Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid

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Stomatal responsiveness to evaporative demand (air vapour pressure deficit (VPD)) ranges widely between species and cultivars, and mechanisms for stomatal control in response to VPD remain obscure. The interaction of irrigation and soil moisture with VPD on stomatal conductance is particularly difficult to predict, but nevertheless is critical to instantaneous transpiration and vulnerability to desiccation. Stomatal sensitivity to VPD and soil moisture was investigated in Semillon, an anisohydric *Vitis vinifera* L. variety whose leaf water potential (Ψᵢ) is frequently lower than that of other grapevine varieties grown under similar conditions in the warm grape-growing regions of Australia. A survey of Semillon vines across seven vineyards revealed that, regardless of irrigation treatment, midday Ψᵢ was dependent on not only soil moisture but VPD at the time of measurement. Predawn Ψᵢ was more closely correlated to not only soil moisture in dry vineyards but to night-time VPD in drip-irrigated vineyards, with incomplete rehydration during high night-time VPD. Daytime stomatal conductance was low only under severe plant water deficits, induced by extremes in dry soil. Stomatal response to VPD was inconsistent across irrigation regime; however, in an unirrigated vineyard, stomatal sensitivity to VPD—the magnitude of stomatal response to VPD—was heightened under dry soils. It was also found that stomatal sensitivity was proportional to the magnitude of stomatal conductance at a reference VPD of 1 kPa. Exogenous abscisic acid (ABA) applied to roots of Semillon vines growing in a hydroponic system induced stomatal closure and, in field vines, petiole xylem sap ABA concentrations rose throughout the morning and were higher in vines with low Ψᵢ. These data indicate that despite high stomatal conductance of this anisohydric variety when grown in medium to high soil moisture, increased concentrations of ABA as a result of very limited soil moisture may augment stomatal responsiveness to low VPD.

**Keywords**: drought responses, Semillon, stomatal regulation, transpiration, VPD, water relations

**Introduction**

Differences between grape (*Vitis vinifera*) varieties in their ability to withstand high evaporative demand when soil moisture is limited can be attributed to a number of morphological and physiological characteristics, including the stomatal behaviour of their leaves. Stomata generally close in response to the steep concentration gradient of water vapour between the leaf and the air and therefore have great influence over plant water status. Stomata are involved in preventing damaging plant water deficits (*Jones and Sutherland 1991*), xylem cavitations and embolisms (*Tyree and Sperry 1988*). It is reasonable to assume that stomata have evolved to balance maximum carbon gain with minimum water loss, averting dehydration and physiological damage.

While there have been advances in understanding the mechanics of stomatal opening and closing, the corresponding physiological mechanisms remain incompletely understood (*Buckley 2005*, *Wilkinson and Davies 2008*). There is evidence, however, that both chemical and hydraulic signals appear to regulate stomatal conductance (*Comstock 2002*). For example, in grapevines the control of stomatal conductance...
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(g) in response to soil water deficits is related to chemical signals, such as abscisic acid (ABA), travelling from the roots to the shoots in the transpiration stream (Loveys 1984a, Correia et al. 1995, Stoll et al. 2000). It was shown that during partial rootzone drying, production of ABA by the drying roots reduced g, despite the maintenance of favourable shoot water status (Loveys et al. 2000). Stomatal conductance can also be regulated by the hydraulic properties of the soil–root–shoot pathway, with a positive correlation between stomatal and hydraulic conductance (Küppers 1984, Meinzer et al. 1988, Schultz 2003). That a hydraulic signal can be a dominant regulator of stomatal behaviour is supported by studies where the reduction in g invoked by soil drying can be reversed by presurization of the root system; once the pressure is released, g will return back to original levels (Fuchs and Livingston 1996, Yao et al. 2001).

Stomatal control over conductance of water in the vapour phase within the leaf air spaces is highly dependent on the vapour pressure gradient between the leaf and air (VPD). In most species, increasing VPD reduces g, but responsiveness ranges widely (Oren et al. 1999, Bunce 2006). For instance, under well-watered conditions, temperate deciduous trees undergo a greater reduction in g in response to increasing VPD than herbaceous species (Franks and Farquhar 1999). A marked stomatal response to VPD is characteristic of isohydric species, while anisohydric species exhibit a less pronounced response and thus higher transpiration rates (Turner et al. 1984, Tardieu and Simonneau 1998). As observed in Helianthus annuus, however, this insensitivity to VPD does not necessarily equate to stomata that are unresponsive to soil moisture (Turner et al. 1985), indicating a complex control mechanism integrating both leaf- and root-derived signals. In Eucalyptus gomphocephala strong stomatal down-regulation in response to VPD could not maintain leaf water potential (Ψ_l) as soil moisture declined throughout the season (Franks et al. 2007). Despite this anisohydric behaviour, E. gomphocephala maintained a constant midday hydrodynamic water potential gradient from roots to shoots, referred to as isohydrodynamic, and this was explained by a stomatal control model integrating plant hydraulic conductance (Franks et al. 2007).

These examples illustrate that variables such as irrigation and soil moisture interact with Ψ_l and add a tier of complexity to predicting stomatal response to VPD. Nonetheless, it is important to be able to understand this response because it affects not only instantaneous transpiration (E) but also long-term whole-plant functioning including growth and reproductive development (Davies and Zhang 1991, Loveys et al. 2004). The genotypic diversity in drought tolerance of grapevines makes this perennial crop an ideal model for studying plant water relations. Some grapevine varieties exhibit isohydric characteristics, but others, usually derived from mesic origins, do not (Schultz 1996, Soar et al. 2006, Rogiers et al. 2009). The anisohydric variety, Semillon, has high transpiration rates and low Ψ_l compared with other varieties when grown under similar conditions (Rogiers et al. 2009). We hypothesize that the high rates of transpiration in Semillon allude to poor stomatal control. In order to gain a better understanding of this variety’s stomatal responsiveness to VPD and soil moisture, our objective was to investigate the water relations of this variety in a number of vineyards operating under contrasting irrigation regimes. A second objective was to investigate the response of Semillon stomata to exogenous ABA application in order to better understand the role of ABA in stomatal control of this variety.

Materials and methods

Description of study sites

The trial sites were located in two Australian grape-growing regions of New South Wales: the Murrumbidgee Irrigation Area (MIA) and the Lower Hunter Valley (HV). Between budburst and harvest, the MIA has a warmer climate than the HV. In Cessnock (HV), the mean long-term maximum temperature during berry development (November to February) ranges from 27 to 30 °C while the mean minimum ranges from 13 to 18 °C. Average monthly rainfall ranges from 65 to 100 mm. In Griffith (MIA), the mean maximum temperature during berry development ranges from 28 to 33 °C while the mean minimum ranges from 13 to 17 °C. The average monthly rainfall is substantially less in Griffith and ranges from 27 to 33 mm. Both the HV and the MIA were in a drought-declared area for the first two seasons of the project (2005–2006 and 2006–2007). In the third season, with rain in the HV, this region was upgraded to satisfactory in terms of its soil moisture status.

The vineyards of the HV are grown on alluvial loam flats and on the surrounding hills. The hills are covered in loam-clays which overlay limestone regolith. The vineyards of the MIA are grown on alluvial soils with a clay, loam or sand topsoil which overlays a reddish-brown clay subsoil.

A total of seven vineyard sites were assessed; four in the MIA and three in the HV. The sites consisted of Semillon (V. vinifera L.) vines that had been characterized with no symptoms of leaf burn to consistent leaf burn over several seasons. These commercial vineyards were irrigated according to the usual practice of the grower/manager of that site. Five vineyards were drip irrigated, one was flood irrigated and one was not irrigated. All vines were on their own roots but the Semillon clone most often was not known. Vine age ranged from 6 to 60 years. Yield between the sites was variable but was generally less in the HV than in the MIA. An electromagnetic mapping (EM-38) survey was used to delineate a low, intermediate and high apparent electrical conductivity soil zone within each site (Table 1). For further detailed information on the site characteristics, see Rogiers et al. (2011).
Weather and soil moisture

Within each site a weather station was placed at canopy height. It was equipped with a temperature and humidity probe (Intercap HMP50, Vaisala, Hawthorn, VIC, Australia) and VPD was calculated from data logged at half hourly intervals. Air VPD as opposed to leaf-to-air VPD was used because the latter is based on leaf temperature which is influenced by transpiration rates and this is dependent on soil moisture, a variable which we wanted to test independently.

Soil moisture was measured with Watermark® 200 SS soil moisture sensors (Irrometer Co., Riverside, CA, USA) placed at 25, 50, 75 and 100 cm depths directly beneath the drippers. In the flood-irrigated and unirrigated vineyards, the sensors were placed at the same distance from the vine as in the drip-irrigated vineyards. A soil core analysis indicated that in the irrigated vineyards most of the roots were located within a 1 m profile; however, we recognize that in the unirrigated vineyard the roots may have extended to deeper layers. The soil water potential at each depth was logged at hourly intervals and was used to calculate relative extractable soil water (REW) according to the method of Granier et al. (2000), but with the following modifications. Soil water content was obtained from the measurements of soil water potential by using the soil moisture characteristic curve given by van Genuchten (1980) with the four parameters in this equation: \( \theta_s = 0.095, \theta_r = 0.41, \alpha = 0.095 \) and \( n = 1.31 \). These parameters were selected by determining the soil type by hand texturing using the methods described by the NSRI, Silsoe, UK and by appropriate selection from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999). The water contents at field capacity and at the refill point were obtained from the UNSODA database (Nemes et al. 1999).

Physiological measurements

Four vines were marked within each EM location and vine water relations were assessed over a 4-month period between pea size and harvest (November to February). A Scholander pressure chamber (ICT international, Armidale, NSW, Australia) fitted with a Model 3015G4 specimen holder was used for leaf water potential (\( \Psi_l \)) measurements. Two mature leaves were taken from each of four vines within each of the three EM locations within the vineyards. An LI-6400 gas exchange system (LI-COR Corporation, Lincoln, NE, USA) was used to monitor assimilation (A), transpiration (E) and stomatal conductance (g) of mature leaves on both sides of the canopy (two leaves per vine). Illumination of the leaf was by an artificial red-blue light source attached to the chamber head, set to ambient light levels to ensure a constant light source in spite of passing clouds. Temperature was not controlled to allow instantaneous measurements under natural conditions and the chamber head was covered in shade-cloth to prevent heating by the sun. A fixed, high flow rate without desiccant scrubbing of the water vapour allowed minimal equilibration time once the leaf was installed into the cuvette.

Stomatal sensitivity

The magnitude of stomatal response to VPD with REW was assessed using the method of Oren et al. (1999). For each vineyard, the g data were partitioned into sequential 0.1 intervals of REW and then fitted to \( g = -m \times \ln(VPD) + g_\alpha \), where \( g_\alpha \) and \( m \) are parameters generated in a least squares regression analysis. The empirical parameter \( g_\alpha \) is the reference conductance as VPD approaches 1 kPa and \(-m\) is the sensitivity of g to VPD.

Abscisic acid

Xylem sap was collected from excised leaf petioles of field- and hydroponically grown vines using the pressure chamber specified above. Pressure was increased until the first appearance of sap. Pressure was maintained at this value (which varied with level of vine water stress) while a standardized volume of sap (100 \( \mu l \)) was collected in the shade using a micropipette and dispensed into a microtube wrapped in foil and immersed in ice. Upon completion of collection, the samples were immediately frozen in liquid nitrogen. For ABA analysis, the xylem sap was thawed at 4 °C and ABA was quantified using the enzyme-linked immunosorbent assay (ELISA) technique (Mertens et al. 1983). The xylem sap was passed through a Sep Pak C\(_{18}\) cartridge (Waters, Inc., Rydalmere, NSW, Australia) preconditioned with 100% methanol and then deionized H\(_2\)O. The sample was loaded in 0.1 M citrate/0.2 M phosphate buffer (pH 2.8). After washing with 10% methanol, the ABA fraction was collected in 100% methanol. The sample was reduced to dryness with a centrifugal evaporator (Labconco Corp., Kansas, MO, USA) and reconstituted in 1 ml of TBS buffer (TRIS-buffered saline; 150 mM NaCl, 1 mM MgCl\(_2\), and 50 mM TRIS, pH 7.5). The sample was analysed by an ELISA (Phyodtek®, ABA Test Kit, Agdia Inc., Elkhart, IN, USA) and processed according to kit instructions. Absorbance was read with a \( \mu \)Quant microplate reader.
spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA). An internal ABA standard was used and results compared well with samples that had been analysed with the high-performance liquid chromatography method.

**Exogenous ABA application**

Dormant single-node canes of Semillon, clone DA16162, were placed in a grid supported on trays of water and allowed to undergo budburst and grow roots over a 2-month period within a glasshouse. Approximately at the 10-leaf stage, the water was removed from the tray and the roots of each plant were immediately immersed into individual 300-ml plastic cups filled with aqueous ABA solutions ranging from 0 to 100 μmol l⁻¹ (six plants for each ABA concentration). The tops of the cups were enclosed with aluminium foil to minimize evaporation of the solution. Gas exchange was measured the following day and 3 days later on two leaves per plant using an LI-6400 gas exchange system as described above.

**Data analysis**

Data were analysed with GenStat statistical software, version 11 (VSN International, Hemel Hempstead, UK) using analysis of variance for vineyard comparisons or simple and multiple linear regressions to explore relationships between VPD, REW, g, Ψᵢ, m, gₛₑ, m/gₛₑ, and ABA concentrations. Three-dimensional mesh plots were created with smoothed data calculated from running averages using SigmaPlot 8.0 graphing and statistics software (SPSS Inc., Chicago, IL, USA).

**Results**

**Predawn and midday Ψᵢ**

Predawn Ψᵢ, as averaged over the three seasons, was up to twice as negative in those vineyards with infrequent flood irrigation or no irrigation as compared with those that were irrigated regularly using the drip system (Table 2). Mean REW differed threefold over the sites while VPD was milder in the HV due to the higher humidity of this coastal region (Table 2).

Predawn Ψᵢ declined with increasing predawn VPD in the drip-irrigated vineyards of the MIA (P < 0.001, R² = 0.72) and HV (P < 0.001, R² = 0.91) (Figure 1a and c). Predawn Ψᵢ extended to −0.8 MPa as predawn VPD reached 1.5 kPa during warm, dry nights. This trend was not as apparent in the infrequently flood-irrigated vineyard (P < 0.01, R² = 0.07) where REW was less than that of the drip-irrigated vineyard (Figure 1a). Conversely, in this vineyard there was a closer correlation of predawn Ψᵢ with REW (P < 0.001, R² = 0.25) than VPD (Figure 1b). The influence of REW on predawn Ψᵢ was most evident in the unirrigated vineyard (P < 0.001, R² = 0.83) of the HV (Figure 1d). As REW diminished to 0.1, predawn Ψᵢ declined to −1.0 MPa in this dry vineyard, without any apparent influence of VPD as observed in the reverse correlation between these two parameters. The interaction of VPD and REW on predawn Ψᵢ was highly significant in each vineyard (P < 0.001). For instance, in the drip-irrigated vineyard of the MIA, 73% of the variance was accounted for by the interaction (Figure 1e). At low REW there was a strong response to VPD while at high VPD there was a marked response to REW. At low VPD, however, there was a poor response of predawn Ψᵢ to REW. As the REW increased and the VPD became smaller, predawn Ψᵢ became less negative (Figure 1e).

Midday Ψᵢ, as averaged over the 3 years of sampling during berry development was 33% more negative in the flood- than in the drip-irrigated vineyard of the MIA and 36% more negative in the unirrigated than in the drip-irrigated vineyard of the HV (Table 2). Midday Ψᵢ was negatively correlated to VPD in each vineyard studied (P < 0.001), with the exception of the one that was not irrigated (P = 0.72). Leaf water potentials declined to less than −2.0 MPa as VPD extended to >4 kPa (P < 0.001, R² = 0.29) (Figure 2a). Relative extractable soil water was also linearly correlated with midday Ψᵢ (P < 0.001); however, there was extensive variation in the relationship (R² = 0.15) (Figure 2b). The interaction of VPD and REW on midday Ψᵢ was highly significant for each individual vineyard (P < 0.001) as well as for all vineyards combined (P < 0.001) (Figure 2c). Lowest midday Ψᵢ values occurred at high VPD and low REW (Figure 2c). Together, these two parameters explained 36% of the variance in the data across all vineyards.

**Stomatal conductance**

In the drip-irrigated vineyard of the MIA, g was not significantly correlated to VPD (P = 0.63) (Figure 3a) and poorly correlated to REW (P < 0.01, R² = 0.03) (Figure 3b). In the infrequently flood-irrigated vineyard, however, both environmental factors had an impact with reduced g under high VPD (P < 0.001, R² = 0.21) or low soil moisture (P < 0.001, R² = 0.19) (Figure 3a and b). In this vineyard, the interactive effect of VPD and REW explained 24% of the variance (P < 0.001) (Figure 3e). Similar to the drip-irrigated vineyard of the MIA, g of the HV vineyard under drip irrigation was poorly correlated to VPD.

### Table 2. Leaf water potential of vines grown under differing irrigation systems in two grape-growing regions of NSW. Relative extractable soil water, VPD, predawn and midday leaf water potential (Ψᵢ) were sampled over three seasons (n = 110–240, SED = standard error of the difference).

<table>
<thead>
<tr>
<th>Vineyard/region</th>
<th>REW</th>
<th>VPD (kPa)</th>
<th>Predawn Ψᵢ (MPa)</th>
<th>Midday Ψᵢ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drip/MIA</td>
<td>0.87</td>
<td>2.07</td>
<td>−0.32</td>
<td>−0.93</td>
</tr>
<tr>
<td>Flood/MIA</td>
<td>0.60</td>
<td>2.62</td>
<td>−0.56</td>
<td>−1.39</td>
</tr>
<tr>
<td>Drip/HV</td>
<td>0.90</td>
<td>1.72</td>
<td>−0.26</td>
<td>−0.98</td>
</tr>
<tr>
<td>Unirrigated/HV</td>
<td>0.27</td>
<td>1.44</td>
<td>−0.58</td>
<td>−1.33</td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>SED</td>
<td>0.02</td>
<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Partitioning the stomatal conductance data into intervals of REW demonstrated that as VPD approached 1 kPa, stomatal conductance \( g_{sr} \) decreased with increasing REW (slope = −0.09, \( P < 0.01, R^2 = 0.62 \)) as averaged across all the sites (Figure 4a). Within individual vineyards, however, this trend was not consistent. In the unirrigated vineyard of the HV a similar negative trend occurred, but in the flood site the trend was in the opposite direction with an increase in \( g_{sr} \) with increasing REW (slope = 0.20) (Table 3). The magnitude of stomatal response (−\( m \)) increased with declining REW as calculated from the combined vineyard data (\( P < 0.001, R^2 = 0.75 \)) (Figure 4a). There was a similar significant relationship of −\( m \) with REW in the unirrigated vineyard of the HV (slope = −2.7) and the drip-irrigated vineyard of the MIA (slope = −0.3), but in the infrequently irrigated flood vineyard stomatal sensitivity decreased with decreasing REW (slope = 0.5, Table 3). The combined data set illustrates that stomatal sensitivity to VPD increased with \( g_{sr} \) (\( P < 0.001, R^2 = 0.82 \)) and the slope was 1.15 (Figure 4b, inset). The regression of \( m/g_{sr} \) with REW was
significant \( P < 0.001, R^2 = 0.78 \) for the combined vineyard data set (Figure 4b) but was not significant for any of the individual vineyards (Table 3), probably because the data sets were not large enough.

Unlike the drip-irrigated vineyard of the MIA, there was a positive correlation of \( g \) with \( \Psi_l \) in the flood-irrigated vineyard \( (P < 0.001, R^2 = 0.43) \), possibly due to the more negative \( \Psi_l \) in this vineyard (Table 2, Figure 5a and b). There was no relationship of \( g \) with \( \Psi_l \) in the drip-irrigated vineyard of the HV; however, in the unirrigated vineyard the relationship was linear \( (P < 0.001, R^2 = 0.51) \) (Figure 5c and d).

**Abscisic acid response**

Daytime trends in the ABA concentrations of leaf xylem sap indicated that highest concentrations were evident at midday, increasing threefold from early morning levels (Figure 6). This increase was concomitant with increases in VPD and decreases in \( \Psi_l \).

Leaf petiole xylem sap collected from vines from the MIA and HV indicated that ABA concentrations were linearly dependent on \( \Psi_l \), and thus highest in sap from plants with lowest \( \Psi_l \) (Figure 7a) \( (P < 0.001, R^2 = 0.73) \). Midday ABA concentrations were 10-fold greater in vines at moderate–severe stress \( (−1.6 \text{ MPa}) \) compared with unstressed vines early in the morning \( (−0.5 \text{ MPa}) \) (Figure 7a). Stomatal conductance was also correlated against the xylem sap ABA concentrations and exhibited a loose exponential relationship \( (P < 0.001, R^2 = 0.44) \) (Figure 7b). Exogenous ABA application to Semillon roots in a hydroponic system resulted in lower \( g \) \( (P < 0.01) \) and \( E \) \( (P < 0.003) \) as compared with control plants (Figure 8).

Abscisic acid applied at 100 \( \mu \text{mol l}^{-1} \) resulted in nearly complete stomatal closure and transpiration rates were fourfold less than control plants.

**Discussion**

**Air vapour pressure deficit and soil moisture effects on leaf water status**

Irrigation regime had an impact on the sensitivity of Semillon predawn \( \Psi_l \) to predawn VPD. In the drier vineyards, subjected to infrequent or no irrigation, predawn \( \Psi_l \) was highly dependent on soil moisture. Conversely, in the drip-irrigated vineyards predawn \( \Psi_l \) was more closely correlated to VPD than to soil moisture. Incomplete rehydration in response to high night-time VPD is indicative of significant night-time transpiration in these field vines. Previous detailed gas exchange measurements during the night have substantiated that grapevines do transpire at night and that the rate is dependent on variety as well as VPD (Rogiers et al. 2009). Predawn \( \Psi_l \) is sometimes used in irrigation scheduling as an indicator of soil moisture because it is assumed that during the night stomata are closed and \( E \) is negligible. Since in this anisohydric variety plant water status at dawn is not always directly related to soil moisture alone, irrigation scheduling will need to take into consideration this extra environmental parameter.

Irrespective of irrigation regime, midday \( \Psi_l \) in Semillon was dependent on both evaporative demand and soil moisture. In
each vineyard, the VPD and soil moisture interaction explained a greater proportion of the variance on midday \( \Psi_l \) than either variable alone. In the unirrigated and infrequently irrigated flood vineyards, soils contained on average lower levels of moisture than the drip-irrigated vineyards. The flood-irrigated vineyard was irrigated every 2–4 weeks and exposed vines, as observed by the mean soil moisture values, to a reasonable amount of water stress. As a consequence, midday \( \Psi_l \) was more negative in these vineyards. Air vapour pressure deficit and soil moisture explained up to 70% of the variance in midday \( \Psi_l \) in the unirrigated vineyard, and 50% in the infrequently flood-irrigated vineyard, indicating that in dry Semillon vineyards plant water stress can be exacerbated by high VPD. A similar interaction between these environmental parameters occurred in the drip-irrigated vineyards, with most negative midday \( \Psi_l \) during low soil moisture combined with high VPD.

The impact of VPD on midday \( \Psi_l \) has been well established in a number of other anisohydric species. For example, \( \Psi_l \) was...
correlated with VPD in prune (McCutchan and Shackel 1992),
sunflower (Tardieu et al. 1996) and barley (Tardieu and
Simonneau 1998). A similar VPD response has also been
reported for four other grape varieties (Thompson Seedless,
Merlot, Cabernet Sauvignon and Chardonnay) grown in a
semiarid environment under full, deficit or no irrigation
(Williams and Baeza 2007). However, not all grape varieties
have unstable $\Psi$. Grenache, in particular, has been classified
as near-isohydric based on its response to soil moisture defi-
cits (Schultz 1996, 2003). The diurnal $\Psi$ response of

Table 3. Linear regressions of $g_w$, $m$ and $m/g_w$ with REW of vines grown under differing irrigation systems in two grape-growing regions of NSW. The data have been fitted to $g = -m \times \ln(VPD) + g_w$ according to Oren et al. (1999), where $g_w$ is the reference conductance as VPD approaches 1 kPa (in mol m$^{-2}$ s$^{-1}$) and $-m$ is stomatal sensitivity to VPD in mol m$^{-2}$ s$^{-1}$ ln (kPa)$^{-1}$. F pr. denotes F probability and an asterisk highlights those F pr. that are <0.05.

<table>
<thead>
<tr>
<th>Vineyard/region</th>
<th>$g_w \times$REW</th>
<th>$-m \times$REW</th>
<th>$m/g_w \times$REW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>F pr.</td>
<td>$r^2$</td>
</tr>
<tr>
<td>Drip/MIA</td>
<td>$-0.08$</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Flood/MIA</td>
<td>0.20</td>
<td>&lt;0.001*</td>
<td>0.82</td>
</tr>
<tr>
<td>Drip/HV</td>
<td>$-0.08$</td>
<td>0.46</td>
<td>–</td>
</tr>
<tr>
<td>Unirrigated/HV</td>
<td>$-1.74$</td>
<td>0.04*</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Figure 4. Dependency of (a) $-m$ and $g_w$ and (b) $m/g_w$ as a function of REW across all vineyards. The data have been fitted to $g = -m \times \ln(VPD) + g_w$ according to Oren et al. (1999), where $g_w$ is the reference conductance as VPD approaches 1 kPa (in mol m$^{-2}$ s$^{-1}$) and $-m$ is stomatal sensitivity to VPD in mol m$^{-2}$ s$^{-1}$ ln (kPa)$^{-1}$. The inset demonstrates the relationship of $-m$ with $g_w$. 
Grenache grown under water stress to VPD indicated a lower \( g \) and less negative \( \Psi \) during the middle of the day compared with Shiraz, a variety of mesic origin (Soar et al. 2006). The sensitivity of midday \( \Psi \) to both VPD and soil moisture in Semillon provides further evidence for the anisohydric nature of this variety.

**Air vapour pressure deficit and soil moisture effects on stomatal conductance**

Stomatal conductance of Semillon field vines was not particularly responsive to VPD or soil moisture when vines were grown under drip irrigation; the stomata were relatively widely open, hence the decline in \( \Psi \), with increasing VPD. Under conditions
of ample soil moisture, the cooling benefit of high $g$ and thus high $E$ is known to prevent leaf injury caused by high temperatures (Gates 1968). Considering that Semillon leaves are quite susceptible to leaf burn in warm climates, the relative openness of stomata under high soil moisture conditions allows transpiration to cool the leaves and help prevent such damage.

Under infrequent irrigation Semillon stomata did respond to VPD. Lowest $g$ was observed in those vines grown at high VPD with very little soil moisture and $g_{sr}$ declined with decreasing soil moisture. Under low soil moisture $E$ is reduced and, therefore, the limited cooling benefit that can be attained by leaving stomata open during the middle of the day may be outweighed by the desiccation injury that could occur under the combined effects of high VPD and dry soil. Low stomatal conductance in high VPD under dry soils was observed in Shiraz, another variety otherwise traditionally classified as anisohydric (Collins et al. 2010). Partial rootzone drying and deficit irrigation resulted in those Shiraz vines behaving in a more isohydric-like manner. In contrast to control vines, total vine transpiration was tightly controlled when VPD increased, and it was suggested that the origin for this was increased stomatal control (Collins et al. 2010). This divergent response to irrigation regime in anisohydric varieties may explain why the magnitude of stomatal response to VPD, i.e., stomatal sensitivity to VPD as defined by Oren et al. (1999), was not consistent across our vineyards. Stomatal sensitivity increased with drying soil in the unirrigated site but there was no such relationship in the drip-irrigated or flood-irrigated vineyards. This inconsistency indicates that irrigation regime has an impact on how stomata respond to VPD, and this may be related to other factors such as rooting depth/volume, hydraulic conductance and resultant leaf water status.

High stomatal sensitivity to VPD in very dry soils but not in moderately dry to wet soils is a major finding of this study. It is interesting to note that the slope of $-m$ versus $g_{sr}$ across the sites was $\sim 1.1$ in our anisohydric variety. In other words, vines with high stomatal conductance at low VPD had greater sensitivity to VPD. This slope is higher than the previously reported value of 0.6 derived from empirical data across a number of species (Oren et al. 1999). In the same study, it was found that two desert species had slopes that were <0.6 while bottomland populations of the mesic species Quercus alba had a greater stomatal sensitivity than an upland population (Oren et al. 1999). The increase in stomatal sensitivity for species derived from wet regions may also be characteristic of anisohydric varieties which tend to have larger values of $g_{sr}$ as compared with isohydric varieties or species. Semillon originates from the mild, humid coastal region of Aquitaine, France, where low values of $g_{sr}$ may not provide an adaptive advantage. However, when such an anisohydric variety is exposed to hot, dry conditions under low soil moisture, stomata do need to respond to prevent catastrophically low plant water status, hence the greater stomatal sensitivity under these conditions. Conversely, in isohydric plants with low values of $g_{sr}$ such a strong relationship with stomatal sensitivity may not necessarily be required. Further work on the relationship between $g_{sr}$ and stomatal sensitivity in both isohydric and anisohydric...
varieties under a range of soil moistures is warranted to better understand how plant water status is maintained.

Stomatal conductance in the infrequently flood-irrigated vineyard was quite variable under moderate soil moisture. Similar to water stress, flooding is known to cause stomatal closure (Jackson 1993) and this may be the result of increased foliar ABA (Jackson and Hall 1987), which does not appear to be root derived (Else et al. 1996, Blanke and Cooke 2004, Jia and Zhang 2008). We did not, however, observe stomatal closure at high soil moisture levels in our field vines. This may simply be because we were unable to make measurements for several days following the flooding event (due to restricted access), and therefore we likely missed the period when g could have responded to hypoxia. Depth of flooding also impacts on stomatal responses (Iwanaga and Yamamoto 2008), and considering that grapevine roots can grow to depths >1 m, these vines may not have experienced extensive stress.

**Leaf water potential and ABA effects on stomatal conductance**

In Semillon, stomatal conductance was actively restricted in leaves with low $\Psi_l$, but the correlation was confined to conditions where vineyard soil moisture was low and $\Psi_l$ was less than $-1.5\, \text{MPa}$. In situations where the decline in $\Psi_l$ is the result of soil moisture stress, ABA signals from the roots are thought to induce stomatal closure rather than the low $\Psi_l$ itself (Zhang and Davies 1989, Davies and Zhang 1991). By applying pneumatic pressure to the roots of wheat and sunflower, it was observed that stomatal conductance declined in drying soil despite the maintenance of fully turgid leaves (Gollan et al. 1986). In that experiment it was proposed that the leaves sensed the drying soil through a signal that was sent up from the roots, and it was this signal rather than the decline in $\Psi_l$ to which conductance was responding to. Similarly, split root irrigation studies confirm the role of ABA signals in controlling stomatal closure in grapevines (Stoll et al. 2000). Our observations of a stomatal response to $\Psi_l$ only at low soil moisture would support a root signalling mechanism in Semillon vines. Abscisic acid was likely one of the messengers because stomatal conductance was lower in vines with high xylem sap ABA concentrations. A similar relationship between g and xylem sap ABA concentrations, with an attendant rise in xylem sap pH, was found in two varieties differing in sensitivity to water stress (Rodrigues et al. 2008). Stomatal closure in response to exogenous ABA applied to the roots confirms that Semillon stomata are able to perceive ABA transported through the transpiration stream.

In situations where the decline in $\Psi_l$ is due solely to extremes in evaporative demand, there is uncertainty in the mechanism inducing stomatal closure (Bunce 2006). It has been proposed that the narrowing of the stomatal aperture that occurs in many species in response to high evaporative demand may be in response to increasing transpiration rather than the increase in VPD itself (Mott and Parkhurst 1991, Monteith 1995). This ‘feedback’ mechanism is based on a decline in leaf water status or an increase in the gradient in water potential between the guard cells and the epidermal cells which could decrease the turgor pressure of guard cells relative to the epidermal cells (Bunce 1996). Diversity in stomatal density and epidermal cell types, however, may lead to variety differences in stomatal sensitivity to a particular $\Psi_l$. We did not observe stomatal closure in well-watered vines during high E induced by high VPD. However, $\Psi_l$ did not reach below $-1.5\, \text{MPa}$ under these conditions, indicating that the decline in leaf water content may not have been severe enough to induce stomatal closure in our anisohydric variety.

A second mechanism by which VPD can induce stomatal closure is by a ‘feedforward’ response through an increase in the concentration of ABA in the leaves and xylem sap (Farquhar 1978, Grantly 1990, Trejo et al. 1995). We found diurnal increases in ABA xylem sap concentrations for moderately water-stressed Semillon vines, as has been reported previously for Riesling and Sylvaner (Loveys 1984a, Loveys 1984b, Loveys and Düring 1984) as well as Shiraz and Grenache (Soar et al. 2006), and concentrations were in the order of what has been reported in these other varieties. Response of g to VPD was inconsistent in our Semillon, however. This can be explained by a model where sensitivity to VPD is dependent on soil moisture. Under low soil moisture, ABA production or mobilization in Semillon was likely stimulated by the roots and transported to the leaves, resulting in a decline in stomatal conductance. High VPD then brought about an additional drop in leaf water status through cuticular evaporation of water from the leaf’s surface, and this concentrated the existing ABA, eliciting a further decline in stomatal conductance. Conversely, under high soil moisture, it is likely that ABA production was not stimulated by the roots. The existing leaf ABA molecules were concentrated by the high VPD but the concentration was not high enough to elicit stomatal closure. Furthermore, alkaline apoplastic and xylem pH appears to reduce g and is thought to provide a pH-based ABA-dependent signalling mechanism that links the aerial microclimate with stomatal aperture (Wilkinson and Davies 2008). Perhaps this pH-based mechanism is not operative under high soil moistures in Semillon vines.

The role of hydraulic signals between roots and leaves under drying soils (Meinzer and Grantz 1990, Domec et al. 2006) also deserves consideration. When xylem pressures drop within the cavitation range in response to dry soil, stomata may close to prevent further declines in pressure, embolism and leaf death (Sperry et al. 1993). In *Betula occidentalis*, it was proposed that stomata may respond indirectly to reduced hydraulic conductance through changes in leaf water status (Sperry et al. 1993). With the additive VPD factor...
driving leaf water status further negative, the two environmental signals may act synergistically to control stomatal conductance. Moreover, it has even been suggested that stomatal conductance is dependent on the integration between hydraulic and chemical signals (Tardieu and Simonneau 1998, Comstock 2002, Jia and Zhang 2008). In the isohydric species Commelina communis, for instance, a hydraulic signal enhanced stomatal sensitivity to root-derived ABA (Tardieu and Davies 1993). In maize and poplar, both isohydric, stomatal response to concentrations of ABA in the xylem sap changed with VPD or leaf water status, but this was not the case for anisohydric sunflower and barley (Tardieu and Simonneau 1998). The irrigation-dependent stomatal response in our Semillon indicates that further work is required to resolve the interaction between chemical and hydraulic signals to understand how they may act synergistically to control stomatal behaviour.

Conclusion

Semillon is anisohydric and predawn $\Psi_l$ was closely correlated to soil moisture in dry vineyards but to night-time VPD in drip-irrigated vineyards, with incomplete rehydration during high night-time VPD. Midday vine water status was also dependent on both soil moisture and evaporative demand. Stomatal conductance was responsive to midday $\Psi_l$ but only if values fell below $-1.5$ MPa. Stomatal response to soil moisture and VPD was inconsistent and dependent on irrigation regime; however, in the unirrigated vineyard stomatal sensitivity to VPD increased with declining soil moisture. Semillon stomata were responsive to exogenous ABA, and ABA concentrations within the petiole xylem sap increased with vine water stress. While the increase in ABA concentrations of leaf petiole xylem sap as a result of drying soil were likely the result of ABA synthesis/mobilization from the roots, the concentration increase as a result of high VPD was likely a by-product of lower vine water status and concentration of existing ABA. Therefore, the increase in stomatal response to high VPD in drying soil as compared with well-watered soils is likely the result of higher leaf ABA concentrations through leaf dehydration combined with the transport and accumulation of root-derived ABA and possibly other hydraulic signals.

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