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Effect of irrigation management on the relationship between stomatal conductance and stem water potential on cv. Cabernet Sauvignon

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Abstract. Water relations in vineyards have been largely studied; giving insides regarding the way grapevine interacts with its environment according to water availability, providing us terminologies like Isohydric and Anisohydric, which have been applied to categorize cultivars and rootstocks. Recently, the use of this terminology has been subjected to discussion regarding its use as a category to describe and separate grapevine cultivars instead of using it as a continuous, where grapevines can behave more iso or anysohidric depending on the environment where they are grown. In this study, using a deficit irrigation (RDI) trial in a commercial vineyard located in Maule Valley in Central Chile, the relationship between Midday Stem Water Potential (ψx), Stomatal Conductance (gs), Soil Water Content (WC), and Vapour Pressure Deficit (VPD) on cv. Cabernet Sauvignon was studied, a grape variety that has been classified as isohydric. The trial was carried out for 3 years from 2018 to 2020 in which four RDI treatments were employed to replenish different portions of evapotranspiration (ET) from pea-size until harvest. These irrigation treatments were conceived as 100% ET, 70% ET, 50-100% ET (50% ET before veraison and 100% ET afterwards) and 35-100% ET (35% ET before veraison and 100% ET afterwards). Findings in this study show how the relationship between ψx and gs is treatment dependent; in treatments where no water deficit was imposed a weaker relationship between the variables was observed, showing a large variation between gs at similar ψx, being gs more dependent on VPD compared to the treatments with a larger deficit on its irrigation. As the deficit irrigation increased, grapevines started to shift to a smaller variation on its gs over large variations on its ψx. This result shows that irrigation management and deficit irrigation strategies impact the way plants react to water availability and leads to different behavior on its gs/ψx relationship.

1 Introduction

The relationship between water potential, either leaf or stem water potential, and the stomatal conductance of grapevines its been widely studied in the last years. This effort had resulted in the broadly used terms to classify grapevine varieties as Isohydric and Anisohydric. Isohydric is used to describe varieties that maintain a constant daily minimal leaf water potential regardless of the pre-dawn soil water potential and anisohydric to describe plants that exhibit progressively lower minimal water potential as a function of lower soil water potential [1]. This classification with time included the relationship between stomatal conductance and plant water potential, where the isohydric plants have a better regulation of their water status due to a tighter response to stomatal closure [2-3].

Different varieties behave differently in terms of this relationship between stem or leaf water potential and stomatal conductance and can be classified at least as near-Isohydric or Near Anisohydric, although this classification might not be absolute and other factors should be take in care, like the water status of the plant [3-4]. This might be one of the reasonswhy Cabernet Sauvignon it's been classified as Isohydric[5-6]and Anisohidric [7-8].

This study aims to contribute to the hypothesis that the water status of grapevines mediated by irrigation impacts the relationship between stem water potential and stomatal conductance, contributing with information on how vineyards modulate their behavior depending on the conditions that are grown, from plants that have been fully irrigated to plants that had been submitted to high levels of deficit irrigation.

2 Materials and methods

2.1 Plant material and study site

The study was performed in a Cabernet Sauvignon commercial vineyard located in the Maule Valley, Chile (lat. 35° 26 'S; long. 71° 48' W; 38 m.s.n.m.) during three consecutive seasons from 2017 to 2020. The vineyard was planted in a vertical shoot position (VSP) trellis system in 2014 and the plant material correspond to a 341 clone grafted on an SO4 rootstock, cane pruned (double Guyot). Vine spacing is 2.3 m between rows and 1.2 between plants, and the orientation is northeast to the southwest. Soil texture is sandy loam with a depth of 1.5-0.9 m.

2.2 Experimental design and treatments

To test the hypothesis a trial of three treatments of different levels of regulated deficit irrigation (RDI) and a

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control with full irrigation were applied in a randomized block design with four replicates. Each replicate consisted of six continuous rows, where only the two center rows of those six were measured, each row had 160 plants. The three treatments of RDI were based on the incomplete replacement of crop evapotranspiration (ETc) from flowering to harvest, a control treatment without RDI where 100% of the ETc was applied, a treatment where only 70% of ETc was applied, a third treatment where 50% of ETc was applied form berry-set to veraison and 100% afterwards and a fourth treatment were only 35% of the ETc was replenish from berry-set to veraison and 100% afterwards. Treatments where codified as X0, X1, X2 and X3; X0: 100% ETc, X1: 70% ETc, X2: 50-100% ETc and X3: 35-100% ETc. Irrigation was provided weekly and calculated based on evapotranspiration measurements done by a Surface Renewal and Eddy Covariance system.

The data used for this assay came from the period where the RDI was applied, from berry-set to veraison, since after veraison two of the treatments X2 and X3 increased their irrigation to100% of the ETc.

2.3 Measurements

Midday stem water potential (Ψx md) was measured using the pressure chamber (PMS 600, PMS Instrument Company, Corvallis, OR, USA) and stomatal conductance (gs) with a handheld steady-state portable porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA.). Measurements were done on a weekly basis the day prior to irrigation when the deficit reach its peak. Two leaves from two different plants per replicate were measured for Ψx md at solar noon, before being covered by an aluminized zipper bag for an hour.

For gs, four leaves from four different plants were measured, including the plants measured for stem water potential and plants right next to them.

The weekly mean value of Ψx md and gs calculated from the measurements for each treatment were used to create the dataset for the analysis.

2.4 Statistical analyses

Results were analyzed with Non-Linear Regressions, and calibration models, using STATGRAPHICS Centurion XVI software (StatPoint Technologies, Virginia, USA).

3 Results and Discussion

The relationship observed in the study between Ψx md and gs responds to the normal behavior described in previous works [2,4,7] either as lineal or non-linearfunctions, where a decrease in the Ψx md is accompanied by a decrease in the gs, all of this mediated by the water available by the plant, the water demand of the environment and the plant physiological response. The relationship observed in this study can be represented as a logarithmic model.

$$Y = a + b*ln(X) \qquad (1)$$

Where Y is the Ψx md and X is the gs.

If all the data from the different treatments is used to model (Fig. 1), the relationship between the variables can be described by the following model:

$$\Psi x\ md = -3.28012 + 0.360123*ln\ (gs) \qquad (2)$$

Where the model and their parameters, intercept and slope, are highly significant with a strongcorrelation coefficient and high adjusted R^2 (Table 1), showing a robust relationship between Ψx md and gs that can be modeled as a logarithmic curve, with a limit on the constant (a) as gs gets lower and closer to 1.

Important is to consider that the values observed are higher than the normal values measured with an Infrared Gas Analyzer (IRGA), this particularity is associated with the equipment used to determine gs, which is a steady state porometer that calculates stomatal conductance derived from relative humidity and temperature measurements in a continuous air path where a desiccant and the leaf are connected through. This is been previously reported by Lavoie-Lamoureux et al. (2017) [2], who found that values of gs measured with a steady state porometer are on average 2.1 timer higher than those measured with an IRGA.

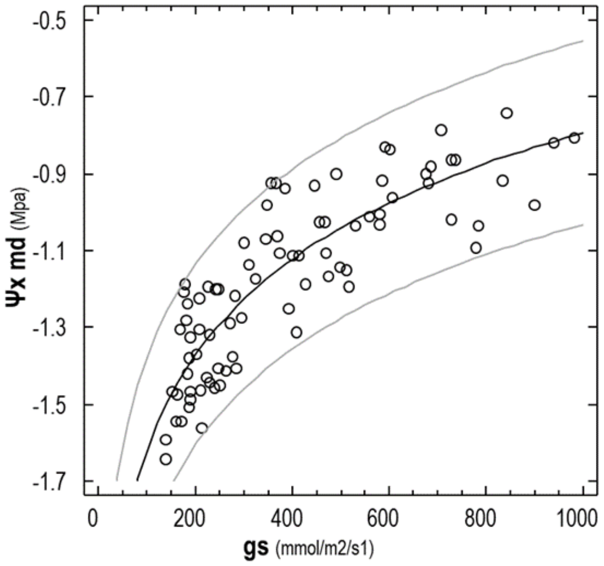

Figure 1. Relationship between Midday Stem Water Potential (Ψx md) and Stomatal Conductance (gs). All Treatments.

When the data is separated and modeled in terms of the different treatments, the behavior of each treatment changes as shown in Figure 2, giving hints of a non-specific classification of the cultivar Cabernet Sauvignon as iso or anisohydric.

Depending on the treatment used the relationship between the variables can be described by the following models:
For 100% ETc

$$\Psi x\ md = -3.53287 + 0.39879*ln\ (gs) \qquad (3)$$

For 70% ETc

$$\Psi x\ md = -3.25847 + 0.348061*ln\ (gs) \qquad (4)$$

For 50-100% ETc

$$\Psi x\ md = -3.30425 + 0.373925 * \ln(gs) \tag{5}$$

For 35-100% ETc

$$\Psi x\ md = -4.45100 + 0.575113 * \ln(gs) \tag{6}$$

Regarding the model and their parameters slope and intercept, all of them were highly significant, p-value < 0.001; indicators of a strong relationship and confident models. Although the adjusted determination coefficient R^2, shows a particular behavior, moving from lower values to higher values from the model of 100% of the ET to 35% of the ET (Table 1). Here is possible to see how much of the variance is explained by the model, in the case of 100% of the ET only 55.7% of the observed Ψx md can be explained and is related to gs. In this case, the Ψx md of the plant that is irrigated to fully replenish his water needs depends less on his stomatal conductance, and other factors as the environment and their VPD might play a more important role compared to the treatments where irrigation is restricted. So as the irrigation is more restrictive the more dependent on gs the Ψx md gets.

Figure 2 (A) shows how in plants irrigated 100% of the ET the gs as a larger variation in a shorter range ofΨx md compared to the other treatments, in this case when aΨx md of -1.0 MPais observed the gs varies from 400 mmol/m2/s to 900 mmol/m2/s indifferently, indicating as it was discussed before, that the relationship between Ψx md and gs is less dependent than for example in 70% of the ET (Fig. 2 B) or 50% of the ET (Fig. 2 C). In those cases, we can observe that as we move to the more water-restrictive treatments the relationship starts to shift and becomes more directly related. In these treatments as the stem water potential goes down, the stomatal conductance response gets more sensitive in terms of closing stomata.

Table 1. Parameters of the models built to relate and predict Midday Stem Water Potential (Ψx md) with Stomatal Conductance (gs).

Analysis of variance	**All treatments**	**100% ETc**	**70% ETc**	**50-100% ETc**	**35-100% ETc**
Source	*P-Value*	*P-Value*	*P-Value*	*P-Value*	*P-Value*
Model	0.0000	0.0002	0.0000	0.0000	0.0000
Logarithmic-X model: Y = a + b*ln(X)					
Parameter	*P-Value*	*P-Value*	*P-Value*	*P-Value*	*P-Value*
Intercept	0.0000	0.0000	0.0000	0.0000	0.0000
Slope	0.0000	0.0002	0.0000	0.0000	0.0000
Correlation coeficient	0.85	0.74	0.79	0.84	0.86
R^2	73.8	55.7	63.3	71.8	74.7
Mean absolute error (MAE)	0.095	0.069	0.091	0.082	0.096

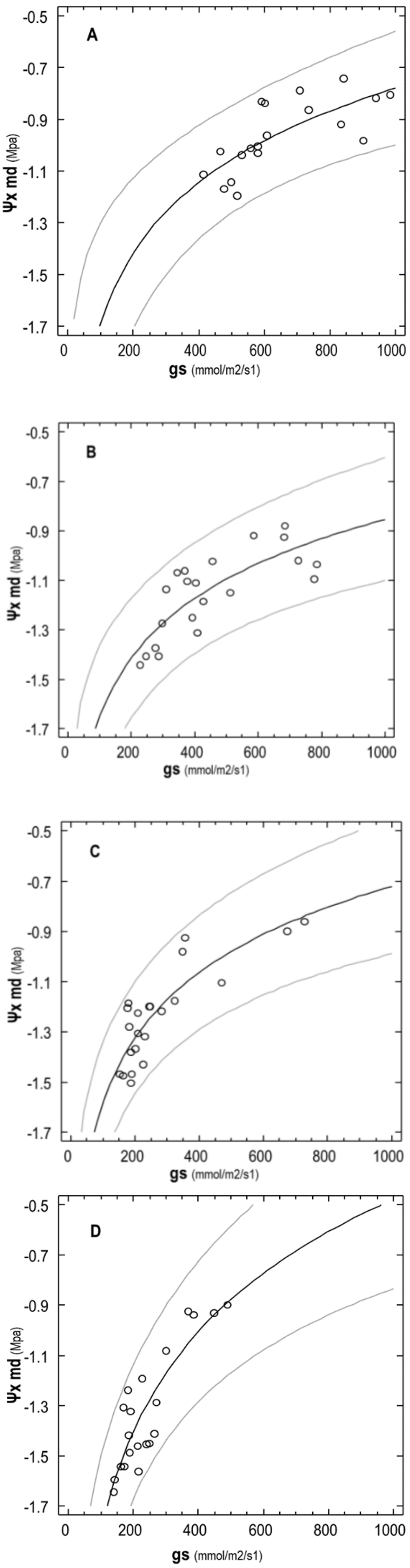

Figure 2. Relationship between Midday Stem Water Potential (Ψx md) and Stomatal Conductance (gs). Models were built with separated data from the treatments. A: 100% ETc; B: 70% ETc, C: 50-100% ETc and D: 35-100% ETc.

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In the case of the treatment of 35% of the ET (Fig. 2 D) the relationship moves to the other extreme, where a large variation in the Ψx md does not impact the gs of the plants, even in conditions where Ψx md reaches values high as 0.9 Mpa. In this case, the stomata might reach a point where most of them stay close, even if the water status of the plant or the water demand from the environment is more favorable.

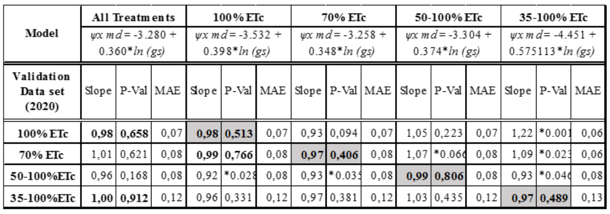
The different behavior of the treatments described before gives more hints of the difficulty to classify Cabernet Sauvignon as either an Iso or Anisohydric variety, since the treatment of 100% of the ET behaves in an Anisohydric way, with poor stomatal control, and hypothetically a water status more dependent on the environment demand. On the other hand treatments like 70% or 50% of the ET which behaves in an Isohydric way, with strong stomatal control, and the treatment of 35% of the ET, where the gs stays low even in conditions of high Ψx md, where other treatments reach a peak of gs.

Regarding the validation of the different models built for each treatment, where a separate dataset of a third year was used. Is possible to say that the behavior described before is stable on time, and the models are more accurate if built and used for each irrigation treatment. This approach gives models that have smaller errors in the estimation as is possible to see in Table 2. The closer to slope to 1 the better the model, and if the *p*-value is higher than 0.05 means that we cannot reject the idea with a 95% of confidence that the slope = 1. In all the cases where the model was validated with a new dataset from the specific treatments that the model was built, the better the fitting it was, with a slope closer to 1, a *p*-value higher than 0.05, and a smaller MAE compared with other models. In some cases, the general model also fits equally or slightly better than the specific model (highlighted in Table 2), and also when using data from treatments closer to each other like 70% ET with the model of 100% ET. But in general terms, the observed data had a better fitting with the specific model built for it. Also the more different the model treatment from the observed dataset of other treatments the worsen the fitting. As seen in Table 2 when using the dataset of 100, 70 or 50% of the ET with the model developed for 35% of the ET, or when using the dataset of 50% of the ET with the model developed for 100 or 70% of the ET (all the validations with * sign). In those cases, the errors are higher and the dataset cannot fit into the model.

4 Conclusion

The behavior observed in this trial show how the relationship between Ψx md and gs can be described with a logarithmic model. This relationship can't be described as Iso or Anisohydric since as shown in this study, is affected by the irrigation management. In this regard the relationship moves from Anisohydric to Isohydric depending on how much water is available to the plants, when irrigation is applied to fully replenish crop evapotranspiration, the plants behave as Anysohydric, with a limited relation between Ψx md and gs and a poor stomata control. As the water available to plants is continuously reduced, the response shifts to a more Isohydric behavior, until a point where the irrigation restriction is so high that the plants seem to maintain the stomata close even when the environmental conditions and the plant water status are not detrimental, in our case 35% of the ET.

Table 2. Model validation. Compared indicators of the model's behavior in predicting different datasets. Slope of the linear relation between the model and the dataset, *p*-value for the null Hypothesis of slope = 1, and Mean Absolute Error of the model compared to the validation dataset.



Model	**All Treatments**			**100% ETc**			**70% ETc**			**50-100% ETc**			**35-100% ETc**		
	$\psi x\ md = -3.280 + 0.360 * ln\ (gs)$			$\psi x\ md = -3.532 + 0.398 * ln\ (gs)$			$\psi x\ md = -3.258 + 0.348 * ln\ (gs)$			$\psi x\ md = -3.304 + 0.374 * ln\ (gs)$			$\psi x\ md = -4.451 + 0.575113 * ln\ (gs)$		
Validation Data set (2020)	Slope	P-Val	MAE	Slope	P-Val	MAE	Slope	P-Val	MAE	Slope	P-Val	MAE	Slope	P-Val	MAE
100% ETc	**0,98**	**0,658**	0,07	**0,98**	**0,513**	0,07	0,93	0,094	0,07	1,05	0,223	0,07	1,22	*0.001	0,06
70% ETc	1,01	0,621	0,08	**0,99**	**0,766**	0,08	**0,97**	**0,406**	0,08	1,07	*0.06	0,08	1,09	*0.02	0,06
50-100%ETc	0,96	0,168	0,08	0,92	*0.028	0,08	0,93	*0.03	0,08	**0,99**	**0,806**	0,08	0,93	*0.04	0,08
35-100%ETc	**1,00**	**0,912**	0,12	0,96	0,331	0,12	0,97	0,381	0,12	1,03	0,435	0,12	**0,97**	**0,489**	0,13

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