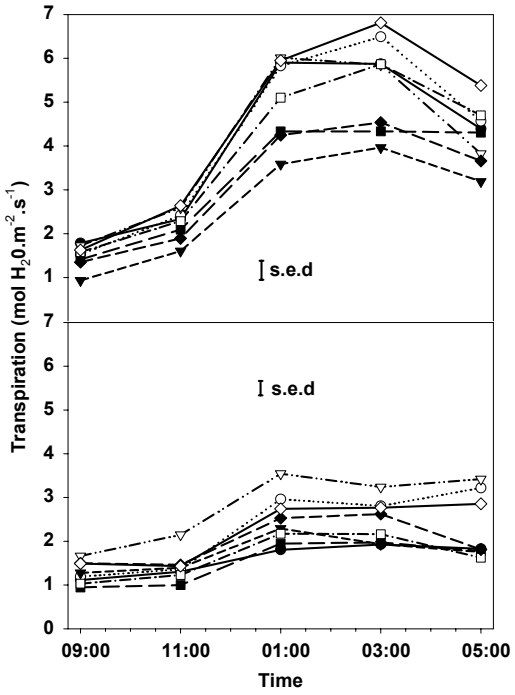

Figure 1. Diurnal change in transpiration rate of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

As expected the ranking of g_s strongly followed the ranking of E in both years (Fig 2a and b). The pattern of change in g_s within a day for all Shiraz/rootstock combinations was dramatically different between the two seasons, clearly indicating the extremes in water availability in the two years. Stomatal conductance in 01/02 increased during the day in all combinations (Fig 2a) most likely in response to increasing irradiance. In 02/03 g_s did not increase with irradiance, but rather had significant negative correlation with increasing VPD in contrast with the 01/02 season. There was more oscillation in g_s between time points in the 02/03 season which may be a consequence of higher sensitivity of the leaves to small changes in conditions throughout the day possibly as a consequence of the lower water availability.

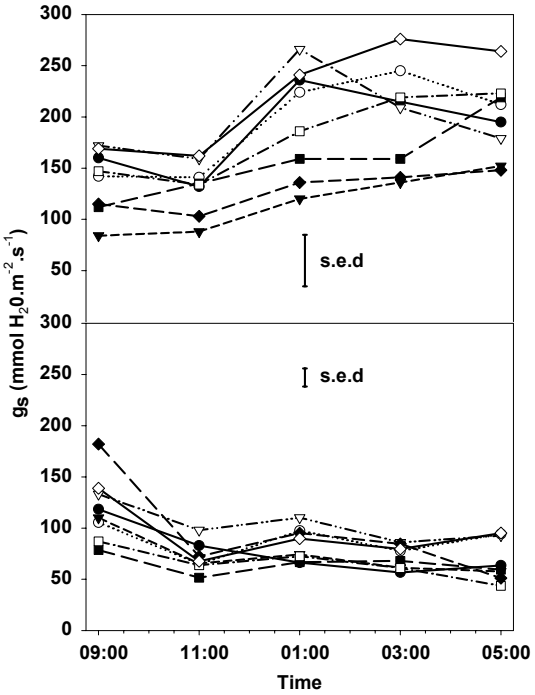

Figure 2. Diurnal change in stomatal conductance (g_s) of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

The CO_2 assimilation data for the 01/02 and 02/03 seasons is presented in Figure 3a and b respectively. In the 01/02 season assimilation increased with irradiance and stomatal conductance under the well-irrigated conditions. Similarly, in 02/03 there was an increase in assimilation rate with increasing irradiance in the morning, however in the afternoon significant limitation of assimilation was evident for all combinations. The differences between combinations were similar in the two seasons with a few exceptions. Shiraz on own roots, Ramsey and Teleki 5C had much higher afternoon assimilation (A) than Shiraz on Ruggeri 140, K51-40 in both seasons. Shiraz on 420a and Schwarzmann had relatively high A in 01/02 but performed much worse in 02/03, such that they were similar to Shiraz on K51-40 and Ruggeri 140. In contrast Shiraz on SO4 had much higher afternoon A in 02/03 than in 01/02.

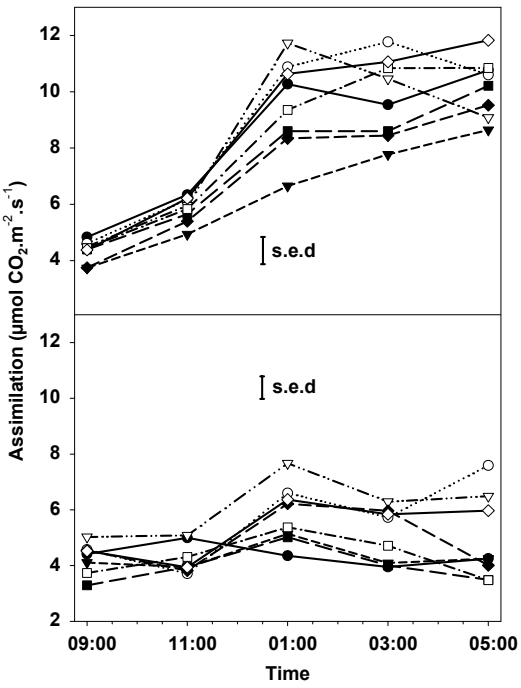

Figure 3. Diurnal change in CO_2 assimilation rate of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

It was expected that these rootstock-induced changes in gas exchange would have net effects on the transpiration efficiency (ratio of CO_2 assimilated to water lost by transpiration; A/E) of the vines. Significant differences in transpiration efficiency between rootstocks were only observed in the morning in both seasons (Fig 4a and 4b) despite there being comparatively small differences in gas exchange between combinations in the morning relative to the afternoon. In general Shiraz on own roots, Teleki 5C and Ramsey had low morning A/E in both years. The other combinations varied in relative morning A/E between seasons with few significant differences in 01/02 but with Shiraz on Ruggeri 140, 420a and Schwarzmann having higher morning A/E than all other combinations in 02/03.

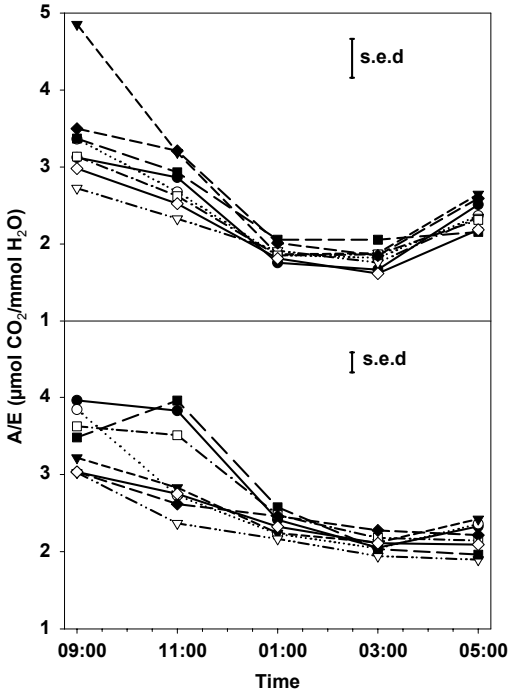

Figure 4. Diurnal change in transpiration efficiency (A/E) of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

Water potentials

The leaf water potential (Ψ_{leaf}) for all combinations decreased with increasing VPD during the day in both seasons (Fig 5a and b). The relative differences between the combinations were greater in the 02/03 season than in 01/02 and the relative order appeared to be positively related to differences in transpiration such that the combinations with higher water potentials also had higher afternoon transpiration rates.

Stem water potential (Ψ_{stem}) was also measured but did not differ significantly from Ψ_{leaf} both in terms of relative differences between rootstocks and pattern of change during the day. Thus there was a strong linear correlation between Ψ_{stem} and Ψ_{leaf} (r^2 = 0.92 and 0.93 for 01/02 and 02/03 respectively) in both seasons with a slope of 0.735 in 01/02 and 0.98 in 02/03 (data not shown) indicating that the leaves from which Ψ_{leaf} were measured were in balance with other leaves in the canopy.

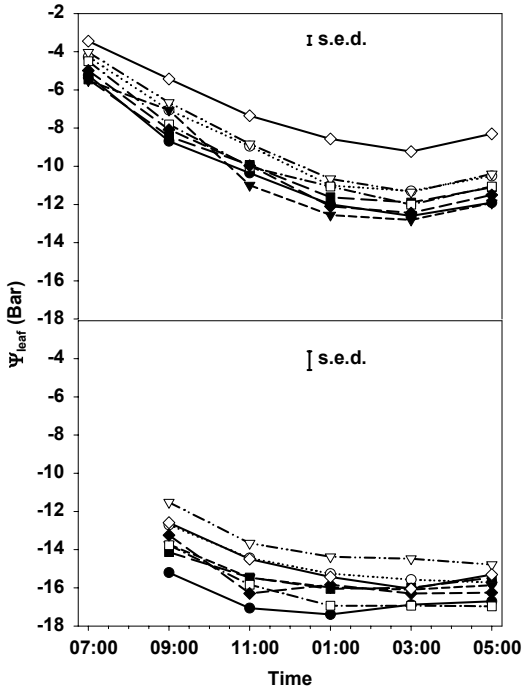

Figure 5. Diurnal change in leaf water potential (Ψ_{leaf}) of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

Sap abscisic acid concentration

Results of the analysis of the abscisic acid concentration in sap expressed from the petiole ($[ABA]_{sap}$) are shown in figures 6a and b for 01/02 and 02/03 respectively. In 01/02, $[ABA]_{sap}$ increased throughout the day in all Shiraz/rootstock combinations and there were no significant differences between combinations before midday with the exception of Shiraz on K51-40 which had much higher $[ABA]_{sap}$ than any other combination (Fig 6a). In the afternoon large differences were observed between several of the combinations, with Shiraz on K51-40 still having the highest $[ABA]_{sap}$ which correlated well with the lowest readings of g_s observed for this combination throughout the day in 01/02 (Fig 2a).

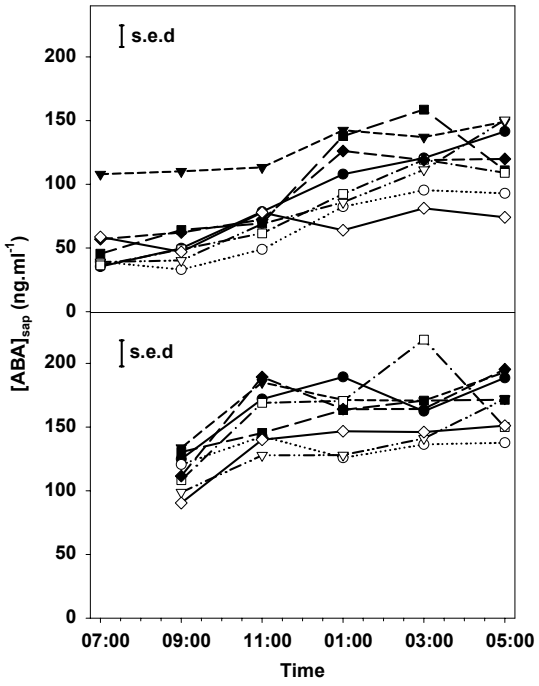

Figure 6. Diurnal change in xylem sap ABA concentration ($[ABA]_{sap}$) of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

Generally, those combinations that had higher $[ABA]_{sap}$ in the afternoon (Fig 7a) had lower afternoon values for g_s (Fig 2a) such that there was a strong correlation between Δg_s relative to Shiraz on own roots and $\Delta[ABA]_{sap}$ relative to Shiraz on own roots for each grafted combination (Fig 7a). There was however no correlation between g_s and $[ABA]_{sap}$ in 01/02 (Fig 7b) despite the expected inverse relationship between these parameters. In the 02/03 season $[ABA]_{sap}$ was higher overall for each combination compared with the previous season which again is in response to the low water availability in 02/03 compared with 01/02. In contrast to 01/02, the only appreciable increase in $[ABA]_{sap}$ occurred between 9:00 and 11:00, after which it reached a plateau in all combinations with the exception of Shiraz on Ramsey in which $[ABA]_{sap}$ continued to climb throughout the day. As observed for 01/02 there was a strong correlation between Δg_s and $\Delta[ABA]_{sap}$ (Fig 7c), however in contrast to the previous season there was also a strong negative correlation between g_s and $[ABA]_{sap}$ (Fig 7d).

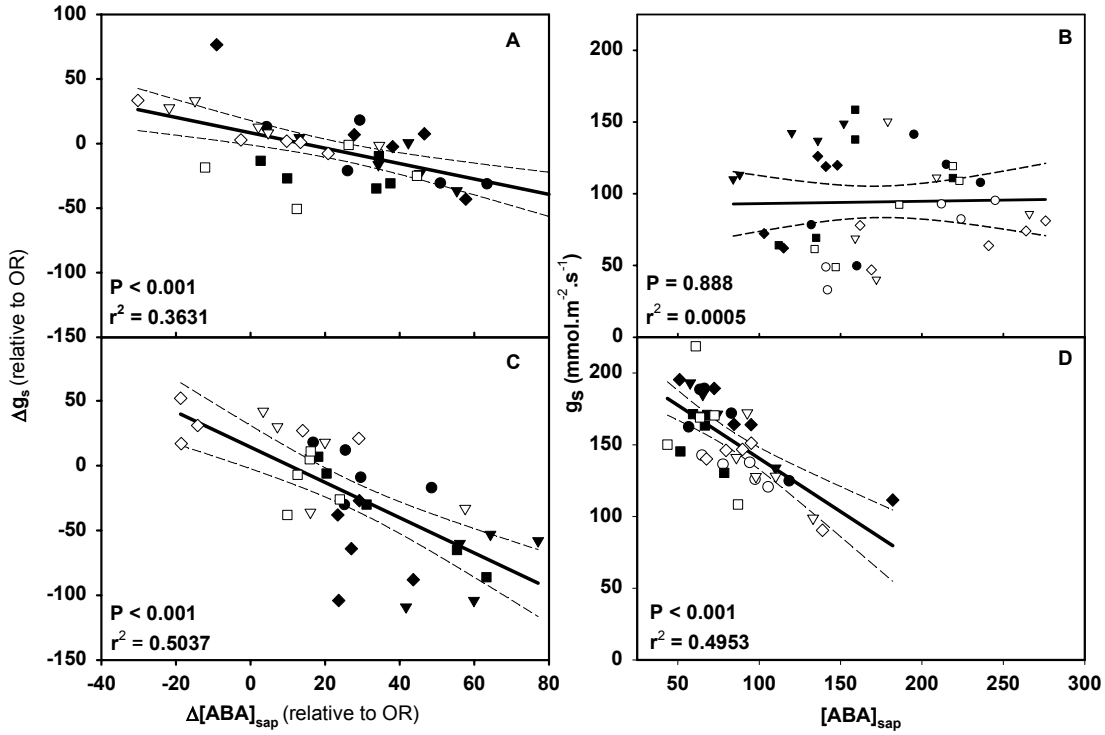

Figure 7. Relationship between sap ABA and stomatal conductance in the 01/02 (A, B) and 02/03 (C, D) growing seasons. A and C show the relationship between $[ABA]_{(grafted)}$-$[ABA]_{(own\ roots)}$ and $g_{s(grafted)}$-$g_{s(own\ roots)}$ for each rootstock whereas B and D show the absolute relationship between [ABA] and g_s. The solid lines represent the regression of the points in each panel with 95% confidence intervals indicated by the dashed lines. P and r2 values are given in the lower left corner of each panel.

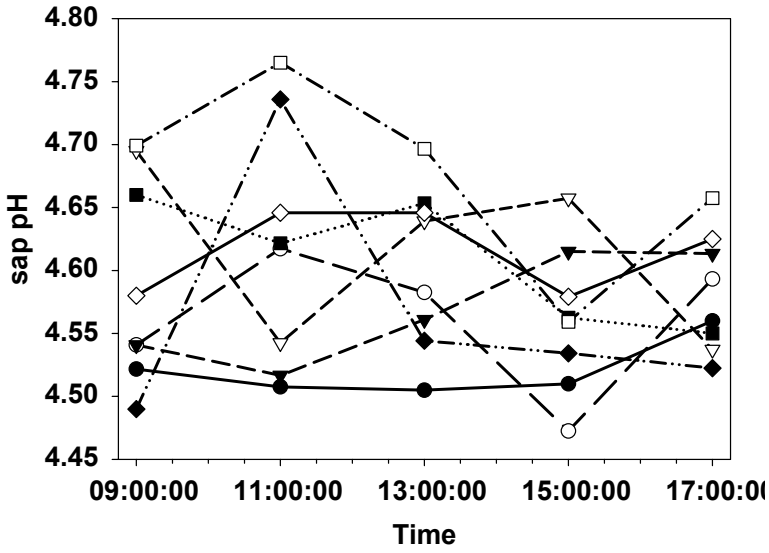

Figure 8. Diurnal change in xylem sap pH of Shiraz clone BVRC12 grafted to 420a (●), K51-40 (▼), Ramsey (▽), 140 Ruggeri (■), Schwarzmann (□), SO4 (◆), Teleki 5C (◇) and an ungrafted control (○) in February of the 01/02 (A) and 02/03 (B) growing seasons.

Sap pH

The pH of xylem sap extruded using the scholander pressure chamber was measured in 01/02 but not in 02/03. No consistent diurnal trend was observed in sap pH and there was no statistically significant difference between rootstocks (Fig 8). The pH of sap extracted by this method proved to be highly variable making it difficult to draw any conclusions from the data. For this reason sap pH was not measured in 02/03. Some rootstocks did display similar diurnal trends, for example Schwarzmann, Teleki 5C and Own Roots, however no relationship between pH and other physiological parameters could be made for any rootstock.

Pruning weights

Pruning weights were generally lower for all rootstock/shiraz combinations in 02/03 compared with 01/02 which was not unexpected given the lower water inputs in the second year of the trial. There were significant differences between the vines with the highest pruning weights and those with the lowest pruning weights in both seasons with the rankings being similar in the two seasons. (Fig 9). The lowest pruning weights were recorded for Shiraz on K51-40 and 420a in both seasons. Shiraz on Teleki 5C and Ramsey were in the top three highest pruning weights in both seasons. Shiraz grafted to Schwarzmann, SO4 and 140 Ruggeri were intermediate in both seasons not being significantly different to the highest or lowest pruning weight combinations. Shiraz on own roots was the only vine to change ranking significantly between the two seasons. Under the high input year of 01/02 Shiraz on own roots had the highest pruning weight, however when vines were stressed in 02/03 the own rooted control had the third lowest pruning weight.

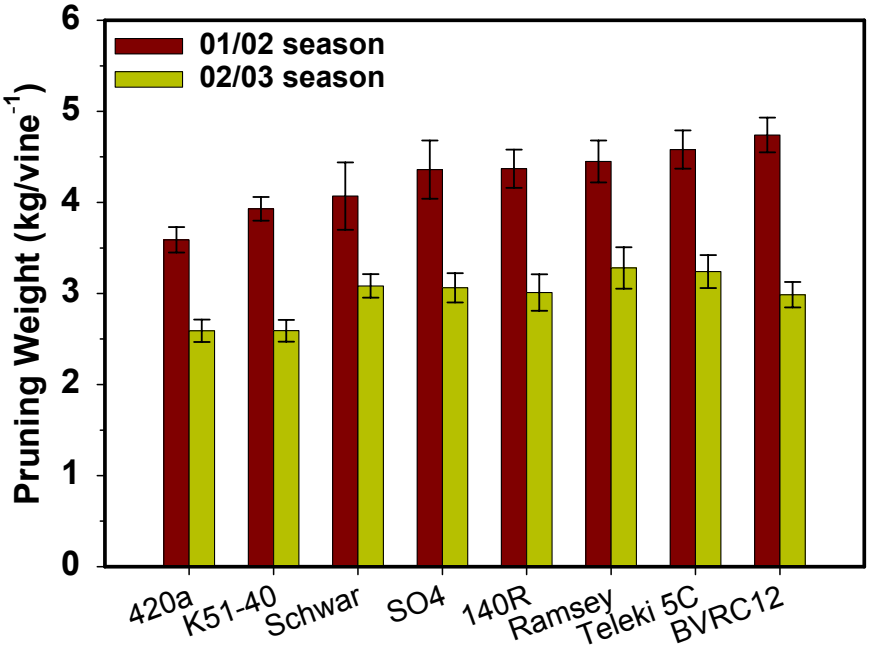

Figure 9. Pruning Weights of *Vitis vinifera* cv Shiraz (BVRC12 clone) grafted to 7 rootstocks and an ungrafted control in the measured in June 2002 and June 2003.

Root distribution studies on potted vines

Shiraz grafted to Ramsey and Own roots had significantly higher root biomass than when grafted to either 420a or Riparia Gloire (Fig 10*A*). Similarly the total length of roots greater than 2 mm diameter was significantly higher for Shiraz on Ramsey and Own roots compared with the other two rootstocks (Fig 10*B*). Shiraz grafted to Riparia Gloire had the lowest total length of roots greater than 2 mm of all rootstocks studied (Fig 10*B*). The mass of roots in each diameter category differed significantly between rootstocks such that Shiraz on Riparia had the highest mass of fine roots (<1 mm diameter) and the lowest mass of thick roots (>2 mm diameter) of all combinations. The distribution of root mass by class of Ramsey was not significantly different to Shiraz on own roots for any class. Shiraz on 420a was in between Shiraz on Ramsey and Shiraz on Riparia Gloire inasmuch as it had a higher mass of fine roots than Ramsey, but less than Riparia, lower mass of course roots (> 2mm) than Ramsey, but more than Riparia. The intermediate root size was unsurprisingly the least variable between rootstocks however there were still significant differences such that Shiraz on Ramsey had higher mass of 1-2 mm roots than Shiraz on 420a, and Shiraz on Own roots had higher mass of 1-2 mm roots than both Shiraz on 420a and Riparia.

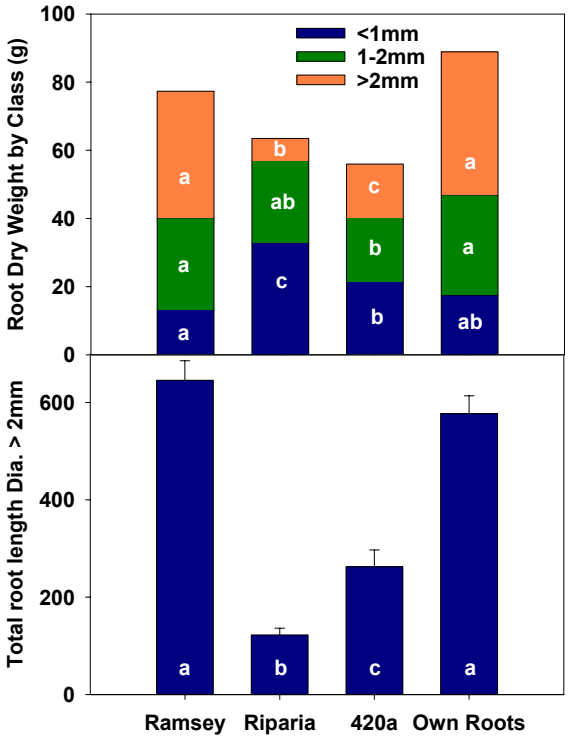

Figure 10. A) Dry weight of roots in different diameter classes and B) Total length of roots greater than 2mm in diameter from 4 different root genotypes (Ramsey, Riparia Gloire, 420a and Shiraz clone BVRC30), grafted to clonal Shiraz (BVRC30). White letters represent significant differences between bars of the same colour as determined by a one factor ANOVA and Fisher's Protected LSD (P<0.05).

A3 Discussion: Physiology of rootstock influence over scion water use

Choice of planting material for Australian vineyards is currently made on the basis of desired wine style and regional suitability of the selected varieties. Historically, differences in water requirements of selected varieties have been addressed through irrigation management. Continued growth of the wine industry in Australia is threatened by a limited supply of quality irrigation water. Declining water availability, coupled with water restrictions, rising water costs and a desire for continued industry growth are forcing the Australian wine grape industry to pursue methods to improve vineyard water use efficiency.

In this study, modification of planting material through the use of grafted vines has been assessed as a means to modify grapevine water use efficiency. Grafting different rootstocks to a single clone of Shiraz dramatically changed the responsiveness of leaf gas exchange to diurnal changes in evaporative demand (figs 1 and 2). For example

grafting of Shiraz to K51-40 resulted in a 39% decrease in leaf transpiration at 3 pm in 01/02 compared to ungrafted Shiraz (Fig 1a). This corresponded with a 44% reduction in leaf stomatal conductance at the same time of day (Fig 2a). The relative differences between graft combinations were smaller in 02/03 compared with 01/02 however Shiraz on K51-40 still had 32% lower leaf transpiration than the ungrafted control at 3 pm in 02/03 (Fig 2b). Thus in this trial it was possible to effect large changes in Shiraz leaf transpiration by grafting to different rootstocks.

Rootstock genotypes have been previously observed to alter the leaf gas exchange of grafted vines in some studies (Candolfi-Vasconcelos et al., 1994; Novello et al., 1997; Peterlunger et al., 1990) but not all (Gibberd et al., 2001). The difference between these studies however was that in the case where rootstock had no effect on gas exchange the vines were well watered whereas in the other studies, including the current study, the vines were grown under normal conditions and experienced some water deficit. This suggests that some degree of soil water deficit is necessary for the differences to be expressed.

A study in the U.S. used a weighing lysimeter to demonstrate that when Sultana vines were not subjected to any soil moisture stress, vine transpiration closely followed irradiance (Williams et al., 2003). A consequence of this was that the vines used very large amounts of water over a growing season, with transpiration closely coupled to atmospheric conditions as described by the Pennman Monteith model for plant transpiration. Under more typical irrigation, where plants frequently experience some degree of moisture deficit, transpiration of Sultana vines was not coupled to the atmosphere (Yunusa et al., 1997a; Yunusa et al., 1997b) suggesting vines require some degree of soil water deficit before the root to shoot signalling mechanisms controlling transpiration come into play. In this study differences between the gas exchange of the different rootstocks were observed in both years however even in 01/02, when water inputs were comparatively higher than 02/03 the vines were still exposed to atmospheric water deficits and soil moisture deficits that were imposed during an irrigation cycle as the vines were watered every 3 days. Thus the effects of rootstocks on vine gas exchange are best observed in the presence of water deficits.

In 01/02 there were significant differences in transpiration efficiency (A/E) at 9:00 and 11:00, but not in the afternoon. Likewise in 02/03 A/E varied greatly depending on graft combination (although not statistically significant) in the morning and much less in the afternoon. The absence of differences in A/E in the afternoon implied that in all combinations the effect of rootstock on E was proportional to the rootstock effect on A. It has been observed that decreases in g_s reduce E more than A to give a net increase in A/E (Condon and Hall, 1997) which is an important component in improving WUE. A study which compared the effect of water stress on photosynthesis in four species, Lupin, Eucalyptus, Sunflower and grapevine, found that in response to water deficits A decreased at a lower rate than g_s in all but grapevine where A and g_s decreased proportionately (Quick et al., 1992). Thus it was concluded that limitation of photosynthesis in response to water deficit in Lupin, Sunflower and Eucalyptus was purely stomatal, whereas in grapevine other non-stomatal limitations impact upon A when vines are under water stress.

Reduction in stomatal aperture can cause an increase in leaf temperature as a result of decreased evaporative cooling, thus increasing the leaf to air vapour pressure difference that drives transpiration. The result of this would be a smaller decrease in E than expected for a given decrease in g_s and thus little change in A/E. However this cannot explain a lack of increase in A/E where decreases in E are observed, as was the case in this study. Thus the smaller than expected increases in A/E in this study must be the result of greater than expected reductions in A. A is temperature sensitive with a temperature optimum of 30ºC found for Sultana grapevines (Kriedemann and Smart, 1971), thus as leaf temperature increases, as a result of increasing ambient temperature and loss of evaporative cooling by transpiration, A can become inhibited. This could further explain why there was less difference in A/E between graft combinations in the afternoon compared with the morning, as ambient temperatures were higher.

The photosynthetic capacity (A_{cap}) of grapevines, and other species have also been shown to be lower in the afternoon relative to the morning (During, 1991) again adding to the smaller differences in A/E in the afternoon. We should not ignore that there could also be differences in photosynthetic capacity between graft combinations; indeed this is likely as differences in A_{cap} between grapevine genotypes has

previously been observed (Gibberd et al., 2001). A graft combination that has lower E but also lower A_{cap} may not end up with better overall A/E (Condon and Hall, 1997).

Ψ_{leaf} changes as a positive function of water supply to the leaf and a negative function of the rate of E. Thus the negative correlation between E and Ψ_{leaf} that can be seen by comparing graphs 4a and b with graphs 1a and b, was expected. Assuming equivalent water supply by the various rootstocks, predawn water potential (not measured for this experiment) should have been the same for all combinations. Then in relative terms, as transpiration increased, combinations with higher E should have had lower Ψ_{leaf}. This was not the case for many of the graft combinations in this study, where higher Ψ_{leaf} accompanied higher E. This implies that water supply to the leaves of the different graft combinations was not the same and as such the different graft combinations would have been experiencing different degrees of stress.

All vines in the trial were watered concurrently on the same drip line, with calibrated drip rate and duration such that available soil moisture should have been equivalent, within the constraints of normal spatial variation, across the experimental site. Therefore any apparent differences in water supply can be attributed to the physiology of the graft combinations.

Differences in root architecture, specifically the suitability of the root to exploit available ground water, can have a sizeable effect on water uptake and thus supply to the canopy. The rootstocks used in this trial differed significantly in canopy pruning weight (Fig 9). Although these vines were hedge-pruned such that differences in leaf area were minimised, root growth was not artificially curbed. Grapevine root growth tends to be in balance with above ground growth (Richards, 1983; Southey, 1992; Southey and Archer, 1988) such that we might expect the more vigorous stocks to have larger root systems. Shiraz on Ramsey and Teleki 5C had the highest pruning weights in both seasons (Fig 9), and also had the highest transpiration, assimilation and leaf water potentials. Thus differences in root volume may be responsible for the rootstock effects on gas exchange and water status.

It is an oversimplification to simply discuss root "vigour" as root diameter, length and density may all influence efficiency of water uptake, and differences in these characteristics may be more or less of an advantage depending on soil environment and soil moisture availability. Differences in root distribution and structure have been observed in grapevine rootstocks grown in a variety of soil types (Southey, 1992; Southey and Archer, 1988; Williams and Smith, 1991). Whilst it has been observed that root distribution is largely dependant on edaphic conditions, and density on genotype (Southey, 1992; Williams and Smith, 1991) there is likely to be interaction of genotype with environment such that root growth will be heavily dependent on the suitability of a given root genotype to the environment, and will be subject to factors such as soil pH (Conradie, 1988), salinity (Southey, 1992; Southey and Archer, 1988), type and structure (van Huyssten, 1988; van Zyl, 1988), presence of diseases and pests (de Clerk and Loubser, 1988; Marais, 1988), and water availability (van Zyl, 1988).

In the present study, differences in the inherent root structure of some of the rootstocks used in the field trial were shown for potted vines. The same trends were observed between ungrafted rootstocks (data not shown) and rootstocks grafted to Shiraz (Fig 10) indicating a dominant influence of rootstock genotype on root architecture. Thus it can be concluded that there are differences in root structure of different rootstocks that may contribute to the observed rootstock effects on vine water status in the field. However the potential of rootstocks to reach their growth potential under physical and chemical restraints imposed in different locations, and the advantages conferred by specific root structures needs further study.

Differences in the transport of water within the plant may also affect water supply to the leaves. Variation in hydraulic conductivity has been observed between ungrafted pot-grown grapevine rootstock clones (Peterlunger et al., 1990) where rootstock canopy vigour was observed to be proportional to root hydraulic conductivity. The rootstock/scion combinations in this study appeared to have greater capacity to supply water to the shoots also had the highest winter pruning weights (Fig 9). Unfortunately the trial used for the present study lacked a self-grafted control, a common failing of this type of field study, and so we cannot deduct graft union effects from our results. However as long as the effects of grafting are consistent for a given rootstock then it

does not impact upon our observations as graft compatibility may still be considered a rootstock effect.

The role of ABA in regulating stomatal aperture has been well documented (Hartung and Heilmeier, 1993; Loveys, 1984; Loveys and Kriedemann, 1974), and xylem sap ABA concentration has been shown to correlate with stomatal closure better than bulk leaf ABA (Zhang and Davies, 1990). In this study an inverse relationship was observed between the relative differences in ABA of a graft combination compared with the ungrafted control versus the relative difference in g_s of the same graft combination compared with the control in both seasons (Fig 7a and 7c). Data points corresponding with the various time points for a particular rootstock tended to cluster together such that rootstock/scion combinations that had higher $[ABA]_{sap}$ than the ungrafted control tended to have lower g_s and vice versa. The correlation between Δg_s and $\Delta[ABA]_{sap}$ was stronger when vines were more water stressed, that is in 02/03.

In terms of absolute ABA concentration and absolute G_s there was no correlation in 01/02 (7b), but a much stronger correlation in 02/03 (Fig 7d). Thus there was distinct seasonal variation in the dependence of stomatal aperture on xylem sap ABA concentration. In 01/02 the vines in the trial showed very little evidence of water stress maintaining highly vigorous canopies throughout the season compared to 02/03.

The leaf water potential values were much lower in 02/03 (Fig 5b) than in 01/02 (Fig 5a) across all combinations. From our data it appears that stomatal sensitivity to ABA was increased when plants were under water stress because the absolute $[ABA]_{sap}$ were lower in 02/03 although the stomatal response to these lower concentrations was greater. This could be due to differences in stress dependent ABA compartmentation in the leaf blade (Zhang et al., 1997) or due to a more direct link between leaf water status and ABA in determining stomatal aperture (Tardieu and Davies, 1992; Tardieu and Davies, 1993).

There is no evidence from the data presented here that any rootstock in this trial had greater capacity to synthesize ABA, than another and to thereby have greater control over water use and higher water use efficiency under well watered conditions. Rather

the elevated $[ABA]_{sap}$ measured for Shiraz grafted to rootstocks such as K51-40, 420a, Ruggeri 140 and SO4 are likely to be due to higher levels of water stress in these combinations, generated by a lower capacity of these rootstocks to supply water to the shoots than Teleki 5C, Ramsey, Schwarzmann and Own Roots in this particular soil type and management system.

This raises the question of whether ABA is coming from the scion and not the stock or that the roots are induced to produce more ABA as a result of changed water relations.

Conclusions

Grafting of rootstocks to commercial varieties of grapevine has the potential to significantly alter the water relations of the whole vine, but not necessarily the transpiration efficiency or water use efficiency. Water use, vigour, assimilation and thus yield will all be influenced by the rootstock's genetic predisposition to extract water from the rhizosphere as well as its suitability to the vineyard soil environment. In this study, reductions in stomatal conductance were accompanied by increases in xylem sap ABA when plants were under stress, but less so when vines were well watered. Differences between rootstocks in terms of gas exchange seem to be the result of different abilities of the rootstocks to supply water, more specifically that rootstocks that endowed lower transpiration and assimilation rates simply had lower capacity to supply water from the soil. The combination of lower leaf water potential and increased ABA concentration resulted in decreases in stomatal conductance and thus transpiration. Improvements in A/E were not observed, as reductions in E were stress related and were accompanied by equivalent decreases in assimilation rate. Xylem sap ABA may be a useful and rapid screen for the relative drought tolerance of rootstocks, providing the vines are subjected to a quantifiable stress prior to sampling. A final word of caution however, that field assessment of rootstocks needs to be performed in a diverse collection of soil types and environments as growth of rootstocks may be limited by the physical and chemical compatibility of the rootstock to the soil.

B2 Results: Physiology of scion varietal responses to water deficit

Gas exchange of ungrafted Chardonnay, Shiraz and Grenache

At low VPD leaf stomatal conductance remained relatively constant in all cultivars over the measured time period (Fig 11). G_s was significantly lower all times in Grenache compared with both Shiraz and Chardonnay which were not significantly different from each other. On days of high VPD stomatal conductance was dramatically lower than at low VPD for all cultivars (Fig 11). As at high VPD, g_s did not change significantly during the day for Chardonnay whilst there was a small yet significant decline in conductance during the day for Shiraz and Grenache. In the morning there was no significant difference between Shiraz and Chardonnay at high VPD, however by 1300 h Shiraz had significantly lower g_s which was maintained for the remainder of the day. Grenache g_s was significantly lower than both Shiraz and Chardonnay g_s at all time points.

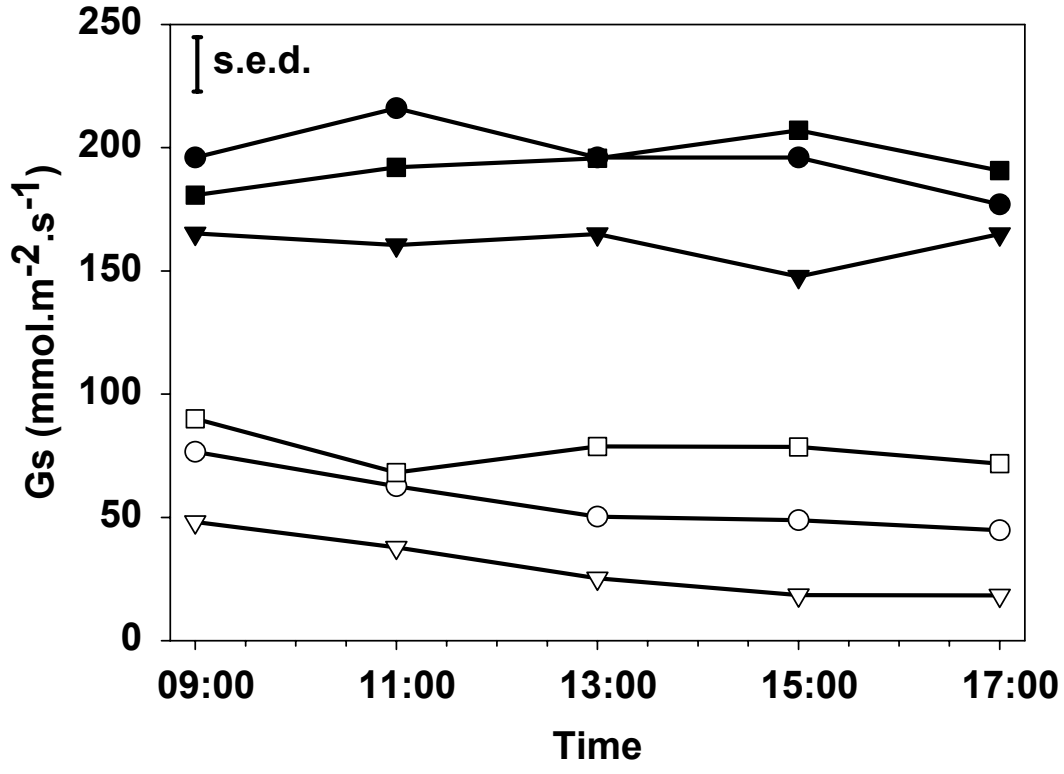

Figure 11. Diurnal change in stomatal conductance during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD in December 2002.

As observed for g_s there was no significant difference in transpiration rate between Shiraz and Chardonnay on days of low VPD (Fig 12), however transpiration rates for Grenache were significantly, although only slightly, lower than the other two cultivars. This corresponded with the lower g_s for Grenache (Fig 11). Transpiration rate increased throughout the day for all cultivars at both high and low VPD despite the lack of change in g_s. Under high VPD transpiration rates for Shiraz were almost identical to that at low VPD with the exception of the 9:00 h time point where the transpiration rate was higher at high VPD (Fig 12). In contrast transpiration rates in Chardonnay were much higher at high VPD compared with low VPD, and in Grenache transpiration was dramatically reduced at high VPD compared with low VPD. At high VPD, Grenache leaf transpiration was decreased to an extent where it was constant throughout the day. There was no significant difference between the leaf area of Chardonnay (9.63 m^2) and Grenache (9.6 m^2), however Shiraz leaf area was on average 13-14% lower (8.32 m^2) than the other two varieties. Due to the large differences observed in leaf transpiration there is no impact of the variations in leaf area on the significance of the differences shown in Figure 12.

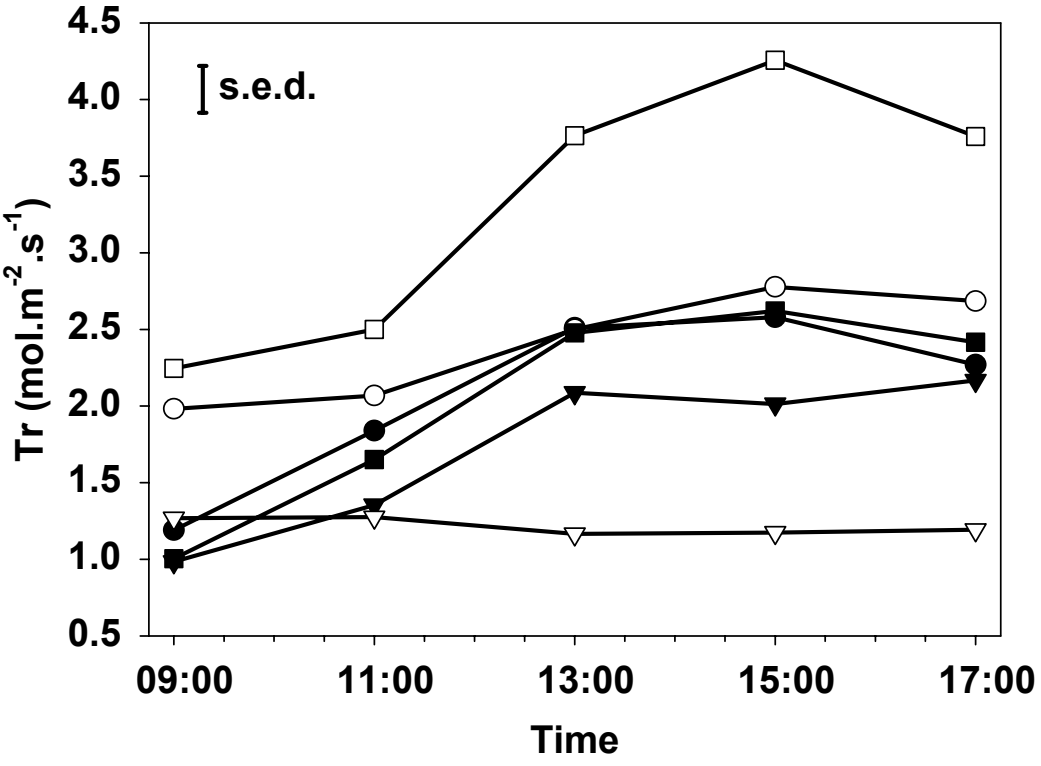

Figure 12. Diurnal change in leaf transpiration rate during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD in December 2002.

Leaf CO_2 assimilation rate was very closely linked to g_s for all varieties at both low and high VPD (Fig 13, c.f. Fig 11). There was no significant difference in assimilation rate between any two varieties at low VPD, with the exception of Grenache versus Shiraz at 11:00. Whilst generally not significant Grenache assimilation was consistently lower than both Shiraz and Grenache throughout the day (Fig 13), as was expected from the lower g_s observed for this variety (Fig 11). At high VPD Grenache assimilation was significantly lower than Chardonnay at all time points, and Shiraz at 9:00, 11:00 and 13:00. Shiraz assimilation was consistently lower than Chardonnay in the afternoon although the difference was only significant at 17:00.

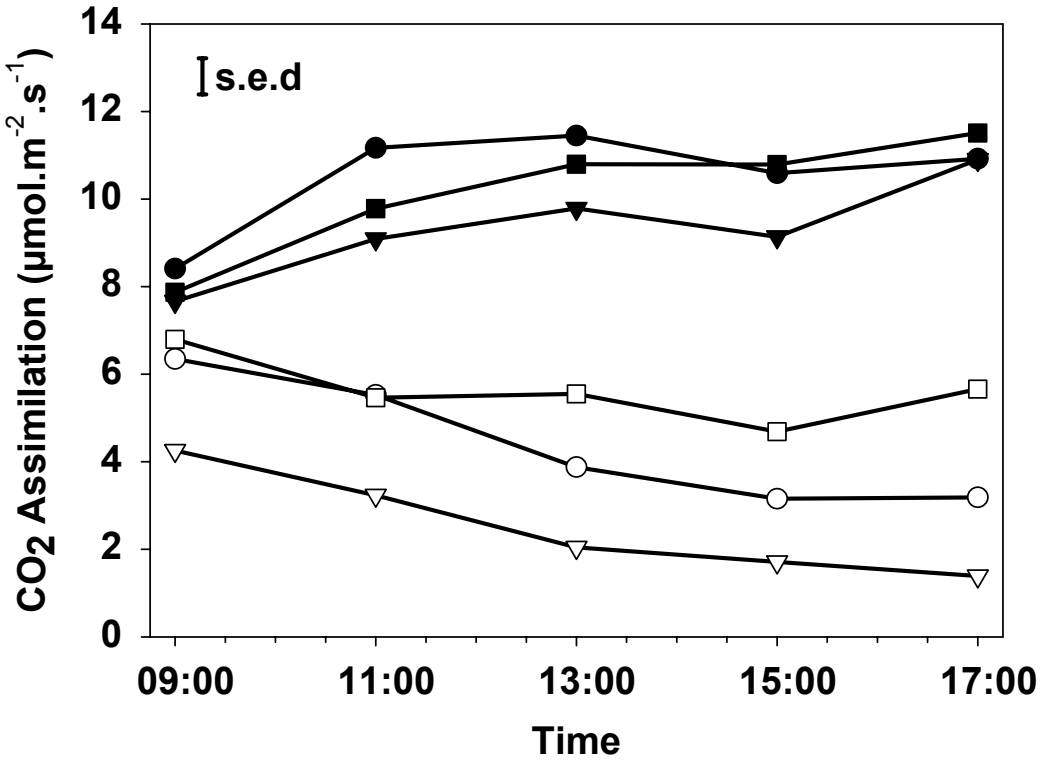

Figure 13. Change in leaf CO_2 assimilation rate during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD.

Despite the dramatic differences in transpiration rate between varieties particularly at high VPD, there was no significant difference in leaf transpiration efficiency (A/E) as decreases in transpiration were matched by changes in assimilation for all three

varieties (Fig 14). There was a significant difference in transpiration efficiency between high and low VPD days, whereby transpiration efficiency was much lower on days of high VPD.

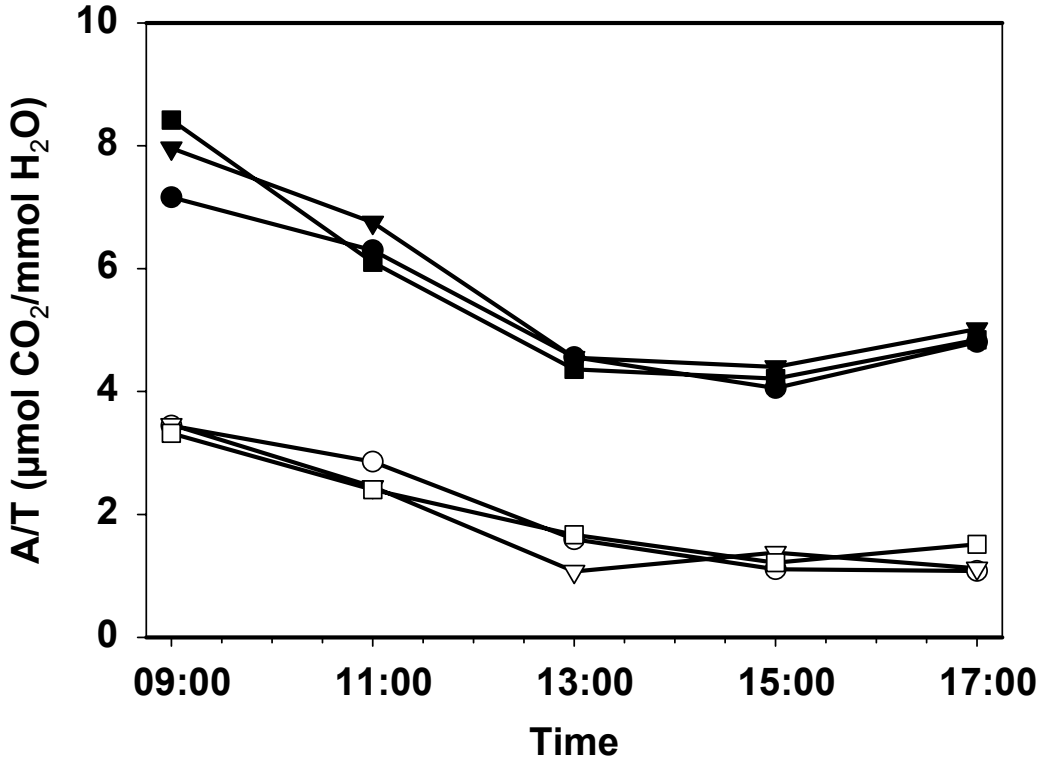

Figure 14. Diurnal change in leaf A/E ratio during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD.

Leaf water potentials

Leaf water potential declined as transpiration rates increased for all cultivars on the low VPD days (Fig 15) with no significant difference between cultivars except between Shiraz and Chardonnay at 17:00 where Shiraz had significantly lower potential. Overall, Ψ_{leaf} dropped for all varieties from the low VPD to high VPD conditions. On the high VPD days leaf water potential also seemed to decrease in proportion with increasing transpiration (Fig 15). Thus the lower transpiration rates observed for Grenache at high VPD relative to the other varieties resulted in conservation of higher leaf water potential. At high VPD there was no difference in leaf water potential between Shiraz and Chardonnay despite the much higher transpiration rates of the Chardonnay leaves.

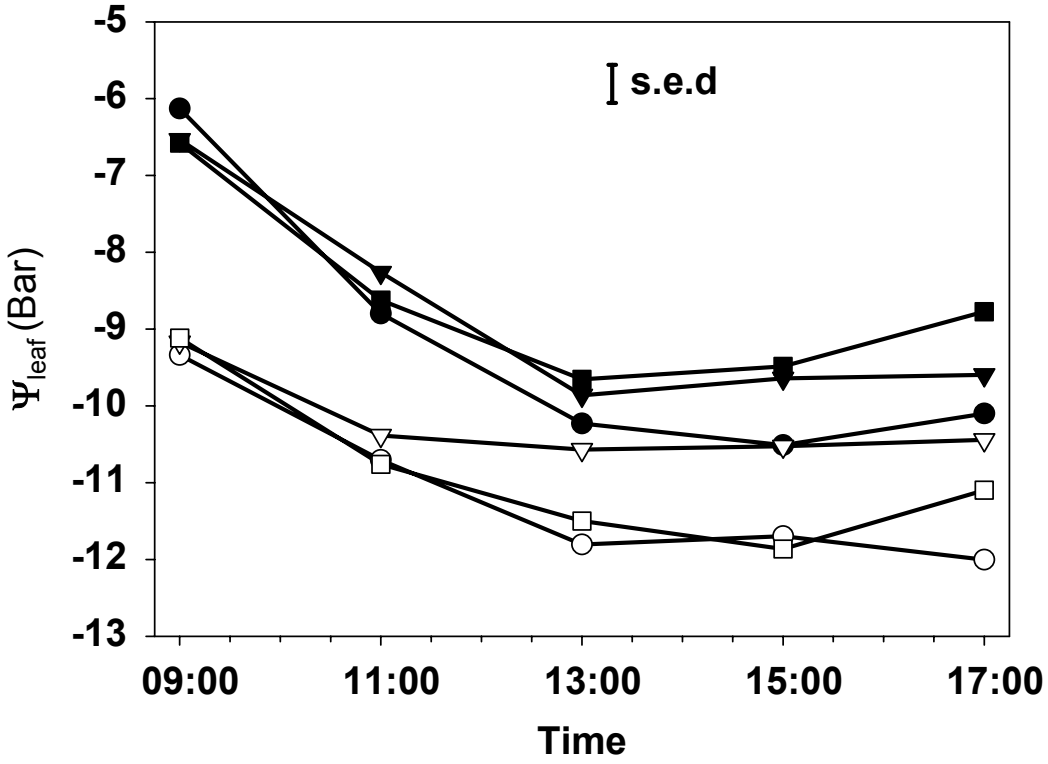

Figure 15. Diurnal change in Ψ_{leaf} during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD.

Xylem Sap ABA

There was no difference in xylem sap ABA between cultivars on low VPD days (Fig 14). Xylem sap ABA increased as expected during the day as leaf water potential decreased for all varieties. $[ABA]_{sap}$ was much higher for all varieties at high VPD with concentrations at 9:00 being very similar to those at 17:00 on low VPD days. The steady increase observed on low VPD days was not apparent at high VPD. $[ABA]_{sap}$ did not change significantly through the day in Shiraz. In Chardonnay the concentration decreased from 9:00 to 13:00 such that by 13:00 the concentration was significantly lower than that of Shiraz and Grenache, but then jumped to similar levels as that observed for the other varieties. Grenache was the only variety that showed an increase in ABA concentration at high VPD but due to the high variability in sap ABA at high VPD the change was not significant. Grenache had significantly ($P<0.05$) higher $[ABA]_{sap}$ than Shiraz at 15:00 (and just insignificant at 13:00) and higher than Chardonnay at 13:00. Likewise Shiraz had higher $[ABA]_{sap}$ than Chardonnay at 13:00. All other differences were not statistically significant.

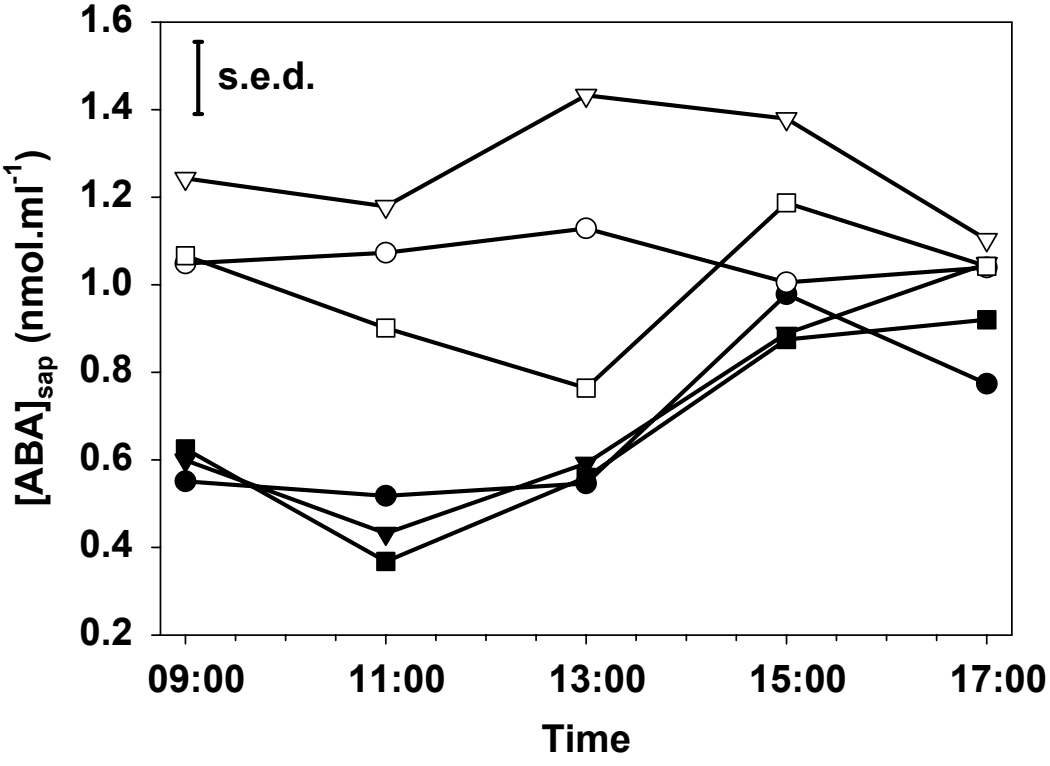

Figure 16. Diurnal change in $[ABA]_{sap}$ during the day of three varieties of *V. vinifera* (Shiraz (●○), Grenache (▼▽) and Chardonnay (■□)) on days of high (open symbols) and low (closed symbols) VPD in December 2002.

Berry development and ripening

Grenache berry development was consistently behind that of Shiraz throughout the season in terms of Total Soluble Solids (Fig 15*B*), juice pH (Fig 15*C*) and juice titratable acidity (Fig 15*D*) however the rate of change was the same for both varieties. Shiraz berries ceased rapid growth earlier than Grenache (11/1/04 compared with 4/3/04) and Grenache attained much higher final berry weights. Despite lower berry sugar concentration (TSS) Grenache had higher sugars per berry in the second half of the ripening period by virtue of the much higher berry weight. The Shiraz was harvested on April 7th 2004 at 26.3 ºBrix, whilst the Grenache was not ready to be harvested until April 29th 2004 with TSS at harvest of 26.1 ºBrix. Grenache yielded 30% higher kg/vine ($P<0.001$) with a mean yield of 14.76 kg/vine compared with only 11.25 kg/vine for Shiraz.

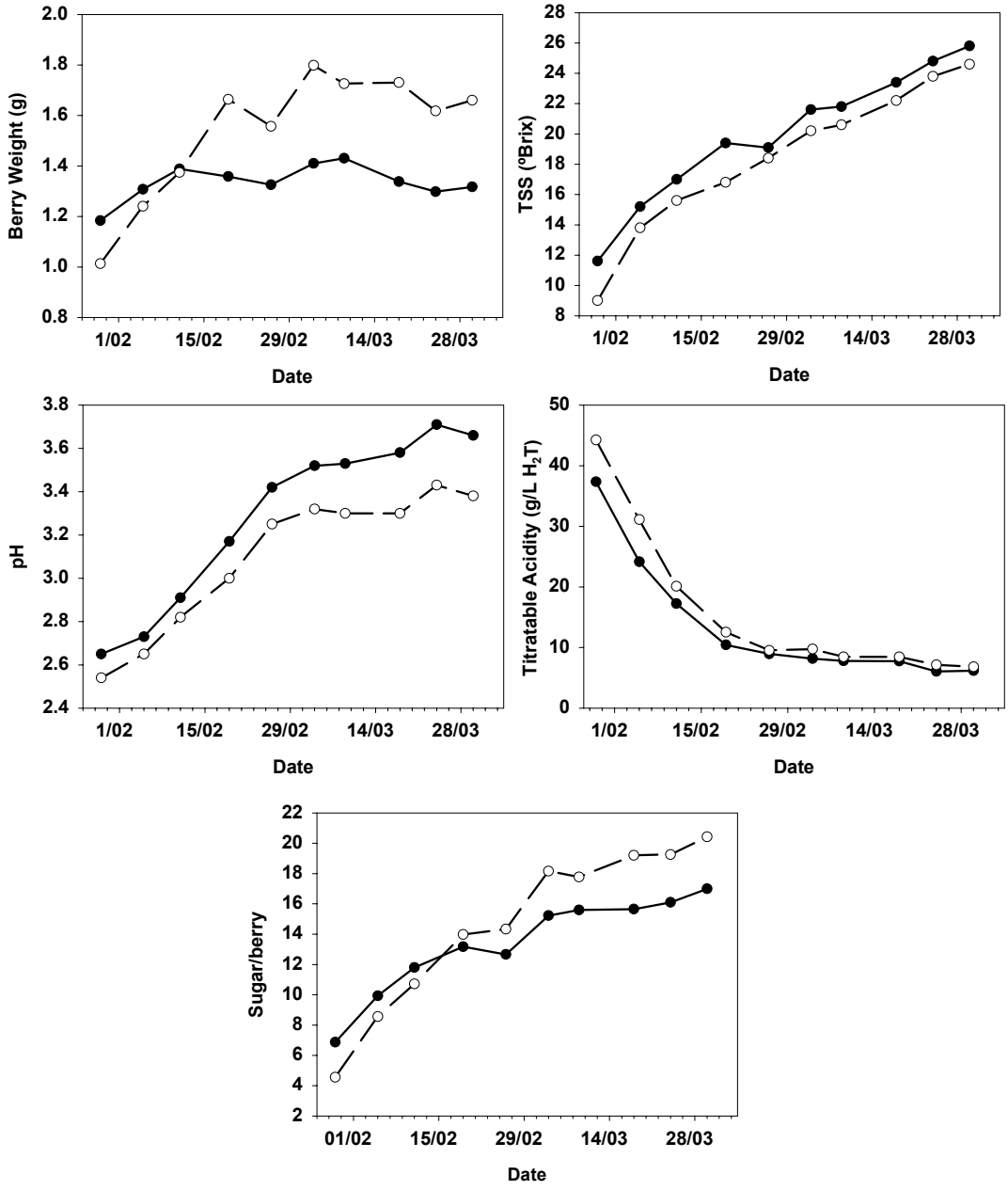

Figure 17. Berry maturation data including berry weight (A), TSS (B), juice pH (C), juice titratable acidity (D) and sugar/berry (E), for Shiraz (●) and Grenache (○) in the 03/04 season. Measurements were made on a pooled 50 berry sample taken randomly across 12 vines for each variety.

B3 Discussion: Physiology of varietal responses to water deficit

In late December 2002 we were fortunate to experience unusual weather patterns in which dramatic swings in temperature occurred in quick succession. Maximum daily temperatures of 40ºC (RH < 20%) were followed by days of 23ºC (RH > 40%) and vice versa. These rapid swings in climatic conditions allowed us to compare the

response of three common grapevine varieties (Chardonnay, Shiraz and Grenache) to extremes in evaporative demand in the vineyard. In a French study, a difference in the response of Shiraz and Grenache to soil moisture deficit had been reported (Schultz, 1997), however the two varieties were not grown on the same rootstock and thus it was not clear whether the differences were scion or rootstock derived. In contrast, the current study assessed the performance of these two varieties and a third, Chardonnay on their own roots, under equal soil moisture in response to significant changes in air/leaf vapour pressure deficit. Scion responsiveness to atmospheric stimuli may prove to be an important component in determining plant water use efficiency. For example there is now some evidence that Partial Rootzone Drying may in part be successful by sensitizing the aerial parts of the grapevine to changes in evaporative demand (Loveys et al., 2004).

The data produced in this project clearly shows that Shiraz, Grenache and Chardonnay differ significantly in response to changing VPD. Under cool non stressful conditions (VPD ~ 1.5 kPa) there were only small differences between the three varieties studied. Shiraz and Chardonnay performed nearly identically at low VPD, whereas Grenache maintained slightly lower rates of g_s, transpiration and CO_2 assimilation. When atmospheric conditions became stressful (VPD ~ 5.8 kPa) very large differences became evident between the three varieties. Grenache was the most conservative or pessimistic of the three, by maintaining significantly lower g_s and thus lower transpiration rates at high demand. In Grenache g_s continued to decrease during the day, maintaining a steady, yet very low transpiration rate of ~1.3 $mmol.m^{-2}.s^{-1}$ throughout the day. Shiraz was considerably less conservative than Grenache inasmuch as it maintained higher g_s, transpiration and assimilation levels under the high stress conditions, however a distinct depression in g_s was still observed indicating that Shiraz was still acting to conserve water. In contrast to both Shiraz and Grenache, Chardonnay was relatively optimistic in its response to the sudden increase in VPD. Whilst the stomatal conductance of Chardonnay at high VPD was much lower than at Low VPD, the absolute values were still higher than the other two varieties, for example at 13:00 Chardonnay g_s was nearly three times that of Grenache and 45% greater than Shiraz. Schultz (Schultz, 1997) did not observe the same degree of difference between Shiraz and Grenache in diurnal transpiration rate as was observed in this study, even under soil moisture stress, however the VPD on the day

the measurements were taken was not reported. However when leaves were detached at the petiole from field vines and subjected to a rapid dry down differences in g_s between Shiraz and Chardonnay were similar to what was observed in the current study (Schultz, 1997). Both studies indicate that Grenache leaves are more sensitive to a drop in water potential than Shiraz.

The change in $[ABA]_{sap}$ through the day was very different at high and low VPD for each variety. In general terms $[ABA]_{sap}$ at low VPD increased as leaf transpiration increased and leaf water potential decreased for each variety. There was no consistent diurnal trend in $[ABA]_{sap}$ at high VPD with all varieties showing much higher ABA at the first three time points compared with at low VPD. The inverse relative differences in $[ABA]_{sap}$ between Shiraz and Grenache related well to the relative differences in transpiration rate and leaf water potential between these varieties suggesting a causative role for ABA in the differential response of these two varieties to VPD. $[ABA]_{sap}$ in Chardonnay was highly variable and whilst lower than Shiraz and Grenache in the morning, it was higher than Shiraz in the late afternoon. The high $[ABA]_{sap}$ in Chardonnay in the afternoon may be a response to the very high transpiration rates for this variety and may well be the cause of the slight decrease in transpiration (Fig 12) and increase in Ψ_{leaf} (Fig 15) observed between 15:00 and 17:00.

Grenache leaf water potential was maintained at a higher level than Shiraz at low VPD and both Shiraz and Chardonnay at high VPD. This can be directly linked to the lower rates of transpiration of Grenache compared with the other two varieties. Chardonnay however maintained higher leaf water potentials than Shiraz at high VPD despite having a ~70% higher transpiration rate. It is possible that Chardonnay at the Nuriootpa field site has higher hydraulic conductivity that Shiraz (and possibly even Grenache) which may be related to root architecture or the inherent conductivity of the water conducting elements with the different vine organs.

Decreases in CO_2 assimilation rate observed for all varieties were proportionate to the decreases in transpiration, and as such no variety had inherently higher transpiration efficiency at the leaf level.

In Australian vineyards Grenache usually ripens later than Shiraz (M.G. McCarthy pers comm.) and this study was no exception. Whilst Grenache was consistently behind Shiraz in terms of TSS, TA and pH, the actual rates of ripening were nearly identical in the two varieties with the major difference arising prior to veraison (before measurements commenced). Thus reduced carbon gain through CO_2 assimilation on hot dry days does not seem to have any negative impact on fruit development in Grenache. Furthermore, the fact that Grenache yielded 30% more than Shiraz, had larger berries with higher total sugars per berry suggests that more carbon is partitioned into the fruit of Grenache than Shiraz. Berry weight and ripening have been shown to be susceptible to post veraison water stress in some grapevine varieties (Esteban et al., 1999; McCarthy, 1997). Thus looking from an alternative perspective it is possible that higher degrees of water stress in Shiraz, as a result of less stringent control over g_s, may have impacted more negatively upon fruit development in Shiraz compared with Grenache in this study. Further work is required to establish a direct link between relative vine water status and berry development between these varieties.

C2 Results: Origins of ABA gradients in grapevine shoots

Differences in sap ABA have been observed throughout this project between shiraz grafted to different rootstocks and also between different ungrafted scion varieties. However it is not possible from the conventional ABA quantitation used in these studies to determine the origin of these ABA signals. Whilst it is clear in the rootstock experiments that differences in $[ABA]_{sap}$ are rootstock derived (the scions were genetically identical), it is possible that the observed differences in $[ABA]_{sap}$ were the results of ABA synthesis in the shoots in response to chemical, or more likely hydraulic signals arising from the roots. Using a combined molecular and physiological approach in collaboration with Jim Speirs (CRV 01/05), the source of gradients in $[ABA]_{sap}$ giving rise to gradients in stomatal conductance along a shoot were studied. The aim of these experiments was to determine the likely source of the ABA, to further our understanding of the key sites of ABA synthesis in plants responsible for stomatal regulation, i.e. the roots or the shoots?

Gradients in stomatal conductance and sap ABA in glasshouse-grown plants

Significant gradients (P<0.001) in stomatal conductance were observed along canes of glasshouse-grown Shiraz that were inversely related to similar significant gradients (P=0.015) in $[ABA]_{sap}$ collected from petioles at corresponding node positions (Fig 16*A* and 16*B*). A regression fitted to $[ABA]_{sap}$ versus g_s indicated an inverse first order relationship between $[ABA]_{sap}$ and g_s ($P = 0.025$, $r^2 = 0.95$). The gradients in $[ABA]_{sap}$ leading away from the apex suggested that the apex may be the source of the sap ABA. The sap flow rate, measured by volume of sap collected per min, was different depending on position on the shoot with the flow rate close to the apex being approximately double the flow rate at the lowest node position (data not shown). This implies that there may be differences in petiole hydraulic conductivity along the stem, however as the flow rate was highest near the apex, where the $[ABA]_{sap}$ was also highest, we can conclude that the observed gradients are not the result of dilution by water in the transpiration stream.

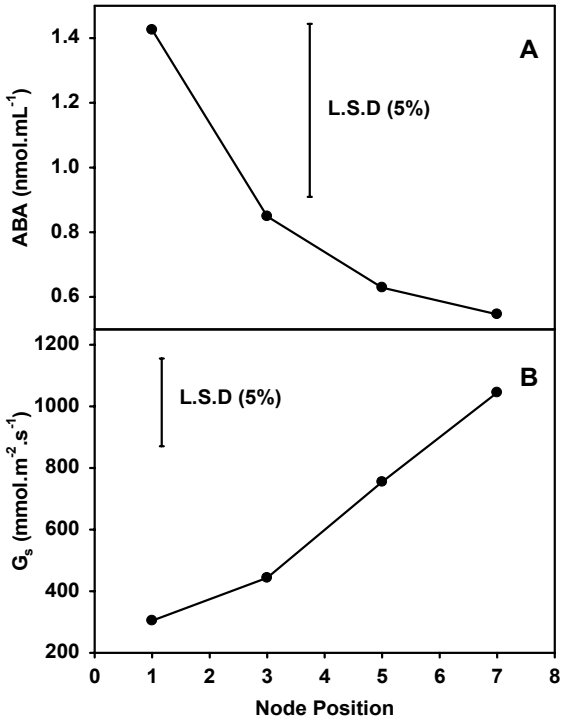

Figure 18. Gradient in xylem sap ABA (A) and leaf stomatal conductance (B) of glasshouse-grown Shiraz. Values are means of five replicates and the error bars represent Fisher's protected LSD ($P \leq 0.05$).

Stomatal conductance and leaf ABA concentration in field-grown vines

In field grown Shiraz similar increasing gradients of stomatal conductance were observed over the first eight leaves in all treatments ($P<0.001$). Neither decapitation nor girdling had any statistically significant effect on stomatal conductance within the first 24 h or 48 h of treatment (Figs 17*A* and 17*B*). While the increasing stomatal conductances were inversely correlated with gradients in $[ABA]_{leaf}$ in leaves three to eight in all treatments at 24 h and 48 h (All inverse first order regressions had $P \leq 0.0035$ and $r^2 \geq 0.59$), this relationship was not evident in the younger leaves. In these leaves ABA levels either increased in leaves one though three (24 h) or remained approximately constant (48 h) in the control canes (Fig 18*A* and 18*B*). Girdling and decapitation treatments resulted in elevated $[ABA]_{leaf}$ in the younger leaves ($P = 0.015$) at both 24 and 48 h with the increases in ABA levels being greatest closer to the sites of injury.

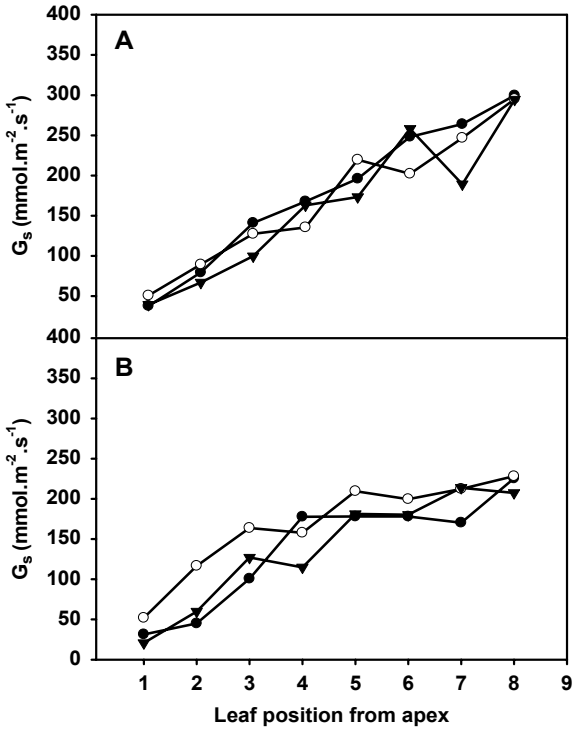

Figure 19. Stomatal conductance of field-grown Shiraz leaves at different node positions 24 (A) and 48 h (B) after treatment. Treatments were control (●), decapitated (○) and girdled (▼). A significant gradient in stomatal conductance ($P<0.001$) was found for the influence of leaf position on stomatal conductance on both days however there were no significant treatment effects on the observed trends.

The ABA content of the apical tissue was measured for both the control and girdled treatments 24 and 48 h after treatment (Fig 18*A* and 18*B* - inset). The ABA concentrations in the apex were higher than were observed for the more mature leaf tissue. There was no significant difference in $[ABA]_{apex}$ between treatments at 24 h, however at 48 h the $[ABA]_{apex}$ in the girdled treatment was approximately three times higher ($P<0.001$) than the control (Fig 18*A* and 18*B* - inset).

The ABA content of the apical tissue was measured for both the control and girdled treatments 24 and 48 h after treatment (Fig 18*A* and 18*B* - inset). The ABA concentrations in the apex were higher than were observed for the more mature leaf tissue. There was no significant difference in $[ABA]_{apex}$ between treatments at 24 h, however at 48 h the $[ABA]_{apex}$ in the girdled treatment was approximately three times higher ($P<0.001$) than the control (Fig 18*A* and 18*B* - inset).

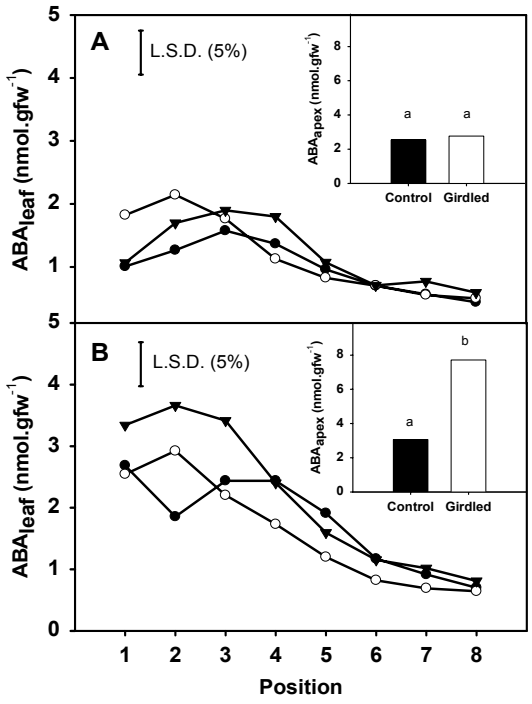

Figure 20. Bulk-leaf ABA concentration of field-grown Shiraz leaves at different node positions 24 (A) and 48 h (B) after treatment, and in the apical tissue (inset.). Treatments were control (●), decapitated (○) and girdled (▼). The Fisher's protected L.S.D indicates significant differences between the three treatments at each individual leaf position on the two days respectively ($P<0.05$) (see statistics section in Materials and Methods). Statistically significant differences ($P \leq 0.05$) between $[ABA]_{apex}$ are indicated by different letters (inset).

After 24 h, small increases in $[ABA]_{leaf}$ relative to controls were found in leaves one and two following decapitation and in leaves two, three and four following girdling. All treatments showed elevated levels of ABA in the younger leaves after 48 h compared with 24 h, possibly due to slightly changed environmental conditions over the two days as the conditions at 48 h (VPD = 1.08 kPa) were warmer and drier than at 24 h (VPD = 0.32 kPa). The enhanced ABA levels of the decapitated and girdled treatments relative to the control were maintained in the decapitated and were enhanced and shifted from leaves two, three and four to leaves one, two and three in the girdled treatment.

Expression of the key genes in the ABA biosynthetic pathway, NCED and Zep
See appendix 5 for detailed results of the molecular work.

In control leaves at 24 h there was a low abundance of *NCED* mRNA in the apical tissues, and an increased abundance in leaves 1 through 5 decreasing again in leaves 7 and 8. A similar profile was evident in control leaves after 48 h but with reduced abundances overall. There appeared to be a loose relationship between *NCED* mRNA abundance and $[ABA]_{leaf}$ in both the 24 h and 48 h treatments, but no obvious association with the higher overall $[ABA]_{leaf}$ observed in the 48 h treatment.

In the girdled treatment *NCED* mRNA levels at 24 h were similar to the levels observed in the control treatment. At 48 h however there was a clear increase in abundance of the *NCED* mRNA in the apex and a lesser increase in leaves 1 and 2 relative to the control leaves at this time. These increases in mRNA at 48 h were consistent with the observed increases in $[ABA]_{leaf}$ in the corresponding leaves.

In the decapitation treatment the profile of *NCED* mRNA levels was similar to that in the control treatment but with an increased abundance of the mRNA in leaves 1, 2 and 3 after 24 h which returned to control levels after 48 h.

A gradient of *Zep* mRNA abundance was observed in all treatments increasing from apical tissue to leaf 8. These reverse gradients, in comparison with the *NCED* mRNA abundances, do not appear to be related to [ABA] in the respective tissues. A general

increase in *Zep* mRNA abundance was evident in leaves of plants that underwent the decapitation treatment after 24 h and 48 h.

C3 Discussion: Origins of ABA gradients in grapevine shoots

ABA gradients along shoots have been reported in cotton leaves (Guinn and Brummett, 1993), castor bean xylem sap (Jokhan et al., 1999) and apple buds (Taylor et al., 1984). To our knowledge ABA gradients of this type have not been reported previously for grapevine. In our initial experiment we observed a pronounced gradient in $[ABA]_{sap}$ of grapevine, decreasing from the apex to leaf seven (Fig 16*A*).

The gradient in $[ABA]_{sap}$ was inversely correlated to a gradient in stomatal conductance (Fig 16*B*) in the leaves of the glasshouse grown grapevines. In the field experiment, gradients in leaf ABA (Fig 20), as opposed to sap ABA, and corresponding inverse gradients in leaf stomatal conductance (Fig 19) were observed in all treatments, and reflected those observed between $[ABA]_{sap}$ and g_s in the glasshouse vines. There was no significant effect of treatment on the gradients in stomatal conductance however there was a significant effect of time ($P = 0.006$) where the stomatal conductances were in general lower after 48 h than at 24 h especially in the more mature leaves. This decrease in g_s was not associated with a corresponding increase in $[ABA]_{leaf}$ at 48 h. However, during the same period a slight change in weather conditions resulted in a rise in VPD of approximately 0.7 kPa and consequently increased evaporative demand on the leaf. An associated drop in leaf water potential is likely to have made the stomata more sensitive to ABA at 48 h (Tardieu et al., 1993).

The expected inverse relationship between $[ABA]_{leaf}$ and g_s did not hold between node positions one to three, perhaps due to leaf immaturity. Leaves 1 and 2 were unlikely to be fully photosynthetically active (Kriedemann, 1968) and as such the stomates may not have been responsive to the same stimuli as in more mature leaves. The basipetal direction of the ABA gradient in both leaf and sap suggested the apical tissue as a logical source of the ABA. To test this, we looked at the effects of removal of the apical tissues and found that this did not significantly decrease [ABA] in the

subtending leaves (Fig 18*A* and *B*). ABA metabolism has been shown to be rapid in leaf mesophyll (Daie et al., 1984; Jia and Zhang, 1997; Jia and Zhang, 1999; Trejo et al., 1993) with a half-life of less than 3 h reported for maize (Jia and Zhang, 1997) and less than 2 h in sunflower (Jia and Zhang, 1999). Assuming similar rates of ABA degradation in grapevine, (Loveys and Milborrow 1984) removal of the apex should result in a rapid decline in the ABA concentration in the more basal leaves if the apical tissue was a major source of the leaf ABA gradient. Thus the absence of an effect of decapitation over 48 h is strong evidence that the apices were not the source of the ABA gradients.

We also looked at the effect of interrupting phloem transport by girdling. There was no appreciable effect of girdling on $[ABA]_{apex}$ at 24 h (Fig 18*A*), however at 48 h the $[ABA]_{apex}$ had significantly increased relative to the control (Fig 18*B*). It is possible that ABA that is normally exported from the apical tissues in control plants had been blocked in the girdled treatment resulting in ABA accumulation above the girdle within 48 h (Fig 18*B*). However we also observed a corresponding increase in *NCED* expression at 48 h after girdling implying that the treatment had induced ABA synthesis in the leaves above the girdle. The increase in gene activity could be due to the action of wound- or stress-induced ethylene in the stimulation of ABA biosynthesis. Hansen and Grossmann (2000) have linked the recent evidence for auxin-induced stimulation of ethylene biosynthesis in sensitive plants with ethylene-triggered increases in the biosynthesis of abscisic acid (ABA) leading to growth inhibition and senescence. In a subsequent paper (Grossmann and Hansen, 2001) they go on to argue that ethylene-induced ABA may, indeed, play a role in natural physiological phenomena, such as root gravireaction and suppression of lateral bud growth in apical dominance. The observed increase in $[ABA]_{apex}$ in this study would be consistent with such an ethylene/ABA cascade event, however we have no evidence at this stage to show a direct link with ethylene.

$[ABA]_{leaf}$ in immature leaves has been shown to be sourced from subtending mature leaves in other species (Zeevaart and Boyer 1984). If acropetal phloem transport was an important contributor to the ABA gradients, girdling would be expected either to have no rapid effect on, or to reduce $[ABA]_{leaf}$ above the injury. The increase in $[ABA]_{leaf}$ observed after 48 h above the girdle (Fig 18*B*) in this study suggests, but

does not fully confirm, that acropetal phloem transport is not essential to elevated concentrations of ABA near the apex. Instead of the expected effects of girdling, the treatment resulted in an accumulation of ABA in the leaves above the girdle that was not observed in the equivalent leaves in the controls and was probably a consequence of wounding (see above). An alternative route of acropetal phloem transport of ABA from mature leaves, through roots and back up to immature leaves via the xylem, has been described in other species (Zeevaart and Boyer 1984) but was not examined in this study.

Changes in the $[ABA]_{leaf}$ in all the leaf position experiments were generally accompanied by similar changes in the abundances of *NCED* mRNA but not of *Zep* mRNA. While increased mRNA abundance is not always linked with increased activity of the enzyme product (see for example Audran et al., 1998; Seo et al., 2000; Thompson et al., 2000), it does appear that the variations in abundance of *NCED* mRNA observed in all the treatments are linked with variations in ABA abundance in the respective tissues, presumably via modified abundance of the NCED enzyme. Such a relationship is consistent with observations on unstressed leaf (Taylor *et al*. 2000, Thompson *et al.* 2000) and more markedly on droughted leaf (Tan *et al*. 1997; Liotenberg *et al.* 1999; Qin and Zeevaart 1999 and Taylor *et al*. 2000).

Hybridisations with the *NCED* probe revealed gradients of intensity declining from the terminal leaves towards the basal leaves, whilst there did not appear to be an association between the 'inverse' gradients of *Zep* mRNA abundance seen in all the treatments and changes in [ABA]

This study has demonstrated that in *V. vinifera* bulk leaf ABA is not purely sourced from the roots as the sap concentration increased along shoots away from the root source. This implies that additional ABA is being added to a total pool from somewhere within the grapevine canopy. The gradients do not appear to be originating from the apex. Removal of the apical tissue in the decapitation treatment did not significantly affect the gradients; likewise the gradients were maintained below the girdle in the girdling treatment. From these observations we conclude that the elevated [ABA] in leaves above the girdle could be a result of synthesis within the leaves closest to the apex, reduced turnover of ABA in the younger leaves or import

of ABA from the more basal leaves of the same shoot, becoming more concentrated towards the apex due to reduced dilution by the transpiration stream. Assuming variations in the abundance of mRNA for *NCED* are related to the regulation of ABA biosynthesis, the mRNA studies suggest that there is elevated ABA synthesis in some of the leaves towards the apex as a result of girdling. Although we have not assessed the levels of ABA catabolism along the shoot, it is likely that combinations of all three mechanisms, *de novo* synthesis, catabolism and transport, are contributing to the observed gradients.

Stomatal conductance appeared to be inversely related to bulk leaf [ABA] in mature leaves (leaf positions 3-8). However in immature leaves, and when rises in ABA were induced by wounding treatments, this correlation did not hold. There is increasing evidence that bulk leaf [ABA] is not necessarily directly correlated with stomatal conductance but rather that it is the subcellular localization of a discrete portion of the ABA that is important. In *Vicia faba* it is the apoplastic ABA that is correlated with stomatal conductance (Zhang and Outlaw 2001) and this ABA accumulates in response to declining soil water status. The lack of correlation that we have observed in some instances may be the result of differential ABA compartmentation.

Overall the implications of this study are that the shoots of *V. vinifera* under low stress conditions have some capacity to regulate ABA concentrations independently of hydraulic or chemical signals from the roots and that stomatal conductance in unstressed mature leaves correlates with bulk leaf [ABA].

7. Outcomes/Conclusions

This project (CRV 99/8) focused on three core areas, understanding the physiology of how rootstocks and rootstock derived chemical signals control scion water use; the role of the scion in control of grapevine water use; and a study to identify the source of intrinsic ABA gradients in grapevine shoots. The first two areas were major objectives of the initial project proposal whereas the shoot position work was a new objective that received significant attention due to its potential importance for understanding sources of ABA pools (shoots versus roots) in grapevine and its potential implications for root to shoot communication of water stress.

The major objectives of this project were all met with the exception of the cytokinin analysis from different rootstocks. An experiment was set up to assess cytokinin and ABA production from four rootstocks grafted to Shiraz and whilst the experiment was harvested in September 2002, they still have not been analysed. There have been a number of factors that have contributed to the non-achievement of this objective however the major disruption was the lack of availability of an LCMS until December 2003. Significant effort has been put into developing a HPLC protocol to separate the cytokinins as well as into sample preparation; however time has still not been available to actually run the samples. This objective will still be met but unfortunately outside of the project timeline.

Ungrafted Shiraz (clone BVRC12) was compared to the same clone grafted to seven different rootstocks (420a, K51-40, Ramsey, 140 Ruggeri, Schwarzmann, SO4 and Teleki 5C) over two successive growing seasons with contrasting water inputs. It was established that rootstock genotype can have a measurable effect on several aspects of vine performance. Relative to all other combinations Shiraz on Teleki 5C and Ramsey had consistently higher rates of water use (leaf and extrapolated vine transpiration) and CO_2 assimilation rate than the other combinations, but were not significantly different to Shiraz on own roots. Shiraz grafted to these same rootstocks had the highest leaf areas in 02/03 (not measured in 01/02) and pruning weights across both seasons, thus high vigour corresponded with high assimilation and transpiration rates. K51-40 and 420a tended to be at the opposite extreme to Ramsey and Teleki 5C, in

that they had the lowest transpiration, CO_2 assimilation and vigour of all the combinations.

Through analysis of xylem sap ABA production of the same field vines used for gas exchange measurements, a correlation was observed between relative $[ABA]_{sap}$ and the ranking of rootstocks on the basis of stomatal conductance. This relationship became stronger when vines were subject to greater water stress. Thus in field vines $[ABA]_{sap}$ can provide a good comparative indication of instantaneous vine water use under prevailing conditions and may prove to be a useful tool for surveying in-ground rootstock collections. In pot experiments no evidence was found to show that any rootstock had the inherent capacity to generate more ABA than another either in well watered or water stressed conditions. Whilst care should always be taken when interpreting data from potted vines, it can be concluded that in a nursery situation it is unlikely that differences in ABA synthesis could be detected and used as a relative indicator of potential vine performance.

Xylem sap ABA concentration is known to increase in many plants in response to increasing water stress and have the potential to alter the compartmentation, and thus apoplastic ABA concentration in contact with the stomatal guard cells (Felle and Hanstein, 2002; Hartung and Radin, 1989; Wilkinson et al., 1998; Wilkinson and Davies, 1997). We assessed the potential of using a simple measure of xylem sap pH to compare rootstocks to indicate relative transpiration efficiency or drought tolerance. Measurement of xylem sap pH on samples recovered using a Scholander pressure chamber proved to be highly variable, and whilst pH was shown to increase with water stress no significant correlation could be shown between pH and stomatal conductance, transpiration, transpiration efficiency, $[ABA]_{sap}$ or leaf water potential. As such, xylem sap pH is not a good candidate as a quick biochemical marker for water use efficiency. The relationship between sap pH and ABA compartmentation in the leaf has potentially important implications for all studies involving correlation of ABA with water use, and thus is worthy of further study. In the current study we used sap expressed from the petiole on which to measure pH. The reason we found little correlation between sap pH and the other physiological parameters measured may be because it is the apoplastic pH in the sub stomatal cavity that will influence ABA compartmentation the most, rather than the ABA delivered in the xylem (measured in

petiole sap). Whilst it was beyond the scope of CRCV 2.1.8 to look at apoplastic pH to this detail, further work in this area will be of benefit to all studies involving ABA signalling of water deficits in the future.

In the rootstock field trial, leaf water potential (Ψ_{leaf}) data in combination with the transpiration data provided the most useful information as to the mechanism driving the differences in gas exchange capacity between the different Shiraz/rootstock combinations. Leaf water potentials were consistently higher (less negative) in the combinations that had the highest transpiration rates and vice versa. Given that all vines in the trial received the same amount of water it was expected that vines with higher transpiration rates would have lower water potential. The fact that this did not occur is suggestive that the capacity of the different rootstocks to supply water to the scion (in this case Shiraz) varies. Thus it is postulated that rootstocks such as Ramsey and Teleki 5C conducted water more effectively to the Shiraz scion than rootstocks such as K51-40 and 420a.

Inherent differences in rootstock root architecture were observed in potted vines grown in glasshouse conditions. Ramsey, a rootstock known to have high vigour, had a higher proportion of thick structural roots and much lower density of fine roots than other lower vigour rootstocks such as 420a and Riparia Gloire. The thicker roots of Ramsey are better adapted to penetration through heavier soils and to greater depth than the finer root systems of the other rootstocks, which is consistent with Ramsey accessing more water throughout the growing season from soil moisture at depth in the Coombe vineyard trial. Therefore it is postulated that differences observed in gas exchange, ABA and leaf water potential in this study were the result of different levels of vine water stress due to differences in root volume at lower soil depths.

Improvements in transpiration efficiency, such as can be achieved through partial rootzone drying, are the result of a disproportionate reduction in transpiration over CO_2 assimilation through a relatively small decrease in stomatal conductance. No rootstock in the present study had the capacity to significantly improve transpiration efficiency as reductions in transpiration were accompanied by an equivalent decrease in CO_2 assimilation, which is again consistent with varying degrees of water stress

(Quick *et al*, 1992) as opposed to inherent differences in transpiration efficiency. Although the rootstocks did not improve vine transpiration efficiency, the more vigorous rootstocks (Ramsey, Teleki 5C) were more drought tolerant and as such may require less irrigation to produce the same yield thus improving water use efficiency if we define it as yield (tonnes) per megalitre of irrigation water applied. Whilst yield of these vines was not measured as part of CRV 99/8 a University of Adelaide masters project (Selpulvida-Adriasola and Dry, unpublished data 2003) assessed the effect of rootstocks on yield in the same trial used for CRV 99/8 in the 02/03 season (see table 1). Sepulvida-Adriasola and Dry observed that in general the Shiraz yields on these rootstocks was proportional to the transpiration, assimilation and pruning weights observed in the CRV 99/8 study, with the exception of Shiraz on 420a which had higher yield than all but Shiraz on Ramsey and Teleki 5C (Table 1). Thus in the 02/03 season of the Coombe rootstock trial WUE (yield/ML water) increased with increasing vine vigour even though total water use also increased.

There were no significant differences in bunch number, bunch mass, berries per bunch or berry mass between rootstocks despite there being significant between rootstock variation for each of these factors. A combination of all yield components led to the observed significant differences in yield, for example Teleki 5C, which had the highest yield per plant, had the highest bunch mass and berries per bunch but did not have the highest bunches per plant or berry mass. Similarly the relatively high yield of 420a appears to have been largely due to a higher number of bunches per plant, rather than a particularly high bunch mass.

Table 1. Yield comoponents at 22,5º Brix of cv. Shiraz grafted on different rootstocks
Significant differences between means whithin columns, where present, are indicated by different leters by Tukey's multiple comparision test. (p=0.05)

Rootstock	Yield per plant (kg)	Bunches per plant	Bunch mass (g)	Berries per bunch	Berry mass (g)
420 A	17,5 ab	138.1	128.1	105.3	1.21
Ruggeri 140	13,7 bc	108.7	136.1	110.5	1.24
K51-40	11,9 c	108.5	110.5	99.2	1.12
Schwarzmann	13,9 bc	117.3	120.9	108.6	1.11
Teleki 5C	19,2 a	131.0	147.7	114.6	1.30
BVRC 12	15,9 abc	131.8	120.7	97.6	1.23
SO 4	14,5 abc	129.3	113.6	95.0	1.20
Ramsey	17,6 ab	127.7	138.1	98.1	1.40

Data used with permission of Cristian Sepulvida-Adriasola and Professor Peter Dry of the University of Adelaide

The ranking of drought tolerance imparted by rootstocks given in the literature varies significantly depending on publication (Carbonneau, 1985; Cavanagh, 1991; Cirami et al., 1994), and this study is no exception. This is not surprising given the hypothesis that the relative performance of the rootstocks in the present study is a likely result of root volume and architecture. A high degree of clonal variation in root structure and density was observed in potted vines in the present study; however the ability of each rootstock to grow to its potential in the vineyard will be heavily influenced by its compatibility with the prevailing soil physics and chemistry.

Our current understanding of how the roots of different grapevine genotypes interact with a range of chemical and physical properties of different soils is currently insufficient. Work in South Africa has shown that root density and architecture vary according to rootstock genotype in the field and is dependent on soil properties (Southey, 1992; Southey and Archer, 1988). However, as for CRV 99/8, their results apply directly to the environment under study; a relatively saline soil. Whilst the results of this study suggest that above ground vigour may be used to indicate the relative drought tolerance of different rootstocks, the dependence on environment should not be overlooked. Thus relative vigour of rootstocks should be assessed in a location with similar soil and atmospheric environment to the target destination vineyard.

In the future there would be benefit in extensive studies to increase understanding of the interaction between physical and chemical soil properties and specific rootstock clone root growth to enable better prediction of rootstock performance at specific locations.

Physiology of varietal responses to water deficit

The SARDI Nuriootpa variety block was used to compare the response of Chardonnay, Shiraz and Grenache to changes in VPD. It has been clearly demonstrated that different grapevine varieties vary significantly in their sensitivity to changes in evaporative demand. The three varieties showed distinctly different levels of control over transpiration through stomatal regulation. Grenache was very conservative, showing high stomatal sensitivity to VPD; Chardonnay showed the lowest stomatal sensitivity to VPD and Shiraz was halfway between the other two

varieties. The higher ABA concentrations in Grenache xylem sap compared to the other two varieties at high VPD correlated well with the lower stomatal conductance and transpiration of this variety, however absolute $[ABA]_{sap}$ did not correlate well with absolute g_s, implying that the stomata were not responding to ABA concentration alone. A recent publication has linked the anisohydric (Shiraz) and isohydric (Grenache) behaviours of these varieties to differences in hydraulic conductivity (Schultz, 2003). The enhanced levels of ABA observed in Grenache may be a hypersensitive response to declining water potential or relative water content in the leaves of Grenache inducing stomatal closure. If differences in hydraulic conductivity are the cause of the differences between varieties in terms of sensitivity to changes in VPD, then varieties such as Grenache may be more responsive to the costs/benefits of grafting to rootstocks with differing capacities to supply water to the scion.

Recent work on PRD has shown that the signals generated by PRD sensitise leaves to changes in VPD (Loveys et al., 2004). Thus from what we have learned about isohydric (Grenache) and anisohydric (Shiraz/Chardonnay) grapevines response to VPD, we can conclude that PRD accentuates the responses which differentiate isohydric and anisohydric genotypes. On this basis it is likely that that the responsiveness of a particular variety to PRD may be a function of its inherent isohydric/anisohydric predisposition. This work will thus be continued on through links to CRCV 2.1.8: “Improving the efficiency of deficit irrigation technologies”.

Gradients in ABA in grapevine shoots – where do they come from?

The rootstock and variety studies performed in this project had significant focus on the role of ABA in the relative performances of the genotypes under water stress. A limitation of the methodology currently available to quantitate [ABA] is that it is difficult to locate the original source of the ABA being measured. ABA is synthesised throughout plants in the roots, shoots, leaves and fruit and as such the ABA in the petiolar sap measured throughout this project may have arisen from any plant organ. Whilst it is known that grapevine roots synthesise ABA in response to drying soil it is possible that the shoots may perceive this ABA signal and/or another chemical or hydraulic signal and respond by synthesizing ABA as well. The gradient experiments discussed here were aimed at gaining a better understanding of the source of ABA in

the shoots and the capacity of the shoots to regulate ABA levels independently of the roots.

Whilst looking at the effects of soil moisture deficit on xylem sap ABA concentration in different glasshouse-grown rootstock clones it was observed that there were gradients in [ABA] along canes that inversely correlated with gradients in g_s. It was found that g_s decreased linearly towards the shoot apex as $[ABA]_{sap}$ increased. The increasing nature of the ABA gradient implied that a significant portion of the ABA producing the observed gradient was either synthesised in the shoots, or possibly the apex, rather than being root derived. This experiment was expanded to look at leaf ABA and stomatal conductance gradients in the field, imposing apex decapitation and shoot girdling treatments to ascertain the source of the ABA giving rise to the gradients. Dr Jim Speirs (CRV 01/05) added greatly to this work through his study on the activity of the key genes involved in the ABA biosynthetic pathway (Zeaxanthin expoxidase, and 9-cis-epoxycarotenoid dioxygenase). This allowed us to look at the change in regulation of ABA biosynthesis in leaves in response to the girdling and decapitation treatments. The results showed that the gradients were likely to be the result of de Novo synthesis in the less mature leaves, demonstrating the ability of leaves to regulate ABA concentrations in the shoots independently of root signals.

These shoot experiments have highlighted the potential importance of non root-sourced ABA in regulation of stomatal conductance and have provided an opportunity to hone the molecular techniques for examining ABA biosynthesis with strong links to vine physiology. Analysis of ABA concentrations in different tissues has previously been devalued by only being able to measure localised instantaneous concentrations with an inability to identify the origins of the ABA being measured. Continued use of this combined approach between vine physiology and Jim Speirs' molecular techniques will be of benefit to future CRCV projects such as CRCV 2.1.8.

8. Recommendations

Selecting rootstock for drought tolerance

There are currently resources available which growers can use to make rootstock selection easier. One of the most comprehensive tools available has been provided by Yalumba nursery; it is their web based rootstock selector which can be found at http://www.yalumbanursery.com/rootstock/public/. Whilst this is an excellent guide, there are some limitations of the database that need to be considered. The recommendations of the database are based on scientific publications from around the world and as such include information collated for rootstocks in a diverse range of environments, management practices, irrigation and scion varieties which the rootstocks are grafted to. This diversity is in some ways a positive attribute of the database however it also leads to some ambiguities, simply because there is inconsistency in the scientific literature. An example of this inconsistency is demonstrated in the reported drought tolerance of Ramsey detailed in the summary information for this rootstock. The drought tolerance is shown as low to moderate, moderate and high. This confusion arises because there is at least one publication that classifies Ramsey into each of these drought resistance categories. The most likely explanation for this confusion is that the actual drought tolerance of a rootstock is heavily dependent on environment, for example the physical and chemical properties of the soil, land management and prehistory.

In this study rootstock drought tolerance was found to correlate best with pruning weight which was an indication that grafted vines with higher vigour and pruning weight were more drought tolerant. Grapevine canopy growth tends to be in balance with roots growth and as such higher vigour rootstocks, such as Ramsey and Teleki 5C in the current study, are able to access ground water at depth throughout more of the season than the lower vigour stocks. Thus selection of higher vigour rootstocks will lead to a lower irrigation requirement and higher water use efficiency in grafted vines. Decision of which rootstocks are highly vigorous should be based on regional experience because rootstocks may not meet their vigour potential under all soil environments. This study has indicated that a major determinant of rootstock drought tolerance is root morphology which will be highly dependent on genotype compatibility with various soil types and chemistry. Whilst we have demonstrated

significant genotypic variation in root structure in pots, the ability of a rootstock to reach its root growth potential will be dependent on its compatibility with the prevailing soil environment.

ABA as a biochemical marker of grapevine water status

Differences in drought tolerance were reflected in xylem sap ABA concentration when grafted vines were subjected to water deficit. Surveying vines for xylem sap ABA concentration should give a reliable indication of relative drought tolerance between rootstocks. The use of ABA as a marker should be restricted to in-ground trials rather than nursery situations where the influence of root architecture on vine water status and thus ABA synthesis may be less evident. Xylem sap [ABA] could be used as a marker for vines at the same locality and management; however comparisons between sites are unlikely to be valid because variations in soil, environment and management between sites may all influence root development and thus nay also effect ABA concentrations in the vine.

pH as a biochemical marker of grapevine water status

Theoretical and experiments involving application of ABA suggested that xylem sap pH may be a quick, easy measure of the effectiveness of a rootstock to supply information about soil water status to the scion and considerable effort was put into testing this hypothesis in this project. However, in contrast to ABA, the variable nature of pH measurement on sap samples expressed under pressure from a cut petiole, makes this an unreliable measure of drought tolerance using the methods currently available. The variability observed in this study was potentially a consequence of the method of sap extraction which may be sensitive to the pressure applied for extraction and interaction with the water status of the leaf being measured. There is sufficient evidence in the literature linking apoplastic pH around the stomatal guard cells with ABA partitioning between apoplastic and symplastic compartments, that this remains a concept worthy of further investigation. At this stage we do not have a simple methodology for measuring apoplastic pH in the leaf blade, thus developing such a methodology could be a focus of future research.

Importance of the scion in the control of grapevine water use

The variety trial at Nuriootpa and the shoot position work demonstrated that the scion has significant control over the response of grapevine to atmospheric and soil moisture deficit. Thus choice of scion variety may have a more significant impact on vineyard water use than choice of rootstock. Despite this finding, when scion variety is kept constant it was demonstrated that rootstocks can be used to further modify vine water use. Thus there is an interaction between rootstock and scion that must be considered when predicting the drought tolerance of a grafted vine. We have produced data that shows the distinctly different isohydric and anisohydric strategies used by Shiraz, Grenache and Chardonnay in response to water deficit. A survey of more varieties would be useful to determine the isohydric/anisohydric status of more varieties such that their performance when grafted to different rootstocks or under different irrigation management can be predicted.

Summary of Recommendations

- Rootstocks can be used to modify the water use of the scion through differential abilities to supply water and by supplying varying amounts of hormonal signals which modify the water use of the canopy. Rootstocks which are themselves vigorous and impart higher canopy vigour also tend to be more drought tolerant. At the Coombe vineyard of the University of Adelaide, for example Shiraz grafted to Ramsey and Teleki 5C displayed the highest vigour and also the highest tolerance to water stress and consequently achieved the highest yields.
- Published data regarding relative drought tolerance of rootstocks are often contradictory, probably because of site specific responses. For example Shiraz grafted to Teleki 5C was observed to have high vigour and drought tolerance in the Coombe vineyard; however published data ranks this rootstock as having moderate vigour and low to moderate drought tolerance. Teleki 5C was thus better suited to the soil conditions in the Coombe vineyard than at the other sites reported in the literature.
- Xylem sap abscisic acid (ABA) concentration can be used as a biochemical marker to indicate relative drought tolerance of grafted vines provided they are exposed to the same soil and atmospheric environment – the best correlation between ABA and drought tolerance is achieved if vines are subjected to water deficit. Xylem sap ABA

concentration was a poor indicator of drought tolerance in pot grown plants in a nursery.

- Measurement of the pH of sap expressed by pressurising the leaf blade is too variable to be useful as a quick marker of drought tolerance. However, the link between apoplastic sap pH and ABA function is well established and thus there is significant merit in developing more effective methodology to quickly sample apoplastic pH in the leaf blade.
- Drought tolerant rootstocks may be less responsive to techniques such as PRD, as large root systems may have access to ground water reducing the effective root proportion exposed to the PRD effect. Thus lower vigour rootstocks such as K51-50, 420a and SO4 may be more useful if planning to use PRD
- Scion variety has predominant control over water use strategy and thus choice of variety will have a large impact on vineyard water use. Surveying vines for isohydric/anisohydric behaviour will improve ability to customise vineyard irrigation to water use strategy of the variety.

Appendix 1: Communications

Communication of the results of this work has to date been largely through presentations given to science and industry groups. One refereed publication on the shoot position work has been accepted for publication and will appear in the June issue of Functional Plant Biology (appendix 5). Two other manuscripts on the rootstock and varietal work are still in preparation for publication in scientific journals.

Refereed Publications

Christopher J. Soar, Jim Speirs, Suzanne M. Maffei, and Brian R. Loveys. (2004) Gradients in stomatal conductance, xylem sap ABA and bulk leaf ABA along canes of Vitis vinifera cv. Shiraz: molecular and physiological studies investigating their source. Functional Plant Biology (in press.).

Manuscripts in Preparation

Both of the following manuscripts are to be submitted to Australian Journal of Grape and Wine Research in 2004

Soar, C. J., M^{c}Carthy, M. G., Dry, P. R. and Loveys, B. R. Rootstock influence on *Vitis vinifera* cv Shiraz water relations, evidence for variation in hydraulic and chemical control over water use.

Soar, C. J., Speirs, J., M^{c}Carthy, M. G., Dry, P. R. and Loveys, B. R. Varietal variation in response to changing VPD in grapevine; the role of ABA and implications for vineyard performance in Australia.

Selected Presentations

2004

Soar C. J. and Loveys, B. R. **"**The Mechanics of grapevine Water Use" Clare Valley Field Days, 28-04-2004

2003

Soar, C. J. "Rootstock influence over grapevine water use" Mildura Field Days, Mildura, 28-05-2003

Soar, C. J., M^{c}Carthy, M. G., Dry, P. R. and Loveys, B. R. "Improving water-use efficiency, canopy structure and grape quality by better matching rootstock and scion physiology to irrigation practice" Orlando Wyndham Viticulturists Meeting, Adelaide 15-10-2003

Soar, C. J., M^{c}Carthy, M. G., Dry, P. R. and Loveys, B. R. "Roots or shoots? - Controlling water use in grapevines" 2nd Australian Grapevine Physiology Workshop, Melbourne, 03-10-2003
Soar, C. J., Speirs, J., Penrose, A. B. and Loveys, B. R. "Regulation of the genes encoding Zep and Nced and changes in ABA concentration and stomatal conductance in grapevine" Combio 2003, Melbourne 29-09-2003.

Soar, C. J., M^{c}Carthy, M. G., Dry, P. R. and Loveys, B. R. **"**Improving water-use efficiency, canopy structure and grape quality by better matching rootstock and scion physiology to irrigation practice" CRCV 5 yr review, Adelaide 05-09-2003

Soar, C.J. and Loveys, B. R. "Rootstocks in viticulture: towards an understanding of how they influence water use and productivity" Invited presentation to the department of Horticulture, Viticulture and Oenology, University of Adelaide, 27-08-2003

2002

Soar, C. J., Loveys, B. R., Dry, P.R. and M^{c}Carthy, M. G. Rootstock influence on grapevine gas exchange: The importance of root to shoot signalling. Combio September-October 2002. Sydney, Australia.

Soar, C. J., Loveys, B. R., and Speirs, J. Root to Shoot Communication and the Control of Water Use in Grapevine. Inaugural Australian Grapevine Physiology Workshop, October 2002 Canberra, Australia

Soar C.J. and Loveys, B. R. Root borne signals and the implications for PRD. CRCV PRD workshop, October 2002, Adelaide, Australia.

Soar, C. J., Loveys, B. R., Dry, P.R. and M^{c}Carthy, M. G., 2002. Project 2.1.1 2002 review. Cooperative Research Centre for Viticulture Program 2 review. June 2002 Merbein, Victoria, Australia.

Appendix 2 - Intellectual Property

There is no patentable intellectual property arising from this project.

Appendix 3 – References

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Appendix 5 – Other relevant material

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