

# A continuum of stomatal responses to water deficits among 17 wine grape cultivars (*Vitis vinifera*)

Alexander D. Levin <sup>A,B,E</sup>, Larry E. Williams<sup>C,D</sup> and Mark A. Matthews<sup>C</sup>

<sup>A</sup>Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA.

<sup>B</sup>Southern Oregon Research and Extension Center, 569 Hanley Road., Central Point, OR 97502, USA.

<sup>C</sup>Department of Viticulture and Enology, University of California, Davis, CA 95616, USA.

<sup>D</sup>Kearney Agricultural Research and Extension Center, 9240 S. Riverbend Avenue., Parlier, CA 93648, USA.

<sup>E</sup>Corresponding author. Email: alexander.levin@oregonstate.edu.

**Abstract.** *Vitis vinifera* L. cultivars have been previously classified as isohydric, near-isohydric, anisohydric or isohydrodynamic, depending on the study. To test the hypothesis that *V. vinifera* cultivars' stomatal behaviour can be separated into distinct classes, 17 cultivars grown in a replicated field trial were subjected to three irrigation treatments to manipulate vine water status across multiple years. Predawn ( $\Psi_{PD}$ ) and midday ( $\Psi_1$ ) leaf water potential and midday stomatal conductance ( $g_s$ ) were measured regularly throughout several seasons. The relationship of  $g_s$  to  $\Psi_1$  was best modelled as a sigmoidal function and maximum stomatal conductance ( $g_{max}$ ), water status at the onset of stomatal closure ( $\Psi_1^{95}$ ), sensitivity of closure ( $g_{sensitivity}$ ) and water status at the end of closure ( $\Psi_1^{25}$ ) were compared. There were no significant differences in  $g_{max}$  among cultivars. Cultivar-specific responses of  $g_s$  to  $\Psi_1$  were broadly distributed along a continuum based on the relationship between  $\Psi_1^{95}$  and  $g_{sensitivity}$ . Season-long cultivar mean  $\Psi_1$  values were positively related to  $\Psi_1^{25}$ . In general, cultivars responded similarly to one another at high and low water status, but their stomatal behaviour differed at moderate water deficits. The results show that *V. vinifera* cultivars possess both iso- and anisohydric stomatal behaviours that depend on the intensity of water deficits, and call into question previous classifications assuming a single behaviour.

**Additional keywords:** genotypic variation, grapevine physiology, stomatal conductance, water potential.

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## Introduction

Increasing demand for fresh water in the face of finite resources is intensifying pressure to improve water use efficiency in agriculture in general, and in crop production in particular. Because regulation of stomatal aperture plays a major role in plant control of water loss in dry environments, it has been the focus of intensive research directed towards improved drought tolerance and water use efficiency. For example, reduced maximum transpiration rate (Sinclair *et al.* 2005) and early stomatal closure (Martin-St. Paul *et al.* 2017) have been proposed as traits of drought avoidance. In both cases, the putative benefit in yield stability arises from postponing dehydration. Indeed, the sensitivity of stomata to water deficits could be viewed as a measure of drought avoidance (Jones 1974; Bates and Hall 1981) and is sometimes described in terms of 'isohydric' vs 'anisohydric' behaviour (Tardieu and Simonneau 1998).

In addition to species variation, it has long been known that genetic variation in stomatal response to water deficits is present within crop species (e.g. in sorghum, Henzell *et al.* 1976; e.g. in rice, Dingkuhn *et al.* 1989). Schultz (2003) provided some evidence of such genetic variation within grapevine, *Vitis*

*vinifera* L., but there have since been contradictory reports of cultivar-specific responses (Chaves *et al.* 2010; Costa *et al.* 2012). Ambiguity over the precise definition of what constitutes an iso- vs anisohydric response (Hochberg *et al.* 2018) has caused difficulties in categorisation of either cultivars within a species (Lavoie-Lamoureux *et al.* 2017) or across many species (Klein 2014; Martinez-Vilalta and Garcia-Forner 2017). However, in general, isohydric species/cultivars will maintain water status similar between droughted and well-watered plants (Tardieu and Simonneau 1998) or that a measure of plant water status (such as leaf water potential) will not decrease below a minimum value on a diurnal or seasonal basis (Schultz 2003). Such a response by a crop species may minimise the usefulness of assessing plant water status via the measurement of leaf water potential. Cifre *et al.* (2005) concluded that if grapevine cultivars respond to soil water deficits in an isohydric manner, then midday leaf water potential cannot be used in an irrigation management program or as one would suspect the validation of other means to assess plant water status (Williams 2017).

Based on observed responses of plant water relations and gas exchange, some grapevine cultivars such as Grenache, have been

classified as isohydric (Schultz 2003; Cifre *et al.* 2005) whereas others, such as Syrah and Thompson Seedless have been classified as anisohydric (Schultz 2003; Soar *et al.* 2006b; Williams and Baeza 2007; Williams 2012). However, the discrepancy among cultivars is not entirely clear. This has led some researchers to modify the binary categorisation (i.e. isohydric vs anisohydric) and propose that cultivars may modulate their stomatal response (Domec and Johnson 2012) based on changes in soil moisture (Collins *et al.* 2010), or differences in leaf to air vapour pressure deficit (VPD) (Soar *et al.* 2006b). In addition, acclimation of stomatal response to water deficits has been shown in grapevine through a modification of leaf hydraulic conductivity and osmotic adjustment (Martorell *et al.* 2015; Hochberg *et al.* 2017a). Accordingly, alternative classifications such as ‘near-isohydric’ and ‘isohydrodynamic’ (a relatively constant difference between predawn ( $\Psi_{PD}$ ) and midday leaf water potential ( $\Psi_l$ ) throughout seasonal moisture cycles) have been offered to account for differences in stomatal response to drought among cultivars and the resulting effects on plant water status (Schultz 2003; Franks *et al.* 2007; Chaves *et al.* 2010; Shellie and Bowen 2014).

Putative cultivar differences in stomatal response would be important for water-conserving vineyard management strategies because  $g_s$  is highly correlated with daily water use (Williams *et al.* 2012), and because moderate water deficits have positive effects on fruit quality (Roby *et al.* 2004). Furthermore, relative to vegetative growth, reproductive growth of irrigated grapevines is less affected when applied water amounts are reduced by up to 40% of crop evapotranspiration (Williams *et al.* 2010a, 2010b). As such, some water deficit is beneficial in production of horticultural crops like wine grapes in contrast to production of agronomic crops like rice or sorghum.

The relationship between  $g_s$  and  $\Psi_l$  in grapevine is affected by numerous factors; predominate among them are rootstock and cultivar, accounting for 19 and 16% of the variability in that relationship, respectively (Lavoie-Lamoureux *et al.* 2017). Presumably, a large proportion of the remaining variation in response would be accounted for by plant-environment interactions (Hochberg *et al.* 2018). As mentioned above, soil moisture, VPD and plant hydraulic properties have all been shown to influence the relationship between  $g_s$  and  $\Psi_l$  across grapevine cultivars. However, since the initial classification by Schultz (2003), and numerous subsequent attempts by others – as reviewed by Chaves *et al.* (2010) – there have been no studies that have directly compared a large set of cultivars grown under the same environment conditions (soil and atmosphere) grafted onto the same rootstock in a replicated field trial.

The goal of this study was 2-fold: to (1) determine if numerous *V. vinifera* cultivars separate into distinct iso- or anisohydric classes in response to soil water deficits from measurements of predawn ( $\Psi_{PD}$ ) and midday leaf water potential ( $\Psi_l$ ); and (2) explore the genetic variation in the relationship of  $g_s$  to  $\Psi_l$  among these cultivars over several seasons. To accomplish this, the study was designed to maximise the effect of cultivar on the relationships in question by controlling for numerous factors that have all been shown to be influential such as rootstock, environment, instrument operator, and sampling methodology. Several grapevine cultivars evaluated in this study have been

previously classified as being isohydric (or near-isohydric) – e.g. Cabernet Sauvignon, Grenache, Tempranillo and Touriga Nacional – while others have been classified as anisohydric – e.g. Cabernet Sauvignon, Grenache, Montepulciano, Syrah (syn. Shiraz) and Touriga Nacional (Schultz 2003; Chouzouri and Schultz 2005; Soar *et al.* 2006b; Williams and Baeza 2007; Chaves *et al.* 2010; Lovisolo *et al.* 2010; Rodrigues *et al.* 2012). Finally, an additional 11, red wine grape cultivars were evaluated representing a broad range of plant material grown in warmer viticultural regions around the world.

## Materials and methods

### *Vineyard site, design, and plant material*

The experimental site used in this study was an existing variety trial that was planted with 1103 Paulsen (1103P) (*Vitis berlandieri* Planch.  $\times$  *Vitis rupestris* Scheele) rootstock in June 2003 at the University of California Kearney Agricultural Research and Extension (KARE) Center near Parlier, California (36°48'N, 119°30'W). The soil was a Hanford fine sandy loam. The total vineyard area was ~0.53 ha. Vine and row spacing was 1.83 and 3.05 m respectively (5.58 m<sup>2</sup> per vine).

The trellis system consisted of a 0.3 m cross-arm atop a stake, 1.7 m above the vineyard floor. Fixed catch wires were positioned at each end of the cross-arms. Vines were trained to bilateral cordons on a single fruiting wire at a height of 1.2 m, and were pruned to spurs. In early spring, shoots were thinned to a density of ~15 shoots per meter of row. The vineyard was drip-irrigated with two, 2 L h<sup>-1</sup> emitters per vine placed 0.5 m on either side of the trunk. The dripline was attached to a wire installed 0.5 m above the vineyard floor.

The vineyard layout consisted of 20 rows with 48 vines per row. In May 2004, 20 different *Vitis vinifera* L. cultivars were field-budded onto the rootstock. Foundation Plant Services (University of California, Davis, CA, USA) supplied budwood for the scion varieties. The cultivars, clonal selection, and sources of budwood are given in Table 1. Carmenere, Tinta Francisca and Tinta Cão were not included in this study due to foliar symptoms of viral infection.

### *Irrigation treatments*

Three irrigation treatments were imposed beginning in the 2012 growing season as detailed below.

- (1) Late deficit (LD) – irrigated at 100% of estimated ET<sub>c</sub> from berry set until the onset of ripening (veraison); no applied water from veraison until harvest.
- (2) Sustained deficit (SD) – irrigated at 50% of estimated ET<sub>c</sub> throughout the entire growing season.
- (3) Early deficit (ED) – no applied water from berry set until veraison; irrigated at 50% of estimated ET<sub>c</sub> from veraison until harvest.

Prior to berry set, all vines were irrigated at 100% of estimated ET<sub>c</sub> to maintain midday leaf water potential ( $\Psi_l$ ) at or above -1.0 MPa. After berry set, irrigation treatments were imposed once  $\Psi_l$  reached the threshold value of -1.0 MPa (averaged across cultivars). Drip emitters were plugged or unplugged to impose the irrigation treatments, and inline water meters were used to

**Table 1. Clone and source of budwood for the 20 cultivars planted at the Kearney Agricultural Research and Extension Centre**

| Cultivar                     | Clone | Source of budwood                  |
|------------------------------|-------|------------------------------------|
| Aglianico                    | 03    | VCR 2 Rauscedo, Italy              |
| Carmenere <sup>A</sup>       | 02    | VCR 702 Rauscedo, Italy            |
| Cabernet Sauvignon           | 08    | #102, Concannon, CA                |
| Cinsault                     | 02    | Black Malvoisie FPS 02, Sonoma, CA |
| Durif                        | 03    | Petite Sirah FPS 03                |
| Freisa                       | 01    | Jackson, CA                        |
| Grenache noir                | 515   | ENTAV-INRA 515, (ENTAV, France)    |
| Malbec                       | 06    | VEN, UC Davis, syn. Cot            |
| Montepulciano                | 02    | VCR 10 Rauscedo, Italy             |
| Petit Verdot                 | 400   | ENTAV-INRA 400 (ENTAV, France)     |
| Refosco                      | 03    | VCR 5 Rauscedo, Italy              |
| Souzao                       | 01    | VEN, UC Davis                      |
| Syrah                        | 07    | France 877                         |
| Tannat                       | 474   | ENTAV-INRA 474 (ENTAV, France)     |
| Tempranillo                  | 02    | AGRO, Spain                        |
| Tinta Amarela                | 01    | Jackson, CA                        |
| Tinta Francisca <sup>A</sup> | 01    | Portugal                           |
| Tinta Madeira                | 01    | Lodi, CA                           |
| Tinto Cao <sup>A</sup>       | 04    | Jackson, CA                        |
| Touriga Nacional             | 02    | Portugal, 1981                     |

<sup>A</sup>Cultivars were not included in the study.

quantify applied water amounts. Veraison timing was averaged across replicates of a cultivar-treatment combination (e.g. separately quantified for Syrah-ED vs Syrah-LD). Therefore, treatment transition (e.g. in the ED and LD treatments) occurred over a period of several weeks across cultivars. After harvest, all vines were irrigated at 50% of estimated  $ET_c$  until leaf fall.

Vineyard  $ET_c$  was estimated using the following equation:  $ET_c = ET_o \times K_c$ , where  $ET_o$  is reference ET and  $K_c$  is the crop coefficient. Prior to the initiation of the experiment, seasonal crop coefficients were developed in this vineyard across seven growing seasons during which vines were kept well watered, so as to not inhibit canopy development (LE Williams, unpubl. data). They were determined by measuring the shade cast on the ground beneath the canopy, and then using the relationship between the percentage of canopy shaded area and  $K_c$  according to Williams and Ayars (2005). Finally, a relationship between  $K_c$  and accumulated growing degree-days (GDD) was established. During the experiment, evolution of the  $K_c$  over the course of the growing season was calculated using accumulated GDD from average budbreak. GDD data were downloaded from the University of California Statewide Integrated Pest Management Project's website ([www.ipm.ucdavis.edu](http://www.ipm.ucdavis.edu), accessed 12 September 2019).  $ET_o$  and GDD data were obtained from the CIMIS weather station at the KARE Center (Parlier #39, California Irrigation Management Information System). GDD were calculated using the single sine method with a lower threshold of 10°C.

#### Vine water status measurements

Vine water status (water potential;  $\Psi$ ) was measured according to (Williams and Araujo 2002) using a pressure chamber (Model 1000, PMS Instrument Co.). Specifically, predawn water

potential ( $\Psi_{PD}$ ) measurements began at ~0400 h Pacific daylight time (PDT) and were completed before sunrise, whereas midday leaf water potential ( $\Psi_1$ ) measurements were generally taken between 12:30 and 13:30 hours PDT.  $\Psi_{PD}$  was only measured in 2012. Leaves chosen at predawn and at midday were fully expanded, mature leaves – and at midday exposed to direct solar radiation. Leaf blades chosen across all times of day were covered with a plastic bag, quickly sealed, and petioles then cut within 1 to 2 s (s). Time between leaf excision and pressurisation was generally between 10 to 15 s.

Stomatal conductance ( $g_s$ ) was measured with a steady-state diffusion porometer (LI-1600, LI-COR Biosciences). The porometer was allowed to warm-up to ambient vineyard conditions (placed in the shade) at least 30 min (min) before the first measurement. The null point humidity was set to ambient after warm-up and before first measurements, and subsequently reset every 20 to 30 min. Measurements were recorded after steady-state conditions were reached (10–20 s). Care was taken to reduce sensor head exposure to direct sunlight in between measurements so as not to increase cuvette temperature. Sampled leaves were of same age and position in canopy as for  $\Psi_1$  measurements described above, thus ensuring light saturation.

During the 2012 growing season, the measurements of  $\Psi_1$  and  $g_s$  were made by LE Williams for all cultivar/irrigation treatment replicates and required two days. In 2013, measurement techniques were refined by AD Levin (data not reported). During the last 2 years of the study,  $g_s$  was measured on the same leaves used for the measurement of  $\Psi_1$ , just before leaf excision. Sample leaves were selected for  $g_s$ , which was measured by LE Williams, and  $\Psi_1$  was measured by AD Levin both years, thus minimising potential operator error (Goldhamer and Fereres 2001). Measurements began at 12:00 hours and were completed by 14:00 hours those 2 years. A single leaf from each three-vine irrigation treatment replicate was measured and used for data analysis ( $n = 3-4$ ). There were two, 16, and four measurement dates for the 2012, 2014 and 2015 growing seasons respectively.

#### Curve fitting stomatal response to vine water status

Stomatal response to  $\Psi_1$  was characterised with the following sigmoidal model:

$$g_s = \frac{g_{max}}{1 + e^{\left[\frac{\Psi_1^{50} - \Psi_1}{b}\right]}} \quad (1)$$

where  $\Psi_1$  is midday leaf water potential,  $g_{max}$  is the asymptote that represents a theoretical maximum stomatal conductance,  $\Psi_1^{50}$  is the inflection point of the curve that represents the value of  $\Psi_1$  at which stomatal conductance is half of the maximum, and  $b$  is a scaling parameter that represents the transition width of the function. From the fitted curves, vine water status values were extracted at fixed values of  $g_s$  relative to  $g_{max}$  ( $\Psi_1^{95}$ ,  $\Psi_1^{75}$ ,  $\Psi_1^{50}$  and  $\Psi_1^{25}$  represent the estimated  $\Psi_1$  at 95, 75, 50 and 25% of  $g_{max}$  respectively) for each cultivar, with  $\Psi_1^{95}$  assumed to be the threshold for the beginning of stomatal closure. The value of  $b$  was determined to be equal to  $(\Delta\Psi_1^{75-25} - 0.002) \times 2.196^{-1}$  by linear regression, where  $\Delta\Psi_1^{75-25}$  is the difference in  $\Psi_1$  (MPa) between 75 and 25% of  $g_{max}$  (data not shown;  $R^2 = 0.99$ ,  $P < 0.001$ ). The instantaneous slope of the fitted sigmoidal

curve was calculated as the first derivative of the function at the inflection point and was taken as a measure of the maximum sensitivity of stomatal closure ( $g_{\text{sensitivity}}$ ;  $\text{mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ).

#### *Experimental design and data analysis*

The experimental design was a randomised complete block with a strip-plot factorial treatment structure and four replications. Experimental blocks were assigned across the rows (i.e. 20 rows per block), and scion cultivars (main plot) were field-budded down the rows in a pattern of 12 vines per cultivar per block (i.e. four cultivars per row). The three irrigation treatments (strip plots) within each block were arranged down the rows in a line-source design (Williams *et al.* 2010a, 2010b), alternating sequentially among blocks from LD-SD-ED to ED-SD-LD such that vines in adjacent blocks received the same irrigation treatment. One non-irrigated vine was included among irrigation treatments within a block, such that an experimental unit consisted of the three remaining vines in the irrigation treatment.

Statistical analyses and graphics were done using *R* statistical software (R Core Team 2018). Linear regression, non-linear regression, and ANOVA were conducted using the functions *lm*, *nls* and *anova*, respectively, from *R* base package *stats*. For the analyses of  $\Psi_1$  and  $g_s$  over time, a linear mixed-effects model was fit using the *lmer* function from the packages *lme4* and *lmerTest* with cultivar, irrigation treatment, and sample date (and all interactions) as fixed factors, and block (and its interactions with the fixed factors) as a random factor (Bates *et al.* 2015; Kuznetsova *et al.* 2017). Calculation of least-squares means and multiple comparisons were conducted using the package *lsmeans* and the Tukey-Kramer adjustment method for multiplicity (Lenth 2016). Prior to non-linear regression analyses of  $g_s$  in response to  $\Psi_1$ ,  $g_s$  values were pooled and means calculated across cultivars at each 0.1 MPa of  $\Psi_1$ . Smoothed cubic regression splines were fitted using the *gam* function from the package *mgcv*. (Wood 2017). Data were plotted using the package *ggplot2* (Wickham 2016).

## Results

### *Relationships between $\Psi_1$ and $\Psi_{PD}$*

Predawn ( $\Psi_{PD}$ ) and midday leaf water potential ( $\Psi_1$ ) were measured on vines in each irrigation treatment on numerous dates during the 2012 growing season. Although there were significant differences among irrigation treatments in both measures of vine water status, there were never significant differences among cultivars within an irrigation treatment. On 24 July 2012, just before changing the irrigation treatments that year, mean  $\Psi_{PD}$  ( $\pm$ s.d.) of vines in the ED treatment averaged across cultivars was  $-1.18 \pm 0.13$  MPa whereas those in the LD treatment averaged  $-0.13 \pm 0.02$  MPa. Averaged across cultivars, midday  $\Psi_1$  of the ED and LD treatments were  $-1.78 \pm 0.02$  and  $-0.98 \pm 0.02$  MPa, respectively, whereas that of the SD treatment averaged  $-1.28$  MPa. Ambient temperature and VPD in the vineyard at the time of measurement (midday) was  $34.9^\circ\text{C}$  and  $4.63$  kPa respectively. Notably, mean  $\Psi_1$  of Cabernet Sauvignon, Grenache and Syrah on that day was  $-1.76 \pm 0.04$ ,  $-1.86 \pm 0.07$  and  $-1.80 \pm 0.05$  MPa, respectively. Stomatal conductance for the ED and LD

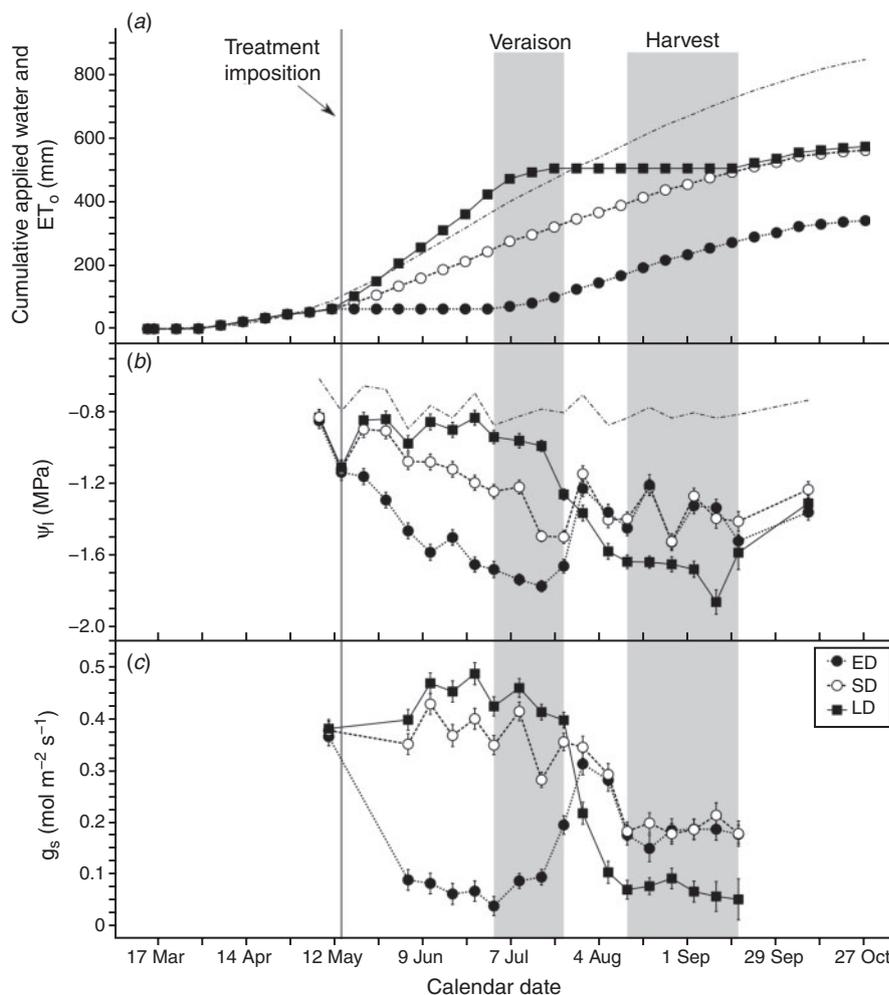
treatments on that day averaged  $0.089$  and  $0.568$   $\text{mol m}^{-2} \text{s}^{-1}$  respectively.

Midday  $\Psi_1$  of the LD treatment averaged across cultivars on 29 August (more than 5 weeks after irrigation had ceased for that treatment) was  $-1.76 \pm 0.05$  MPa. Midday  $\Psi_1$  of two vines of each cultivar (located at the end of the row on the east side of the vineyard) still being irrigated at 100% of estimated  $\text{ET}_c$  averaged  $-0.92 \pm 0.01$  MPa. Midday  $\Psi_1$  of vines irrigated at 50% of  $\text{ET}_c$  averaged  $-1.45$  MPa. Ambient temperature and VPD at the time of measurement were  $33.9^\circ\text{C}$  and  $4.11$  kPa, respectively. On the last measurement date in 2012 (5 and 6 September) mean  $\Psi_{PD}$  and midday  $\Psi_1$  across cultivars were  $-0.73 \pm 0.01$  and  $-1.79 \pm 0.02$  MPa respectively. Midday  $\Psi_1$  of Cabernet Sauvignon, Grenache and Syrah on those dates were  $-1.84$ ,  $-1.78$  and  $-1.80$  MPa respectively. The slope of the linear relationship between midday  $\Psi_1$  and  $\Psi_{PD}$  using data from Cabernet Sauvignon, Grenache and Syrah across the 2012 growing season was  $0.784$  with an  $R^2$  value of  $0.73$  ( $P = 0.001$ ). Notably, the individual slopes of the three cultivars did not differ significantly from one another.

### *$\Psi_1$ and $g_s$ seasonal responses to irrigation treatments*

In general, seasonal responses of  $\Psi_1$  and  $g_s$  to irrigation treatments were sensitive and consistent over the course of each growing season. The seasonal pattern of applied water amounts and responses of both water status variables ( $\Psi_1$  and  $g_s$ ) during 2014 is representative of those for the other growing seasons (Fig. 1). Prior to treatment imposition, all vines received 62 and 41 mm of applied water in 2014 and 2015 respectively. Following treatment imposition (12 and 11 May in 2014 and 2015 respectively), vines were irrigated at previously defined rates with applied water amounts illustrated by the curves of each treatment (Fig. 1a).  $\Psi_1$  and  $g_s$  were significantly affected by the irrigation treatments, with three levels of water stress established before veraison, and two levels of water stress established after veraison in each year of the study (Fig. 1b, c). While  $\Psi_1$  and  $g_s$  responded similarly to the irrigation treatments, there were small, but notable differences in each variable's response to an irrigation treatment during each phenological period.

Before veraison,  $\Psi_1$  and  $g_s$  responses were like one another in the ED and LD irrigation treatments, but they responded differently to the SD treatment. Both  $\Psi_1$  and  $g_s$  values in LD vines remained high early in the season, with  $\Psi_1$  closely following the calculated non-stressed baseline  $\Psi_1$  value, and  $g_s$  increasing slightly from berry set to a maximum near the end of June (just before veraison). Similarly,  $\Psi_1$  and  $g_s$  values in ED vines both declined immediately following treatment imposition and were significantly lower compared with SD and LD at every measurement date from berry set until veraison (Fig. 1b, c). In contrast, while  $\Psi_1$  for SD vines declined after treatment imposition and was significantly different than that of LD vines from the third measurement date after treatment imposition,  $g_s$  response was less sensitive compared with  $\Psi_1$ , and remained high in SD relative to LD for a longer period before veraison (Fig. 1b, c). Overall, preveraison  $g_s$  was less sensitive to the SD irrigation treatment and remained high relative to that of LD vines despite a constantly declining  $\Psi_1$  until veraison.



**Fig. 1.** Seasonal time course of applied water (a), leaf water potential ( $\Psi_l$ ) (b), and stomatal conductance ( $g_s$ ) (c) for each irrigation treatment in 2014 (representative of all years in the study). (b, c) Data are means ( $\pm$ s.e.) calculated at each time point across all 17 cultivars. (a) Dot-dashed line without points is the estimated  $ET_c$ . (b) Dot-dashed line without points is a non-stressed baseline  $\Psi_l$ . (c) First group of points are from 2015. See 'Materials and methods' section for definition of irrigation treatments.

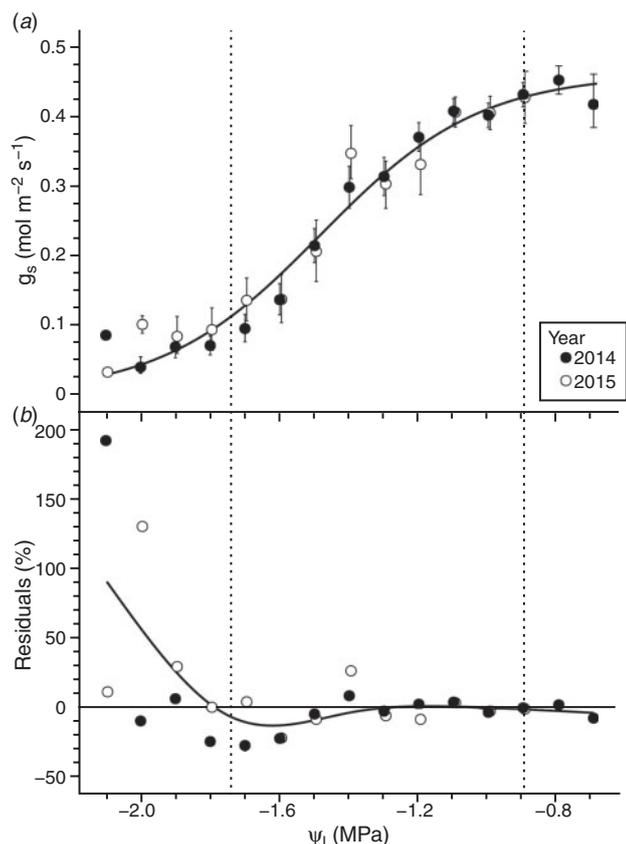
Following veraison,  $\Psi_l$  and  $g_s$  recovered in ED vines once irrigation water was reapplied, and both variables declined in LD vines when water was withheld (Fig. 1b, c).  $\Psi_l$  of LD vines was significantly lower than that of SD and ED beginning with the first measurement postveraison and remained so until harvest.  $\Psi_l$  and  $g_s$  of ED vines recovered to values like those of SD vines and were not significantly different from SD for the remainder of the season. In general, postveraison  $\Psi_l$  was less stable week-to-week compared with postveraison  $g_s$ , whereas preveraison  $\Psi_l$  was more stable week-to-week compared with preveraison  $g_s$ .

#### *The response of $g_s$ to $\Psi_l$ : across and among cultivars*

The response of  $g_s$  to  $\Psi_l$  is summarised for all cultivars analysed together and individually in Figs 2 and 3 respectively. Because year was not a significant factor in either analysis – for all cultivars together or for all cultivars individually – data from the 2014 and 2015 growing seasons were analysed together.

When all cultivars were analysed together in a composite analysis,  $\Psi_l^{95}$  and  $\Psi_l^{25}$  were estimated to be  $-0.89$ , and  $-1.74$  MPa respectively (Fig. 2a). Across all cultivars, the response of  $g_s$  to  $\Psi_l$  was well characterised by the sigmoidal function at  $\Psi_l$  values greater than  $\Psi_l^{25}$ , with relative residuals generally varying within  $\sim 30\%$  from the fitted curve, and in most cases within  $\sim 10\%$ . Below  $\Psi_l^{25}$ , the response of  $g_s$  to  $\Psi_l$  diverged from the fitted curve with a corresponding increase in residuals to values greater than 30% of the fitted values (Fig. 2b).

The three-parameter logistic function was well fit to all cultivars individually as well (Fig. 3), with adjusted  $R^2$  values ranging from 0.72 (Durif) to 0.94 (Aglianico) (Table 2). From the fit sigmoidal functions, parameter estimates and modelled values at various levels of vine water status were compared across cultivars (Tables 2, 3). Excluding Syrah, the coefficient of variation (CV) in estimates of  $g_{max}$  was less than 7% across cultivars, ranging from 0.398 (Grenache) to 0.489  $\text{mol m}^{-2} \text{s}^{-1}$  (Refosco). The estimate of  $g_{max}$  ( $\pm$ s.e.) for Syrah ( $0.595 \pm 0.130$



**Fig. 2.** (a) Stomatal conductance ( $g_s$ ) at various leaf water potentials ( $\Psi_1$ ) averaged across all 17 cultivars with data collected in 2014 and 2015. Within a given year,  $g_s$  values were pooled and means calculated across cultivars at each 0.1 MPa of  $\Psi_1$ . Data are means  $\pm$  95% confidence intervals. The solid line is a non-linear regression line fit with a sigmoidal function (adjusted  $R^2 = 0.96$ ). (b) The plot of residuals as percent deviation from the fitted curve. Curve is fit using a smoothed cubic regression spline. Vertical dotted lines represent  $\Psi_1$  values at  $\Psi_1^{25}$  and  $\Psi_1^{95}$  according to the sigmoidal function in the top panel.

$\text{mol m}^{-2} \text{s}^{-1}$ ) was 1.2 times larger than the next greatest estimate (Refosco;  $g_{\text{max}} = 0.489 \pm 0.029 \text{ mol m}^{-2} \text{s}^{-1}$ ), but with a large standard error (Table 2). Interestingly, the range and standard deviation (s.d.) of measured  $g_{\text{max}}$  values among cultivars – measured range =  $0.151 \text{ mol m}^{-2} \text{s}^{-1}$ ; measured s.d. =  $0.0428 \text{ mol m}^{-2} \text{s}^{-1}$  – was smaller than those of estimated  $g_{\text{max}}$  values among cultivars – estimated range =  $0.196 \text{ mol m}^{-2} \text{s}^{-1}$ ; estimated s.d. =  $0.0461 \text{ mol m}^{-2} \text{s}^{-1}$ . In either case – when comparing estimated or measured values – there were no significant differences in  $g_{\text{max}}$  among cultivars.

For several cultivars, the estimated asymptotic  $g_{\text{max}}$  value from the sigmoidal fit was greater than the highest actual measured mean  $g_{\text{max}}$  value (e.g. Syrah and Tinta Madeira,) or there were few data points near the estimated  $g_{\text{max}}$  (e.g. Tinta Amarela) (Fig. 3). One consequence was that the derived  $\Psi_1^{95}$  values fell outside of the measured values for Syrah and Tinta Madeira, or that the estimated  $g_{\text{max}}$  value was a poor representation of a cultivar's actual  $g_{\text{max}}$ . Since subsequent

analyses depended upon correct determination of  $g_{\text{max}}$ , the estimated  $g_{\text{max}}$  values were regressed on the highest mean measured  $g_{\text{max}}$  (taken at the highest  $\Psi_1$ ). There was a statistically significant relationship between estimated and measured  $g_{\text{max}}$  ( $P < 0.05$ ), though there were some notable outliers (e.g. Syrah and Durif) that weakened the relationship ( $R^2 = 0.31$ ). Nevertheless, the estimated slope of the relationship was not significantly different than unity (slope  $\pm$  95% confidence interval =  $0.599 \pm 0.493$ ). Thus, it was assumed that the estimated  $g_{\text{max}}$  value from the sigmoidal fit was representative of actual  $g_{\text{max}}$  for all subsequent analyses.

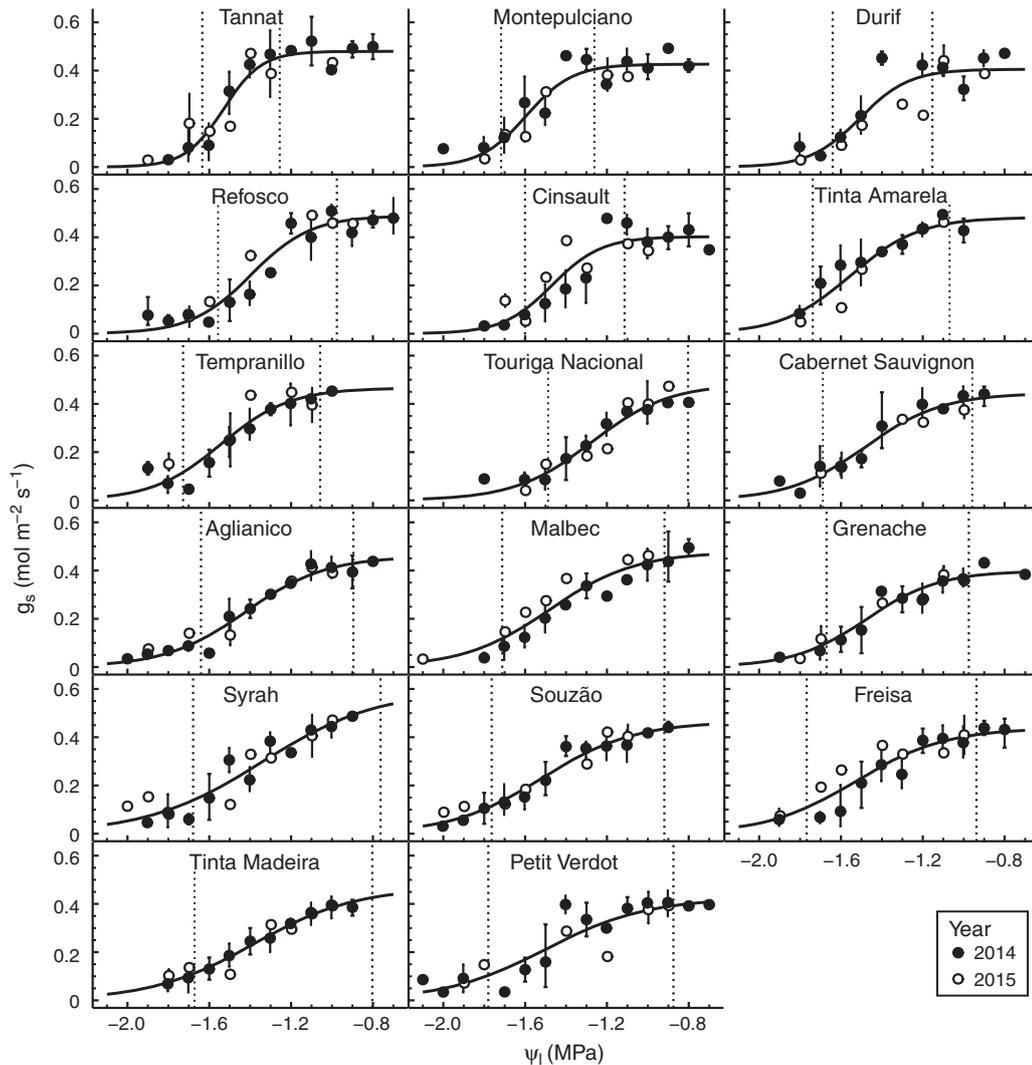
The CV was similarly low (6%) across cultivars for estimates of  $\Psi_1^{50}$ , which ranged 0.32 MPa from  $-1.27$  (Touriga Nacional) to  $-1.59$  MPa (Montepulciano) with no differences among cultivars. In contrast, the unitless scalar parameter  $b$  – the transition width of the function related to the slope – varied broadly among cultivars (CV = 29%). Accordingly, there was broad variation in estimates of  $g_{\text{sensitivity}}$  (CV = 32%) among cultivars, with values ranging 3-fold from 0.428 (Petit Verdot) to  $1.283 \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$  (Tannat). Further,  $g_{\text{sensitivity}}$  was 0.562 and  $0.525 \text{ mol m}^{-2} \text{s}^{-1} \text{ MPa}^{-1}$  for Grenache and Syrah respectively (Table 3).

When comparing modelled  $\Psi_1$  and  $g_s$  values among cultivars at each extracted vine water status value (i.e.  $\Psi_1^{95}$ ,  $\Psi_1^{50}$  and  $\Psi_1^{25}$ ), the across-cultivar CV for modelled  $\Psi_1$  decreased with vine water status (i.e.  $\Psi_1^{95} > \Psi_1^{50} > \Psi_1^{25}$ ), whereas across-cultivar CV for modelled  $g_s$  remained constant at each value (i.e.  $\Psi_1^{95} = \Psi_1^{50} = \Psi_1^{25}$ ) (Table 3). This was not surprising given that the model was fit to minimise variation in  $g_s$ . The across-cultivar mean modelled  $g_s$  and  $\Psi_1$  at high water status (i.e.  $\Psi_1^{95}$ ) was more than  $0.4 \text{ mol m}^{-2} \text{s}^{-1}$  and  $-0.99$  MPa, respectively, whereas the across-cultivar mean modelled  $g_s$  and  $\Psi_1$  at low water status (i.e.  $\Psi_1^{25}$ ) was near  $0.1 \text{ mol m}^{-2} \text{s}^{-1}$  and  $-1.68$  MPa. Furthermore, the cultivars with high  $g_s$  at high vine water status ( $\Psi_1^{95}$ ) tended to have similarly high  $g_s$  at low vine water status. For example, Syrah had the highest  $g_s$  among all cultivars at  $\Psi_1^{95}$  and at  $\Psi_1^{25}$ , whereas Grenache had the lowest  $g_s$  at both  $\Psi_1^{95}$  and  $\Psi_1^{25}$  (Table 3). However, their  $g_{\text{sensitivity}}$  values were similar (as stated above).

There was a significant negative relationship between  $g_{\text{sensitivity}}$  and  $\Psi_1^{95}$  across all cultivars (Fig. 4). As the water status threshold for the onset of stomatal closure ( $\Psi_1^{95}$ ) decreased, sensitivity to further closure increased across the entire range of  $\Psi_1^{95}$  values. Moreover, all 17 cultivars were broadly distributed along the curve and did not cluster together. Notably, Grenache and Syrah were distributed similarly on the vertical axis ( $g_{\text{sensitivity}}$ ) and were separated only on the horizontal axis ( $\Psi_1^{95}$ ).

#### Relationship of cultivar mean $\Psi_1$ and $\Psi_1^{25}$

Overall cultivar mean  $\Psi_1$  (averaged across treatments and years) was positively related to  $\Psi_1^{25}$  both pre- and post-veraison (Fig. 5). Petit Verdot was removed from the analysis as an outlier due to its large absolute residual value in both phenological periods. There were no significant differences in response between phenological periods (slopes not significantly different), except that cultivar mean  $\Psi_1$  values were significantly



**Fig. 3.** Response of  $g_s$  to  $\Psi_l$  for all 17 cultivars used in this study, arranged from top-left to bottom-right in order of decreasing  $g_{s\text{sensitivity}}$  (see ‘Materials and methods’ section for definition). Data are means  $\pm$  95% confidence intervals of  $g_s$  for samples that were pooled at each 0.1 MPa of  $\Psi_l$  in 2014 and 2015. Vertical dotted lines represent  $\Psi_l$  values at  $\Psi_l^{25}$  and  $\Psi_l^{95}$  according to the fitted curves.

lower post-veraison by 0.21 MPa (likely due to the lack of a fully irrigated post-veraison treatment). Nevertheless, in both phenological periods a higher  $\Psi_l^{25}$  resulted in a higher cultivar mean  $\Psi_l$  with nearly a 1:1 ratio based on the slopes of the linear regression lines. The linear regression model was highly significant, and  $\Psi_l^{25}$  explained 83% of the variation in cultivar mean  $\Psi_l$  when the model included both phenological periods.

### Discussion

Since the paper published by Schultz (2003) suggesting that *V. vinifera* cultivars may differ in their stomatal behaviour in response to water deficits, there has been great interest among researchers in unravelling the causes and underlying mechanisms of the an/isohydric phenomenon across cultivars

(Soar *et al.* 2006b; Collins *et al.* 2010; Gerzon *et al.* 2015; Hochberg *et al.* 2015; Coupel-Ledru *et al.* 2017; Charrier *et al.* 2018). However, the obvious experimental difficulty in conducting large, field-scale experiments across many cultivars has limited previous investigations to only a few cultivars at a time or to the comparison of various cultivars grown in different vineyards or locations. As such, much work has been done on potted vines whose responses may not be representative of field performance – particularly in terms of plant water relations (Passioura 2006) – and on cultivars selected based on *a priori* classification as either iso- or anisohydric from few data – notably Grenache and Syrah. Several reviews have been published on the topics of irrigation management and/or water relations of grapevine that have attempted to synthesise the existing literature on across-cultivar stomatal behaviour (Cifre

**Table 2. Parameter estimates (Est.), associated standard errors (s.e.), and regression fit statistics (Adj. R<sup>2</sup>) from non-linear regression analyses of  $g_s$  to  $\Psi_1$** 

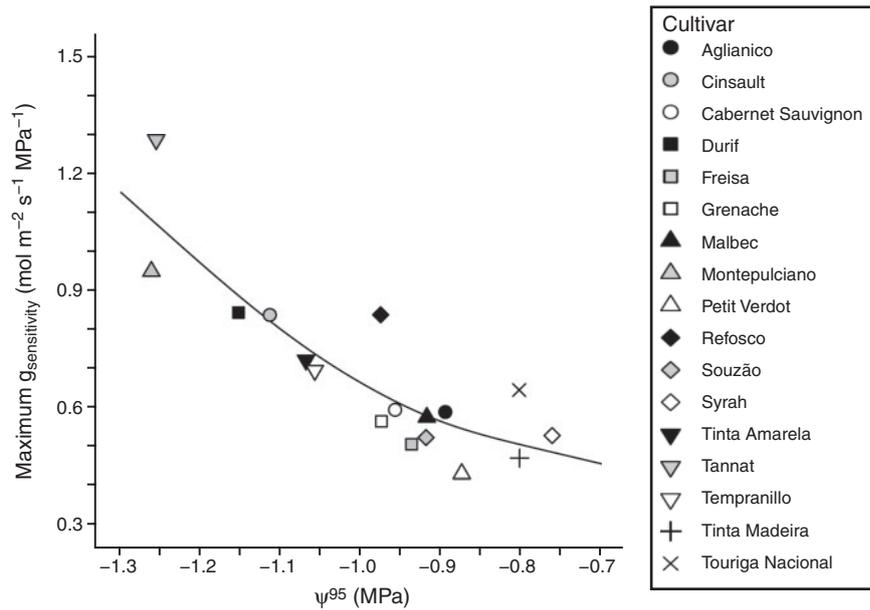
The parameter  $g_{\max}$  represents the curve asymptote, i.e. a theoretical maximum  $g_s$ . The parameter  $\Psi_1^{50}$  represents the inflection point of the curve, i.e. the  $\Psi_1$  (MPa) at which  $g_s$  is 50% of  $g_{\max}$ . The parameter  $b$  characterises the transition width of the function, i.e. the difference in  $\Psi_1$  from 25 to 75% of  $g_{\max}$  and is equal to  $(b \times 2.196) + 0.002$ . Adj. R<sup>2</sup> is a bias-corrected R<sup>2</sup> accounting for the number of parameters in the non-linear regression model

| Cultivar           | $g_{\max}$ (mol m <sup>-2</sup> s <sup>-1</sup> ) |       | $\Psi_1^{50}$ (MPa) |      | $b$ (unitless) |      | Adj. R <sup>2</sup> |
|--------------------|---|-------|---------------------|------|----------------|------|---------------------|
|                    | Est.  | s.e.  | Est.                | s.e. | Est.           | s.e. |                     |
| Syrah              | 0.595   | 0.130 | -1.34               | 0.15 | 0.28           | 0.08 | 0.83                |
| Refosco            | 0.489   | 0.029 | -1.40               | 0.04 | 0.15           | 0.03 | 0.91                |
| Tinta Amarela      | 0.483   | 0.045 | -1.55               | 0.05 | 0.17           | 0.05 | 0.85                |
| Touriga Nacional   | 0.483   | 0.053 | -1.27               | 0.06 | 0.19           | 0.04 | 0.89                |
| Tannat             | 0.480   | 0.026 | -1.53               | 0.03 | 0.09           | 0.02 | 0.86                |
| Malbec             | 0.477   | 0.043 | -1.48               | 0.06 | 0.21           | 0.05 | 0.88                |
| Tinta Madeira      | 0.468   | 0.055 | -1.38               | 0.07 | 0.25           | 0.04 | 0.93                |
| Tempranillo        | 0.466   | 0.050 | -1.54               | 0.06 | 0.17           | 0.05 | 0.83                |
| Souzão             | 0.465   | 0.038 | -1.51               | 0.05 | 0.22           | 0.04 | 0.93                |
| Aglianico          | 0.459   | 0.031 | -1.42               | 0.04 | 0.20           | 0.03 | 0.94                |
| Cabernet Sauvignon | 0.444   | 0.033 | -1.48               | 0.05 | 0.19           | 0.03 | 0.93                |
| Freisa             | 0.438   | 0.045 | -1.52               | 0.07 | 0.22           | 0.06 | 0.82                |
| Montepulciano      | 0.426   | 0.024 | -1.59               | 0.03 | 0.11           | 0.03 | 0.84                |
| Petit Verdot       | 0.424   | 0.052 | -1.50               | 0.10 | 0.25           | 0.07 | 0.78                |
| Durif              | 0.406   | 0.036 | -1.51               | 0.05 | 0.12           | 0.05 | 0.72                |
| Cinsault           | 0.402   | 0.031 | -1.47               | 0.04 | 0.12           | 0.04 | 0.73                |
| Grenache           | 0.398   | 0.025 | -1.47               | 0.04 | 0.18           | 0.03 | 0.92                |
| All cultivars      | 0.461   | 0.021 | -1.48               | 0.03 | 0.23           | 0.02 | 0.96                |

**Table 3. Maximum slope and vine water status values modelling the relation of  $g_s$  to  $\Psi_1$  for each cultivar**

Vine water status values ( $\Psi_1^{95}$ ,  $\Psi_1^{50}$  and  $\Psi_1^{25}$ ) were extracted from the fitted curves using parameters from Table 2. Maximum slope ( $g_{\text{sensitivity}}$ ) was determined from the first derivative of each fitted curve

| Cultivar           | $g_{\text{sensitivity}}$<br>(mol m <sup>-2</sup> s <sup>-1</sup> MPa <sup>-1</sup> ) | $\Psi_1^{95}$     |   | $\Psi_1^{50}$     |   | $\Psi_1^{25}$     |   |
|--------------------|--|-------------------|---|-------------------|---|-------------------|---|
|                    |  | $\Psi_1$<br>(MPa) | $g_s$<br>(mol m <sup>-2</sup> s <sup>-1</sup> ) | $\Psi_1$<br>(MPa) | $g_s$<br>(mol m <sup>-2</sup> s <sup>-1</sup> ) | $\Psi_1$<br>(MPa) | $g_s$<br>(mol m <sup>-2</sup> s <sup>-1</sup> ) |
| Tannat             | 1.283  | -1.26             | 0.456   | -1.53             | 0.239   | -1.63             | 0.120   |
| Montepulciano      | 0.946  | -1.26             | 0.405   | -1.59             | 0.213   | -1.72             | 0.107   |
| Durif              | 0.840  | -1.15             | 0.385   | -1.51             | 0.202   | -1.64             | 0.101   |
| Refosco            | 0.835  | -0.98             | 0.462   | -1.40             | 0.243   | -1.56             | 0.122   |
| Cinsault           | 0.834  | -1.11             | 0.382   | -1.47             | 0.201   | -1.60             | 0.101   |
| Tinta Amarela      | 0.719  | -1.07             | 0.457   | -1.55             | 0.241   | -1.74             | 0.120   |
| Tempranillo        | 0.692  | -1.06             | 0.441   | -1.54             | 0.232   | -1.73             | 0.116   |
| Touriga Nacional   | 0.642  | -0.80             | 0.446   | -1.27             | 0.235   | -1.49             | 0.118   |
| Cabernet Sauvignon | 0.591  | -0.96             | 0.418   | -1.48             | 0.220   | -1.69             | 0.110   |
| Aglianico          | 0.586  | -0.90             | 0.430   | -1.42             | 0.226   | -1.64             | 0.113   |
| Malbec             | 0.572  | -0.92             | 0.447   | -1.48             | 0.235   | -1.71             | 0.118   |
| Grenache           | 0.562  | -0.98             | 0.376   | -1.47             | 0.198   | -1.67             | 0.099   |
| Syrah              | 0.525  | -0.76             | 0.527   | -1.34             | 0.277   | -1.68             | 0.138   |
| Souzão             | 0.520  | -0.92             | 0.434   | -1.51             | 0.229   | -1.76             | 0.114   |
| Freisa             | 0.503  | -0.94             | 0.410   | -1.52             | 0.216   | -1.77             | 0.108   |
| Tinta Madeira      | 0.468  | -0.80             | 0.426   | -1.38             | 0.224   | -1.67             | 0.112   |
| Petit Verdot       | 0.428  | -0.88             | 0.392   | -1.50             | 0.207   | -1.78             | 0.103   |
| Range              | 0.855  | 0.50              | 0.151   | 0.32              | 0.079   | 0.29              | 0.039   |
| Standard deviation | 0.215  | 0.15              | 0.037   | 0.08              | 0.020   | 0.08              | 0.010   |
| Mean               | 0.679  | -0.99             | 0.429   | -1.47             | 0.226   | -1.68             | 0.113   |
| CV (%)             | 32   | 15                | 9   | 6                 | 9   | 5                 | 9   |



**Fig. 4.** Relationship of maximum stomatal sensitivity ( $g_{\text{sensitivity}}$ ) to onset of stomatal closure ( $\Psi_1^{95}$ ) for all 17 cultivars. A non-linear regression was fit through the data ( $y = 0.11e^{-1.80x}$ ; adj.  $R^2 = 0.73$ ).

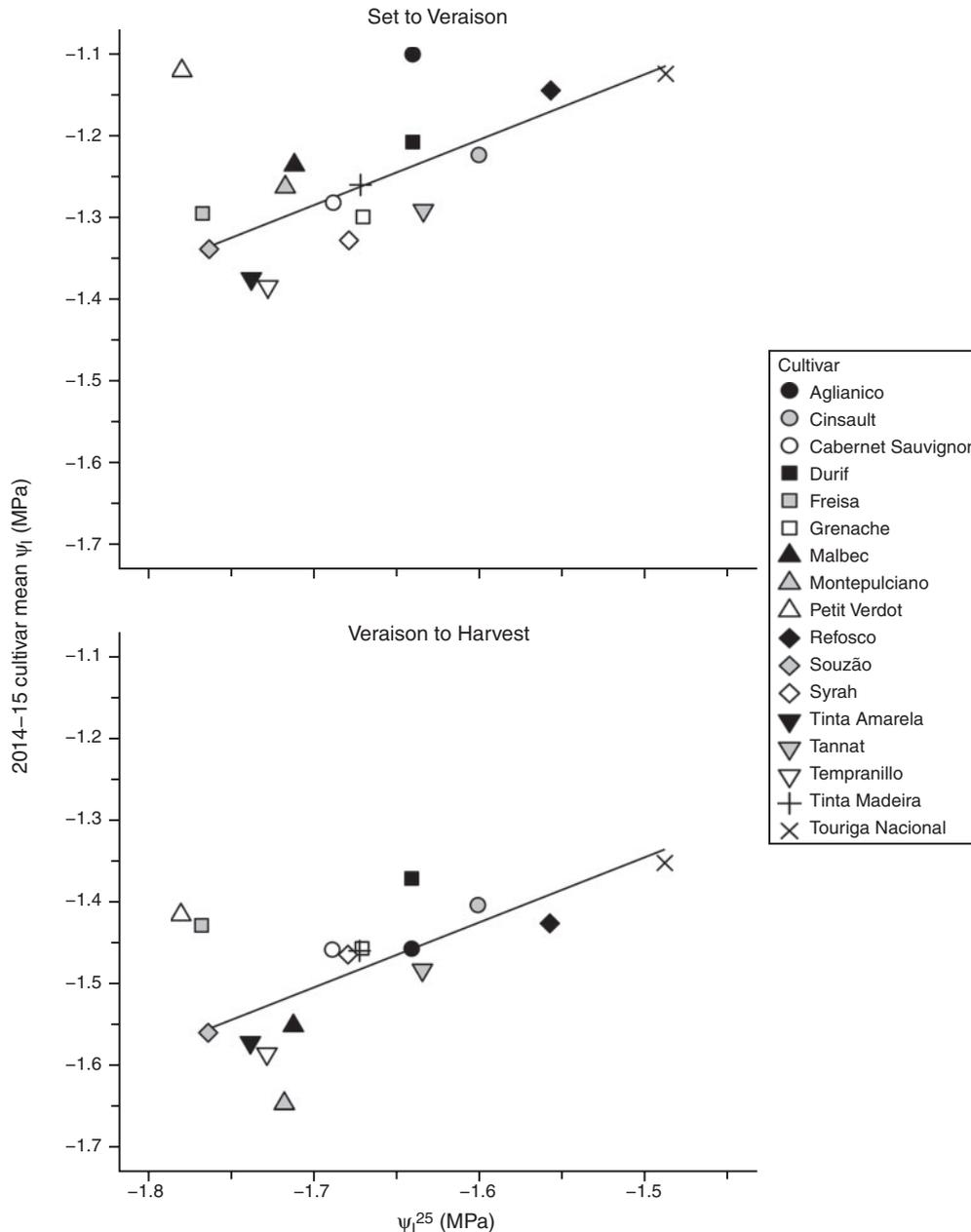
*et al.* 2005; Chaves *et al.* 2010; Lovisolo *et al.* 2010; Costa *et al.* 2012; Domec and Johnson 2012). Unfortunately, differences in plant material, environmental conditions, and experimental methodology and techniques have limited the ability of past reviews to characterise various cultivars' responses and have ultimately resulted in inconsistent classification. In an attempt to resolve these problems, Lavoie-Lamoureux *et al.* (2017) conducted a meta-analysis of 40 publications across 28 *V. vinifera* cultivars and hypothesised that rather than separating into two distinct groups, cultivars' stomatal behaviours were distributed along a continuum. A similar result was found by Klein (2014) in a meta-analysis across 70 plant species.

This study explored genetic variation in vine water status and stomatal behaviour across 17 *V. vinifera* cultivars (clone wood and source known) grafted onto the same rootstock (1103P), grown in a replicated field trial in a high VPD area (San Joaquin Valley of California), and subjected to three season-long deficit irrigation treatments over the course of multiple seasons. Therefore, soil type, weather conditions and cultural practices were the same for all cultivars. Midday  $\Psi_1$  was measured by the same individual on each date in 2014 and 2015 using the technique whereby leaf blades were covered with a plastic bag just before leaf excision therefore minimising potential technique (Williams and Araujo 2002; Williams 2017) and operator errors (Goldhamer and Fereres 2001). Unfortunately, this was not one of the factors considered in the meta-analysis by Lavoie-Lamoureux *et al.* (2017) and even now the exact method used or a reference provided to describe how  $\Psi_1$  was measured is not given (Charrier *et al.* 2018). The timing interval between the two measurements only had to be within two hours in the meta-analysis of Lavoie-Lamoureux *et al.* (2017), and it is unknown to what degree this would affect their results. Stomatal

conductance was measured with the same LI-COR porometer in each year on the same leaf used for the measurement of  $\Psi_1$  just before its excision. Thus, the design and techniques used in this study should have maximised the effect of cultivar on the parameters in question. The hypothesis that cultivars do not separate into clear iso- or anisohydric groups, but rather are distributed along a continuum was tested by (1) establishing a range of plant water deficits using variable amounts and timings of applied water, (2) measuring  $\Psi_{PD}$  and  $\Psi_1$  the first year and (3) measuring  $g_s$  and  $\Psi_1$  on the same leaf samples the third and fourth years, and then comparing response curves among cultivars.

#### *Isohydric, near-isohydric, anisohydric or isohydrodynamic?*

In general, an isohydric plant will maintain its water potential value similar between water stressed and well-watered cohorts (Tardieu and Simonneau 1998). The results from the first year of the study clearly demonstrate that none of the cultivars in this study responded to soil water deficits in an isohydric manner but were more anisohydric-like, in agreement with that of (Soar *et al.* 2006b). Midday  $\Psi_1$  of vines in the three irrigation treatments differed significantly from one another – midday  $\Psi_1$  of well-watered vines across cultivars were always much greater than the other irrigation treatments and as the water in the soil profile was depleted, midday  $\Psi_1$  decreased for all cultivars including that of Grenache. None of the cultivars examined in this study maintained a minimal midday  $\Psi_1$  plateau ( $> -1.5$  MPa) as reported previously for near-isohydric grape cultivars (Schultz 2003; Prieto *et al.* 2010). In addition, the slope of the relationship between midday  $\Psi_1$  and  $\Psi_{PD}$  reported here did not differ among cultivars, similar to that observed by Charrier *et al.* (2018) for



**Fig. 5.** Cultivar mean  $\Psi_1$  and its corresponding  $\Psi_1^{25}$  for each of the 17 cultivars from set to veraison and from veraison to harvest. Cultivar mean  $\Psi_1$  values were computed across 2014 and 2015 and across irrigation treatments. Cultivar  $\Psi_1^{25}$  values were calculated from fitted curves (Fig. 4). Petit Verdot was excluded from the regression because it was an outlier. Equations for each phenological period were:  $y = 0.07 + 0.80x$  for set to veraison, and  $y = -0.14 + 0.80x$  for veraison to harvest. Fit and significance of regression model was  $R^2 = 0.83$  and  $P < 0.001$ .

Grenache and Syrah. Lastly, the cultivars did not appear to respond to soil water deficits in an isohydrodynamic manner (Franks *et al.* 2007; Shellie and Bowen 2014).

#### Exploring the curve: cultivar differences in the relation of $g_s$ to $\Psi_1$

A sigmoidal  $g_s$  relation to  $\Psi_1$  was present in all 17 cultivars and in a composite analysis. A similar result was found by Charrier *et al.*

(2018). Linear relationships between  $g_s$  and  $\Psi_1$  (and  $\Psi_{\text{stem}}$ ) have been commonly reported in grape (Williams and Araujo 2002; Shackel 2007; Williams 2012; Williams *et al.* 2012). However, nonlinearity may have been overlooked in those studies. The reported ranges of  $\Psi_1$  or  $\Psi_{\text{stem}}$  were narrower (~0.5 to 1.0 MPa) than that reported herein (1.4 MPa), thus may have only represented the linear portion of the entire response curve. Furthermore, there are few data in the aforementioned studies at low vine water status ( $\Psi_1$  or  $\Psi_{\text{stem}} < -1.5$  MPa), and the

reported data at high vine water status ( $\Psi_1$  or  $\Psi_{\text{stem}} > -1.0$  MPa) show increasing vertical scatter. Indeed, when a broader range of vine water status was evaluated, non-linear relationships between  $g_s$  and  $\Psi_1$  (and  $\Psi_{\text{stem}}$ ) have been shown for *V. vinifera* cv. Sauvignon blanc (Naor *et al.* 1994, 1997), and between  $g_s$  and  $\Psi_{\text{PD}}$  across five cultivars (Prieto *et al.* 2010). Recently, sigmoidal functions were also well fit to data relating grapevine leaf petiole percent loss of conductivity to xylem tension in potted grapevine (cv. Merlot) (Hochberg *et al.* 2017a). Finally, sigmoidal relations of  $g_s$  to  $\Psi_1$  has been reported for woody and non-woody crops alike, including: *Populus* spp. (Silim *et al.* 2009), *Olea europaea* (Ennajeh *et al.* 2008), *Oryza sativa* (Dingkuhn *et al.* 1989), *Sorghum bicolor* (Henzell *et al.* 1976); and across a broad range of tree species (Klein 2014). Nevertheless, in this study the nature of the sigmoidal relationship between  $g_s$  and  $\Psi_1$  depended greatly upon cultivar.

#### No cultivar differences at high water status

Under well-watered conditions, there were no cultivar differences in  $g_{\text{max}}$ . While both high  $g_{\text{max}}$  (Henzell *et al.* 1976) and low  $g_{\text{max}}$  (Sinclair *et al.* 2005) have been proposed means to improve productivity under dry conditions, there was surprisingly little variation in  $g_{\text{max}}$  under well-watered conditions among cultivars over two years. Measured and estimated  $g_{\text{max}}$  values in this study ( $\sim 0.4$  to  $0.6$  mol m<sup>-2</sup> s<sup>-1</sup> across cultivars) were similar to other reported values for well-watered grapevines (Williams and Baeza 2007; Bota *et al.* 2016), and there were no significant differences among cultivars. Interestingly, measured  $g_{\text{max}}$  values among cultivars were more similar than were estimated  $g_{\text{max}}$  values, further supporting the point that cultivars behave similarly under well-watered conditions. Thus, no prospects were revealed for improved efficiency at either high water status (Price *et al.* 2002) or at low water status (Silim *et al.* 2009).

The lone  $g_{\text{max}}$  outlier – Syrah, who's estimated  $g_{\text{max}}$  was more than  $0.1$  mol m<sup>-2</sup> s<sup>-1</sup> greater than measured  $g_{\text{max}}$  – was likely due a lack of data density in the well-watered range ( $> -1.0$  MPa) that ultimately resulted in a poor estimate of the asymptote by the sigmoidal fit. This was also true for Tinta Madeira, though the difference between measured and estimated  $g_{\text{max}}$  was smaller. The question remains whether or not higher  $g_s$  values would be measured for those cultivars – and a more accurate estimate for  $g_{\text{max}}$  would be obtained – at higher  $\Psi_1$  values. Although it is not biologically impossible – Soar *et al.* (2009) reported  $g_s$  values for Shiraz (syn = Syrah) near  $0.8$  mol m<sup>-2</sup> s<sup>-1</sup> – it is unlikely under field conditions, primarily because those values were obtained through artificial heating and under high relative humidity. Also, significantly higher mid-season  $\Psi_1$  values ( $> -0.7$  MPa) would also be difficult to reach under field conditions (Williams and Baeza 2007).

Generally speaking, the role of  $g_{\text{max}}$  in terms of drought tolerance has been evaluated almost exclusively in the context of crop species that are grown for agronomic purposes (yield/biomass production) – e.g. *Populus* spp., *O. sativa*, and *S. bicolor*. Considering that grapevine water use is highly correlated with  $g_s$  (Williams *et al.* 2012) and canopy percent shaded area/canopy coverage (Williams and Ayars 2005),

similar  $g_{\text{max}}$  across cultivars would suggest that canopy percent shaded area alone – ultimately determined by relative cultivar vigour and canopy training/trellising system – determines total consumptive water use in grapevine. However, understanding the importance of high or low  $g_{\text{max}}$  in wine grape production is further complicated by the common horticultural purposes (fruit quality) in which water deficits are often beneficial (Roby *et al.* 2004).

#### Cultivar differences become apparent at moderate water status

As water deficits increased, differences among cultivars in the onset of stomatal closure ( $\Psi_1^{95}$ ) became apparent, ranging from Syrah at  $-0.76$  MPa to Tannat at  $-1.26$  MPa. There are few reported data regarding the  $\Psi_1$  at the onset of closure for grapevines, primarily due to the predominant practice of fitting linear curves through the data (as discussed above), or simply not conducting a regression analysis. Williams and Baeza (2007) suggest a  $\Psi_1$  value of  $-1.2$  MPa as signifying when grapevines are no longer responsive to changes in VPD, but rather to changes in soil moisture. In their recent meta-analysis of the literature, Lavoie-Lamoureux *et al.* (2017) suggest using  $-1.214$  MPa as the water status level at which to compare cultivars. It is likely that this value –  $\Psi_1 = -1.2$  MPa – varies by cultivar, and indeed falls in the lower end of the across-cultivar  $\Psi_1^{95}$  range obtained herein. The data of Hochberg *et al.* (2017b) show that  $\Psi_1^{95}$  for Chardonnay was closer to  $\Psi_1 \approx -0.8$  MPa, and Hochberg *et al.* (2017a) show a 12% loss of conductivity (i.e.  $\Psi_1^{88}$ ) in Merlot occurring at  $\Psi_{\text{stem}} \approx -0.66$  to  $-0.91$  MPa – which would correspond to a  $\Psi_1 \approx -0.97$  to  $-1.20$  MPa (Williams and Araujo 2002). Finally, a similar range in the onset of stomatal closure has been reported among Poplar clones (Silim *et al.* 2009) and across 70 other tree species (Klein 2014). Therefore, the cultivar-specific water potential values for the onset of stomatal closure in this study are within the expected range for *V. vinifera*, and moreover seem to be highly conserved across other woody perennial plant species as well.

Like the onset of stomatal closure,  $g_{\text{sensitivity}}$  was cultivar-specific, revealing a 3-fold difference in slopes (Fig. 4). Prevailing definitions of iso- and anisohydry state that isohydric plants maintain  $\Psi_1$  almost constant as soil dries due to more sensitive stomata, whereas anisohydric plants maintain  $g_s$  and allow  $\Psi_1$  to decline with soil drying (Tardieu and Simonneau 1998; Jones 2014). Using this definition, cultivars in this study with high  $g_{\text{sensitivity}}$  (e.g. Tannat, Durif and Montepulciano) could be categorised as isohydric/near-isohydric, whereas those with low  $g_{\text{sensitivity}}$  (e.g. Petit Verdot, Tinta Madeira, Freisa) could be categorised as anisohydric/near-anisohydric.

In the present study, Grenache (often considered the archetypal isohydric cultivar) and Syrah (often considered the archetypal anisohydric cultivar) had almost the same  $g_{\text{sensitivity}}$  (maximum slope of the linear part of the curves), and did not segregate based on this criterion. However, simple observation of the sigmoidal functions fit to Grenache and Syrah clearly shows that they behave differently at moderate water status – Grenache is more sigmoidal with a clear asymptotic levelling off of  $g_s$  at high  $\Psi_1$  values, whereas Syrah is less sigmoidal without a clear

asymptotic levelling off of  $g_s$  at high  $\Psi_1$ . If  $g_{\text{sensitivity}}$  were calculated at moderate water status values (e.g.  $\Psi_1^{95} > \Psi_1 > \Psi_1^{50}$ ) instead of in the linear portion (e.g. at  $\Psi_1^{50}$ ), it is possible that Grenache and Syrah would segregate clearly based on this criterion. Simply looking at this constrained dataset supports some previous classification of these two cultivars. Yet the fact that their behaviour – and ultimate classification – depends on how the stomatal sensitivity parameter is defined calls into question its usefulness for classification.

In their comparison of five cultivars, Prieto *et al.* (2010) reported that a stomatal sensitivity factor,  $k$  – calculated using the Ball, Woodrow, and Berry model – significantly differed between Grenache and Syrah, but their level of significance for this parameter was  $\alpha = 0.10$ . Furthermore, they show regressions of  $k$  to predawn water potential for Syrah and Ekigaina (as examples of extreme cases), but do not show the comparison data for Grenache. Recent work has also shown that there are no statistically significant differences between Grenache and Syrah with respect to sensitivity of transpiration rate to declining soil water availability under both field and greenhouse conditions (Charrier *et al.* 2018). Therefore, other factors affecting stomatal behaviour such as leaf hydraulics or environment may be more important to consider for cultivar classification going forward.

It is notable that Grenache and Syrah – the two cultivars first compared by Schultz (2003) – differed in this study only in their  $\Psi_1^{95}$  value, but had almost the same  $g_{\text{sensitivity}}$  (Fig. 4). Although widely cited as the foundational evidence for the an/isohydric phenomenon within *V. vinifera* cultivars, it is seldom discussed that Schultz (2003) actually used Grenache and Syrah scions grafted onto different rootstocks, 140 Ruggeri and 110 Richter respectively (Schultz 1996). *Vitis* spp. rootstocks differ in their effects on vine water relations and gas exchange (Padgett-Johnson *et al.* 2003), and have also been shown to significantly affect scion gas exchange (Soar *et al.* 2006a) as well as scion growth and productivity (Williams 2010). Lavoie-Lamoureux *et al.* (2017) reported that rootstock generally accounts for 19% of the variability in scion  $g_s$  response to water deficits. When grafted onto the same rootstock, similar rates of stomatal closure between Grenache and Syrah in response to  $\Psi_{\text{stem}}$  (ranging from  $-0.5$  to  $-1.5$  MPa) have been reported in a recent three-year field study (Bota *et al.* 2016). It did appear that Grenache and Syrah behaved differently at moderate water status in this study, in contrast to Schultz (2003) who showed a greater divergence between the two cultivars at lower water status. Well-watered conditions have been shown to increase variation in stomatal response across Grenache-Syrah progeny (Coupel-Ledru *et al.* 2017). Indeed, it should be noted that Syrah maintained a higher  $g_s$  compared with Grenache at all water status levels in this study. Nevertheless, whether considering Grenache and Syrah separately, or all the cultivars together in this study, there were more differences among them in stomatal behaviour at moderate water status relative to low water status.

#### *Few cultivar differences at low water status*

Analysis of  $g_s$  at  $\Psi_1^{25}$  and the similarity of  $\Psi_1$  among cultivars at  $\Psi_1^{25}$  suggests a common water status limit to low  $g_s$  among

grapevine cultivars. At  $\Psi_1^{25}$ ,  $g_s$  was already down to 0.08 to 0.1 mol m<sup>-2</sup> s<sup>-1</sup> and declined gradually thereafter. Grapevines have been previously classified as severely stressed when  $g_s < 0.05$  to 0.1 mol m<sup>-2</sup> s<sup>-1</sup> (Cifre *et al.* 2005; Lovisolo *et al.* 2010). The lack of apparent cultivar differences at low water status may have been due to the relatively poor fit of the sigmoidal functions at these levels (shown by increasing homoscedasticity in residuals in Fig. 2b). However, all extracted  $\Psi_1^{25}$  values were above these poorly fit regions of the dataset. Brodrribb and Holbrook (2003) regarded  $\Psi_1^{20}$  as the point of stomatal closure in their analysis of stomatal behaviour across four tropical tree species, and Klein (2014) used  $\Psi_1^{25}$  as a functional parameter by which to compare stomatal behaviour of 70 woody perennial plant species. Hence, it is reasonable to regard the value of  $\Psi_1^{25}$  as the functional lower limit of stomatal contribution to leaf gas exchange and overall vine performance.

Stomata function in part to protect the hydraulic integrity of the xylem (Tyree and Sperry 1988; Brodrribb and Holbrook 2003), but also close in response to reduction in leaf turgor (Shackel *et al.* 1987). Thus, a reduction in  $g_s$  is both an *impact of* and a *protective response to* declining plant water status. This inherently complicates behavioural interpretation. Choat *et al.* (2010) showed that xylem cavitation commences at much lower water status than previously accepted, and Hochberg *et al.* (2017b) recently showed that stomata close well in advance of xylem cavitation in grapevine. In the latter work,  $g_s$  was less than 10% of  $g_{\text{max}}$  before the first embolisms were detected in the xylem ( $-1.46 > \Psi_1 > -1.75$  MPa). Those  $\Psi_1$  values at the appearance of the first embolisms closely correspond to the  $\Psi_1^{25}$  values found among cultivars in this study ( $-1.5 > \Psi_1 > -1.8$  MPa; Fig. 5). Therefore, our data indicate most stomata were closed for all cultivars at or below their  $\Psi_1^{25}$  value, and that the cultivar-specific  $\Psi_1^{25}$  value may be related to the appearance of first embolisms in the xylem. Thus, each cultivar-specific  $\Psi_1^{25}$  value may indicate a hydraulically determined water status limit, which may be governed by cultivar differences in vascular anatomy (Gerzon *et al.* 2015; Hochberg *et al.* 2015).

#### *Potential physiological mechanisms to explain cultivar differences*

It is likely that the different stomatal behaviours observed among cultivars were mediated by hydraulic or turgor-dependent signals, which are closely associated with incipient stomatal closure (Brodrribb and Holbrook 2003). In their modelling work on partitioning stomatal responses to drought, Rodriguez-Dominguez *et al.* (2016) found that leaf turgor-dependent mechanisms accounted for a large majority of the decline in  $g_s$  to soil drought across three woody perennial crop species, including grapevine. Reduced hydraulic conductivity and increased osmotic adjustment made up a large proportion of the overall stomatal response in that study.

In general, differences in stomatal closure among plant species may arise in part from differences in osmotic adjustment (Hsiao 1973), leading to acclimation of stomatal response and leaf hydraulic conductivity to low water status (Matthews and Boyer 1984; Martorell *et al.* 2015). Differences in osmotic adjustment have also been closely associated with differences in stomatal behaviours between tree species

(Nolan *et al.* 2017). Grape leaves also go through significant osmotic adjustment (Schultz and Matthews 1993), and there is recent evidence for differences in osmotic adjustment among *V. vinifera* L. cultivars (Martorell *et al.* 2015). Differences in osmotic adjustment have also been previously reported among cultivars in wheat, sorghum, and cotton (Morgan 1984), and in *O. europea* (Ennajeh *et al.* 2008). Thus, genetic variation in the osmotic adjustment response may play an important role in the wide range of cultivar responses found in this study.

## Conclusions

The results of this study provide clear evidence in support of an across-cultivar continuum of stomatal behaviour, and call into question previous classifications of *V. vinifera* cultivars as having either exclusively iso- or anisohydric stomatal responses. First, cultivars did not separate into distinct iso- or anisohydric groups based on their  $\Psi_1$  v.  $\Psi_{PD}$  relationships. Moreover, a deeper exploration into genetic variation among cultivars revealed that instead of separating into distinct groups, cultivars were distributed along a continuum based upon the negative relationship between the sensitivity ( $g_{\text{sensitivity}}$ ) and the onset ( $\Psi_1^{95}$ ) of stomatal closure in the face of declining vine water status. Notably, this continuum was well defined at moderate status values, but cultivars behaved similarly at high and low water status.

At high vine water status, cultivars had similar  $g_{\text{max}}$  values, suggesting that overall vine water use would simply be a function of canopy size under well watered and saturating light conditions. As  $\Psi_1$  decreased from well-watered conditions, all cultivars responded similarly to one another by maintaining a high  $g_s$  – an anisohydric characteristic. This was despite several of the cultivars having been assigned to the isohydric/near-isohydric category in other studies. However, closer examination of the  $g_s$  vs  $\Psi_1$  relationship showed that as vine water status declined from well-watered conditions, stomata of various cultivars responded differently to minimise water loss – an isohydric characteristic. Finally, at low water status,  $g_s$  was the similar for all cultivars ( $<0.1 \text{ mol m}^{-2} \text{ s}^{-1}$ ), but the water status value at the end of stomatal closure ( $\Psi_1^{25}$ ) differed among them, indicating that there is a cultivar-dependent water status limit to stomatal functioning.

The large and significant differences in midday  $\Psi_1$  values measured as a function of irrigation treatment across all cultivars in this study would indicate that midday  $\Psi_1$  provides an accurate assessment of vine water status, and would therefore be useful to validate other measures of monitoring vine water status, or as a tool in an irrigation management program. In terms of cultivar-specific stomatal behaviour, all cultivars examined herein responded in an anisohydric manner at high water status by keeping stomata open as  $\Psi_1$  decreased. This was despite several of the cultivars having been assigned to the isohydric/near isohydric category in other studies. However, closer examination of the  $g_s$  vs  $\Psi_1$  relationship indicates there may be times – at moderate water deficits – in which stomata of various cultivars may respond differently to minimise water loss, an isohydric response. This would indicate that *V. vinifera* cultivars have both iso- and anisohydric stomatal responses to water deficits, and that any future classification should

incorporate a holistic evaluation of the entire response curve. Finally, differences in osmotic adjustment among cultivars may play the primary role in determining the observed differences in stomatal behaviour.

## Conflicts of interest

Larry E Williams, Mark A Matthews and Alexander D Levin conceived of and designed the research. ADL conducted experiments, analysed data, and wrote the manuscript. All authors read and approved the manuscript. The authors declare no conflicts of interest.

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