Gas Exchange Relations of Ungrafted Grapevines (cv. Carménère) Growing Under Irrigated Field Conditions

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A study was carried out to evaluate the leaf gas exchange relations and water-use efficiency (WUE) of ungrafted Carménère (*Vitis vinifera* L.) grapevines growing under field conditions and different levels of water stress. Stomatal conductance (g_s), transpiration (E), net CO_2 assimilation (A_N) and stem water potential (Ψs) were measured at midday in a drip-irrigated commercial vineyard located in the Maule Valley (Chile) during three growing seasons (2005 to 2009). In addition, the instantaneous (A_N/E) and intrinsic (A_N/g_s) water-use efficiencies and stomatal sensitivity factor (*k*) were estimated for the own-rooted grapevines. In this study a significant non-linear relationship was observed between A_N and g_s ($r^2 = 0.82$), with values of A_N decreasing from 14.9 to 3.5 µmol/m^2/sec as g_s diminished from 0.5 to 0.05 mol/m^2/sec. This resulted in a progressive increase in WUEi (intrinsic water use efficiency). A significant linear relationship was observed between Ψs and g_s ($r^2 = 0.39$) for measurements taken before and after véraison, with an increasing scattering from -1.6 to -0.4 MPa. Finally, *k* decreased as water stress increased, with values of 234 and 120 for no and severe water stress respectively, while *k* ranged from 264 to 480 and 255 to 297 for the measurements taken before and after véraison respectively. Based on the results obtained in the present study, the cultivar Carménère could be classified as drought tolerant at low water potentials, with a large range of physiological parameters changing in response to water stress.

INTRODUCTION

'Carménère' is an emblematic grapevine cultivar in Chile, and has recently been reintroduced to commercial production after its disappearance from Europe during the 19th century. Because the Carménère grapevines are not spread worldwide, few studies on its leaf gas exchange relations and water-use efficiencies have been published. Furthermore, studies of cv. Carménère growing under field conditions and natural variability become an important topic, especially when taking into account the exceptional sanitary conditions in Chile (i.e. absence of Phylloxera), which allow grapevines to grow without grafting and to express their genotypic characteristics. Any knowledge about leaf gas exchange relations generated under field conditions could lead to the development of irrigation tools oriented to optimise water productivity and improve berry composition (Basile *et al*., 2012; Fernandes de Oliveira *et al.*, 2013; Shellie, 2014).

Under semi-arid conditions, with no or a reduced water supply (i.e. regulated deficit irrigation), vine water stress gradually increases as both soil water depletion and atmospheric water demand are augmented during the growing season. In this case, photosynthetic activity decreases due to stomatal and non-stomatal limitations (Moutinho-Pereira *et al*., 2004). This situation occurs very slowly until a certain level of water deficit, after which the photosynthetic activity decreases rapidly (Pellegrino *et al*., 2006). Stomatal closure in response to chemical signals synthesised in the dehydrating roots (including abscisic acid) is generally accepted to be the main factor for the depletion of photosynthesis (A_N) and gas exchange under mild to moderate water stress (Chaves *et al*., 2010). When the drought period is lengthened, dehydration is more severe, or other environmental stresses are superimposed and a decline in mesophyll photosynthesis may occur because of a down-regulation process (Medrano *et al*., 2002). If this condition persists, irreversible reductions in dry matter accumulation and failure of grape maturation may occur. Flexas *et al*. (2002) have suggested that the stomatal conductance (g_s) could be an integrative indicator of water stress intensity in relation to photosynthesis (via a coupled A_N-g_s relationship), as well as serving to unify data from different experiments and allowing the examination of a continuous gradient of water stress. Also, the A_N-g_s relationship could be an informative tool to demonstrate how other types of stress may influence photosynthesis and

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water-use efficiency (Naor & Wample, 1995). Cifre *et al*. (2005) suggest that three phases of photosynthesis response can be identified along a water stress gradient using stomatal conductance as a physiological indicator. In this regard, g_s values are from 0.7 to 0.15, 0.15 to 0.05 and < 0.05 mol H_2O/m^2/sec for the mild, moderate and severe water stress phases, respectively.

Since the water scarcity in viticultural areas is increasing progressively, the need for better water-use efficiency in grapevines is increasingly urgent. Moreover, additional knowledge of the mechanisms involved in grapevine tolerance to water stress and a better understanding of its genetic variability will be relevant to future breeding improvement (Chaves *et al*., 2010; Flexas *et al*., 2010). In this sense, water-use efficiency to leaf level has been studied in several grapevine cultivars around the world under very different growing conditions. Under different soil water and atmospheric conditions, stomatal regulation allows plants to reduce fluctuations in the water status of tissues and hold xylem integrity (Prieto *et al*., 2010). According to their stomatal control, vines can be classified physiologically into two categories (Tardieu & Simonneau, 1998; Schultz, 2003; Prieto *et al*., 2010): (i) isohydric or near-isohydric vines that maintain tight stomatal regulation of the transpiration rate, resulting in nearly constant leaf water potential in drought-exposed and well-irrigated conditions. In contrast, (ii) anisohydric vines display lower stomatal control of transpiration, resulting in large fluctuations in leaf water potential under soil water deficit and high evaporative demand. These conditions allow the vines higher rates of gas exchange and assimilation.

Also, stomatal sensitivity and the degree of coupling of stomatal conductance and photosynthesis generally have been considered in relation to single variables, mostly because interactions between factors have been difficult to quantify in a simple way. In this regard, Ball *et al*. (1987) demonstrated that g_s as a function of the 'Ball factor' (see Equation 4) would yield a linear relationship between g_s and A_N measured under different levels of CO_2 concentration, light intensities, vapour pressure deficit and temperature. The slope of this relationship is named the sensitivity factor (*k*), and it has been used successfully to evaluate stomatal responsiveness to water stress in several *Vitis* species and cultivars (Schultz *et al*., 1999; Schultz, 2000).

Unpublished field data suggest the Carménère cultivar presents an anisohydric-like behaviour, since stomatal conductance remains relatively high with moderate to strong water stress and saturating light conditions. As working hypothesis, the cv. Carménère has the ability to maintain a significant assimilation rate and stomatal conductance under increasing water stress. Thus, the objective of this study was to assess leaf gas exchange relations and water-use efficiency of ungrafted Carménère grapevines (*Vitis vinifera* L.) growing in a drip-irrigated commercial vineyard.

MATERIALS AND METHODS

Field characterisation

Field data were collected from a drip-irrigated Carménère commercial vineyard in the 2005/2006, 2007/2008 and 2008/2009 growing seasons. The vineyard, with 11-year-old plants at the beginning of the experiment, was located in the Talca Valley, Maule Region, Chile (35º25.170' S, 71º33.228' W, WGS 84; 137 m.a.s.l.). The own-rooted vines were trained on a Geneva double curtain (GDC) (spacing 3.3 m between rows and 1.5 m within the row) and pruned into two bilateral spur cordons (12 buds per cordon), with the fruiting zone at 1.8 m above ground and vine rows oriented 330º NW. The canopy geometry of this training system was conserved after bloom (about mid-November) by hedging two or three times during the summer. The soil texture was clay loam with a rooting depth of 150 cm. It is classified as Talca series (fine family, mixed, thermic Ultic Haploxeralf). From 0 to 150 cm, the bulk density, field capacity, wilting point and available water were 1.36 g/cm^3, 0.31 m^3/m^3, 0.13 m^3/m^3 and 0.18 m^3/m^3, respectively. Standard agronomical practices were performed during each season for sanitary care and productivity.

Water requirements and irrigation

Vineyard evapotranspiration was calculated as:

$$ET_v = Kc \times ET_0 \quad (1)$$

where ET_v is the actual evapotranspiration of the vineyard (mm/day), ET_0 is the reference evapotranspiration (mm/day) computed using the Penman-Monteith model (Allen *et al*., 1998), and Kc is the crop coefficient assigned according to phenological stage (dimensionless). Kc values were 0.11, 0.24, 0.61 and 0.49 for budburst, setting, véraison and near to harvest respectively. These Kc values were obtained using measurements of midday stem water potential (Ψs) and soil water content during a three-year experiment (unpublished data), and were similar to those indicated by Acevedo-Opazo *et al*. (2010) and Ortega-Farias *et al*. (2005).

Water was supplied to the vineyard making use of two 2.0 L/h drippers per plant at a frequency of two times per week. Also, the irrigation timing varied according to atmospheric demand and the application of regulated deficit irrigation with the aim of improving berry quality. Due to the high soil water-holding capacity of the rooting zone (270 mm), the first irrigations were applied in late December or early January, which corresponded to the phenological stages between post-setting and before véraison. In this case, the farmer applied about 2 990, 2 570 and 3 140 m^3/ha/season, while the yield was 9.0, 9.2 and 11.0 t/ha for the 2005/2006, 2007/2008 and 2008/2009 growing seasons respectively. The increase in water application was associated with a higher atmospheric demand observed during the last season

Climatic variables

Standard weather variables used to calculate ET_0 from a reference grass were collected at 30-min intervals using an automatic meteorological station (Adcon Telemetry, model A730, Klosterneuburg, Austria), located about 8 km NW from the experimental site. Air temperature (T), relative humidity (RH), solar radiation (R_s), precipitation (P), wind velocity (Wv) and direction (Wd) were measured from 1 May to 30 April of each year.

Growing degree-days and seasonal dryness characterisation

To characterise the climatic conditions at the experimental site, growing degree-days (GDD, °Cd) and dryness index (DI, mm) were estimated for each growing season. The GDD were calculated as:

$$\mathrm{GDD} = \sum_{i=1}^{n} [0.5 \times (\mathrm{T_{max}} + \mathrm{T_{min}}) - \mathrm{T}_b] \quad (2)$$

where T_{max} and T_{min} are daily maximum and minimum temperatures (° C) respectively, and T_b is the base temperature (10 °C). The phenological stages of the vines were observed in 18 representative vines across the experimental field using the modified Eichhorn and Lorenz system (Coombe, 1995). Two fruit-bearing shoots per plant were selected and labelled for the successive readings.

For the three seasons, the dryness index (DI, mm) was calculated following the methodology proposed by Tonietto and Carbonneau (2004):

$$\mathrm{DI} = W_0 + \sum_{01.10}^{31.03} (P - T_v - E_s) \quad (3)$$

where W_0 is the initial useful soil water reserve available at the rooting depth (200 mm), P is the monthly precipitation (mm), T_v is the monthly potential transpiration in the vineyard (mm) and E_s is the monthly direct evaporation from the soil (mm). Using DI, climates are considered to be humid (DI > 150 mm), sub-humid (150 > DI > 50 mm), moderately dry (50 > DI > -100 mm) or very dry (DI < -100 mm).

Leaf gas exchange measurements

An infrared gas analyser (ADC BioScientific Ltd., model LC*i*, Hoddesdon, UK) was used to estimate midday values of photosynthetically active radiation (PAR), stomatal conductance (g_s), transpiration (E), net CO_2 assimilation (A_N) and CO_2 concentrations both internal (Ci) and external (Ca) to the atmosphere. CO_2 mesophyll conductance (g_m, A_N/Ci), intrinsic (WUEi, A_N/g_s) and instantaneous (WUE, A_N/E) water-use efficiencies also were calculated. Mesophyll conductance to CO_2 was calculated as the ratio of A_N/(Ci-Cc), assuming that the CO_2 partial pressure at the site of carboxylation (Cc) is zero (Candolfi-Vanconcelos & Koblet, 1991; Moutinho-Pereira *et al*., 2004).

A total of 362 fully expanded, mature, sun-exposed, healthy leaves were chosen from the mid-zone of the canopy (6th leaf from the tip on fruit-bearing shoots). The measurements of leaf gas exchange were made between 12:00 and 14:00 local time (solar zenith) on fully sunny days without changing the original position of the selected leaves in the canopy and maintaining the same light regime, ensuring a saturating sunlight exposure of the leaves (PAR > 800 µmol/m²/sec).

Plant water status

To evaluate vine water status, midday stem water potential (Ψs) was measured using a pressure chamber (PMS Instrument Co., model 600, Corvallis, Oregon, USA). Fully expanded, healthy leaves were wrapped with plastic transparent film and aluminium foil at least 2 h before taking the measurements, allowing an equilibrium between leaf and plant xylem water status to be achieved (Scholander *et al*., 1965; Begg & Turner, 1970; Choné *et al.*, 2001). Care was taken to minimise water loss during the transfer of the leaf to the chamber, so less than 30 sec were allowed to pass between the excision of the petiole and the measurement. The measurements of Ψs and leaf gas exchange were taken every 20 days from December (one month before véraison) to March (near harvest) in each growing season.

Several thresholds of plant water status to define water stress levels have been developed for different grapevine cultivars (Carbonneau & Costanza, 2004; Sibille *et al*., 2007; Van Leeuwen *et al.*, 2009). To define the level of water stress in this study, data were divided into ranges according to the thresholds proposed by Van Leeuwen *et al*. (2009): i) no water deficit (Ψs > -0.6 MPa), ii) weak water deficit (-0.9 MPa < Ψs < -0.6 MPa), iii) moderate to weak water deficit (-1.1 MPa < Ψs < -0.9 MPa), iv) moderate to severe water deficit (-1.4 MPa < Ψs < -1.1 MPa) and v) severe water deficit (-1.4 MPa > Ψs).

Stomatal sensitivity factor

Cultivar-specific stomatal sensitivity was analysed using the model approach of Ball *et al*. (1987) that allows the evaluation of the stomatal conductance response to the combined effects of climatic variables through a stomatal sensitivity factor (*k*) calculated by a linear regression analysis (Prieto *et al*., 2010):

$$g_s = g_0 + k \times A_N(\mathrm{RH} / \mathrm{Ca}) \quad (4)$$

where g_0 is a residual stomatal conductance (mmol/m²/sec); A_N is the net CO_2 assimilation (µmol/m²/sec); RH is the relative humidity (%); and Ca is CO_2 concentration at the leaf surface (µmol/mol). Also, the factor 'A_N (RH/Ca)' is termed the 'Ball factor'. Schultz *et al*. (1999) indicated that a higher value of *k* means less stomatal responsiveness to water stress (inverse relationship between *k* and stomatal closure control), consequently leading to lower WUE and WUEi.

Data filtering and analysis

The whole database of gas exchange was submitted to an exploratory data analysis to determine the existence of measurement errors. Data were removed from the dataset when (i) the temperature within the infrared gas analyser chamber was over 42 ºC (Jacobs *et al*., 1996; Jara-Rojas *et al*., 2009), and (ii) when g_s and/or Ci where close or equal to zero under saturating sunlight conditions (PAR > 800 µmol/m²/sec; Prieto *et al*., 2010).

Statistical analysis

Non-linear regressions between A_N and g_s, Ci and g_s, g_m and g_s, A_N/g_s and g_s and A_N/E and g_s were developed for different levels of vine water status. For these levels, linear regressions between g_s and the 'Ball factor' also were done to calculate the values of *k* before and after véraison. It is important to indicate that the stomatal sensitivity factor corresponds to the slope of the linear regression. The coefficient of determination (r^2) was used to evaluate how well the regression line represents the data (percentage of the data that is the closest to the line of best fit.

Furthermore, a statistical analysis of climatic data was performed using the Kruskal-Wallis range test, while that for vine water status was done using a two-way ANOVA. The mean separation was done using Tukey's test at a 95% confidence level (P-value < 0.05).

RESULTS

Local growing conditions

In general, the seasonal mean values of T_{max}, VPD and GDD were statistically higher during the 2008/2009 season than during the other seasons (Table 1). Also, the seasonal T_{min} was lower during the 2007/2008 season in comparison with the other seasons, but there was not a significant difference in R_s values among the seasons. The highest atmospheric demand was observed between setting and véraison, with the mean values of T_{max} and T_{min} ranging between 29.3 and 30.3°C and 12.5 and 13.1 ºC, respectively. The mean seasonal values of VPD ranged between 0.6 and 0.9 kPa, while those of R_s were between 21.4 and 22.6 MJ/m^2/d. The seasonal values of GDD were between 1 632 and 1 793°C, which, according to Tonietto and Carbonneau (2004), indicates that there were good climatic conditions to reach appropriate berry maturation. Table 2 indicates that the cumulative values of ET*v* and ET_0 ranged between 315 and 349 mm and

TABLE 1
Mean values of climatic variables for the main phenological stages of a drip-irrigated Carménère vineyard.

	Leaf fall to budburst	Budburst to setting	Setting to véraison	Véraison to harvest	Season (Oct–Apr)
T_{max} (° C)					
2005/2006	14.4 a	23.8 b	29.3 b	25.7	25.8 b
2007/2008	12.9 b	23.7 b	29.9 a	26.2	26.1 b
2008/2009	14.4 a	25.2 a	30.3 a	27.0	27.1 a
Significance	*	*	*	ns	*
T_{min} (° C)					
2005/2006	5.8 a	9.6 a	13.1	9.8	10.5 a
2007/2008	2.2 c	8.5 b	13.0	9.6	9.9 b
2008/2009	4.8 b	9.4 a	12.5	10.1	10.4 a
Significance	*	*	ns	ns	*
VPD (kPa)					
2005/2006	0.2	0.5	0.9 b	0.6 b	0.6 c
2007/2008	0.2	0.7	1.2 a	0.7 b	0.8 b
2008/2009	0.2	0.7	1.3 a	0.9 a	0.9 a
Significance	ns	ns	*	*	*
R_s (MJ m^2/d)					
2005/2006	8.5	22.1 b	28.5	17.1	21.4
2007/2008	9.2	24.7 ab	27.4	18.4	22.6
2008/2009	8.4	23.2 a	26.9	18.6	22.1
Significance	ns	*	ns	ns	ns
GDD (° Cd)					
2005/2006	135 a	483.5 ab	518.7	648	1650.5 b
2007/2008	45 b	438.5 b	534.6	659	1631.9 b
2008/2009	118 a	537.7 a	530.9	725	1793.2 a
Significance	*	*	ns	ns	*

T_{max} = maximum temperature; T_{min} = minimum temperature; VPD = average leaf-to-air vapour pressure deficit; R_s = average solar radiation; GDD = growing degree-days.
* Columns with different letters denote statistical differences (P-value < 0.05); ns = non-significant differences.

TABLE 2
Cumulative values of precipitation (P), actual (ET_v) and reference (ET_0) evapotranspiration and dryness index (DI) from October to March.

Seasons	P (mm)	ET_v (mm)	ET_0 (mm)	DI (mm)
2005/2006	63	315	893	-115
2007/2008	77	332	945	-130
2008/2009	46	349	983	-151

between 893 and 983 mm respectively. Moreover, the DI was between -115 and -151 mm, indicating that the area was very dry. Under these atmospheric conditions, the mean values of Ψs were maintained between -0.69 and -1.05 MPa from setting to véraison, indicating that the farmer maintained the Carménère commercial vineyard under a water status from moderate to weak water deficit (Fig. 1) (Van Leeuwen *et al.*, 2009). It was only in the 2005/2006 growing season

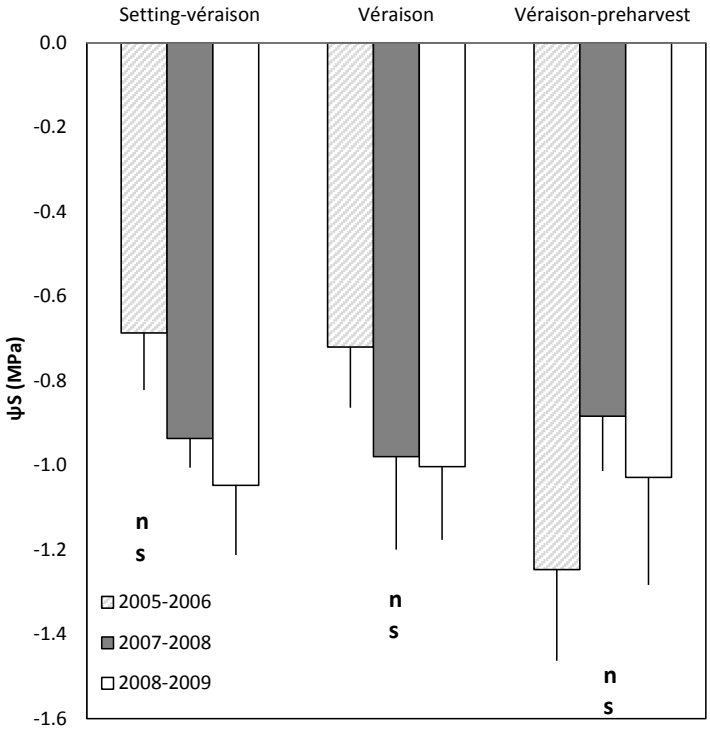


FIGURE 1

Mean values of midday stem water potential (Ψs) during setting-véraison, véraison and véraison-pre-harvest of a drip-irrigated Carménère vineyard. Each bar represents a line indicating the standard deviation (n > 24 for each set). ns indicates that there were not significant differences between seasons or phenological stages.

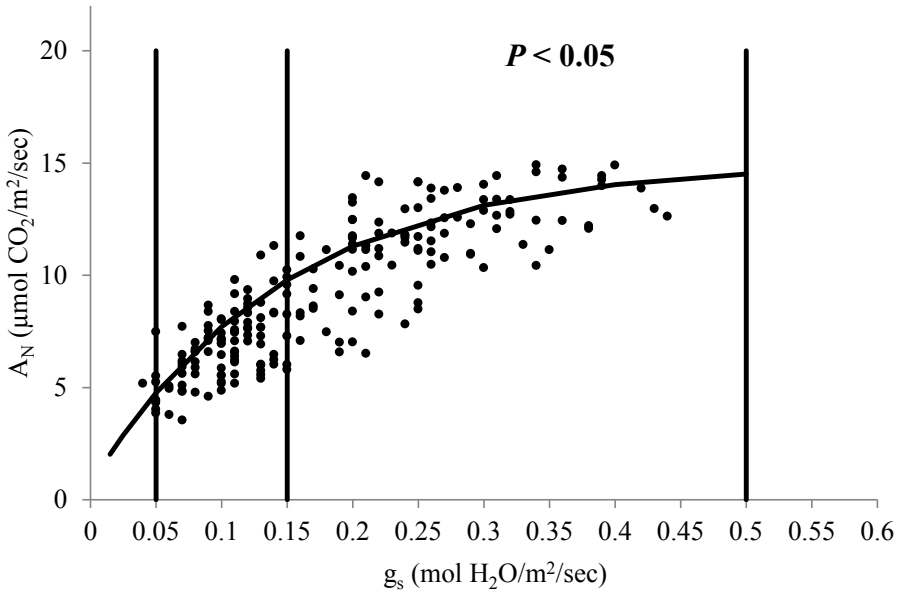


FIGURE 2

Relationships between net CO_2 assimilation (A_N) and stomatal conductance (g_s) of grapevines (cv. Carménère) growing at field conditions. Solid vertical lines represent the threshold values proposed by Cifre *et al*. (2005) to define water stress using stomatal conductance as an index: g_s values from 0.5 to 0.15, 0.15 to 0.05 and < 0.05 mol $H_2O/m^2/sec$ correspond to mild, moderate and severe water stress phases, respectively.

that the vineyard showed values of Ψs < -1.4 (severe water stress) during post-véraison and pre-harvest; during the other seasons, the vineyard was always maintained under Ψs > -1.1 MPa. It is important to indicate that there were no significant differences in mean Ψs between seasons and phenological stages.

Gas exchange performance

For the field-grown grapevines, a significant non-linear regression was observed between A_N and g_s ($r^2 = 0.82$), in spite of the large scattering observed in this study (Fig. 2). Also, the results show that the net CO_2 assimilation was between 5.8 and 14.9 and 3.5 and 11.3 µmol CO_2/m^2/sec for Carménère vines under mild (g_s values from 0.5 to 0.15 mol/ m^2/sec) and moderate (g_s values from 0.15 to 0.05 mol/m^2/sec) water stress respectively. In Fig. 3A, a significant non-linear relationship between Ci and g_s ($r^2 = 0.54$) can be observed, with an important scattering of the g_s value ranging from 0.05 to 0.15 mol/ m^2/sec. The mean values of Ci were 233.4 (± 16.9) and 211.2 (± 25) µmol CO_2/mol air for the mild and moderate water stress phases respectively. In Fig. 3B, a significant non-linear regression can be observed between the mesophyll conductance of the leaf ($g_m = A_N/Ci$) and g_s ($r^2 = 0.49$), with a very large scattering of g_s, ranging between 0.15 and

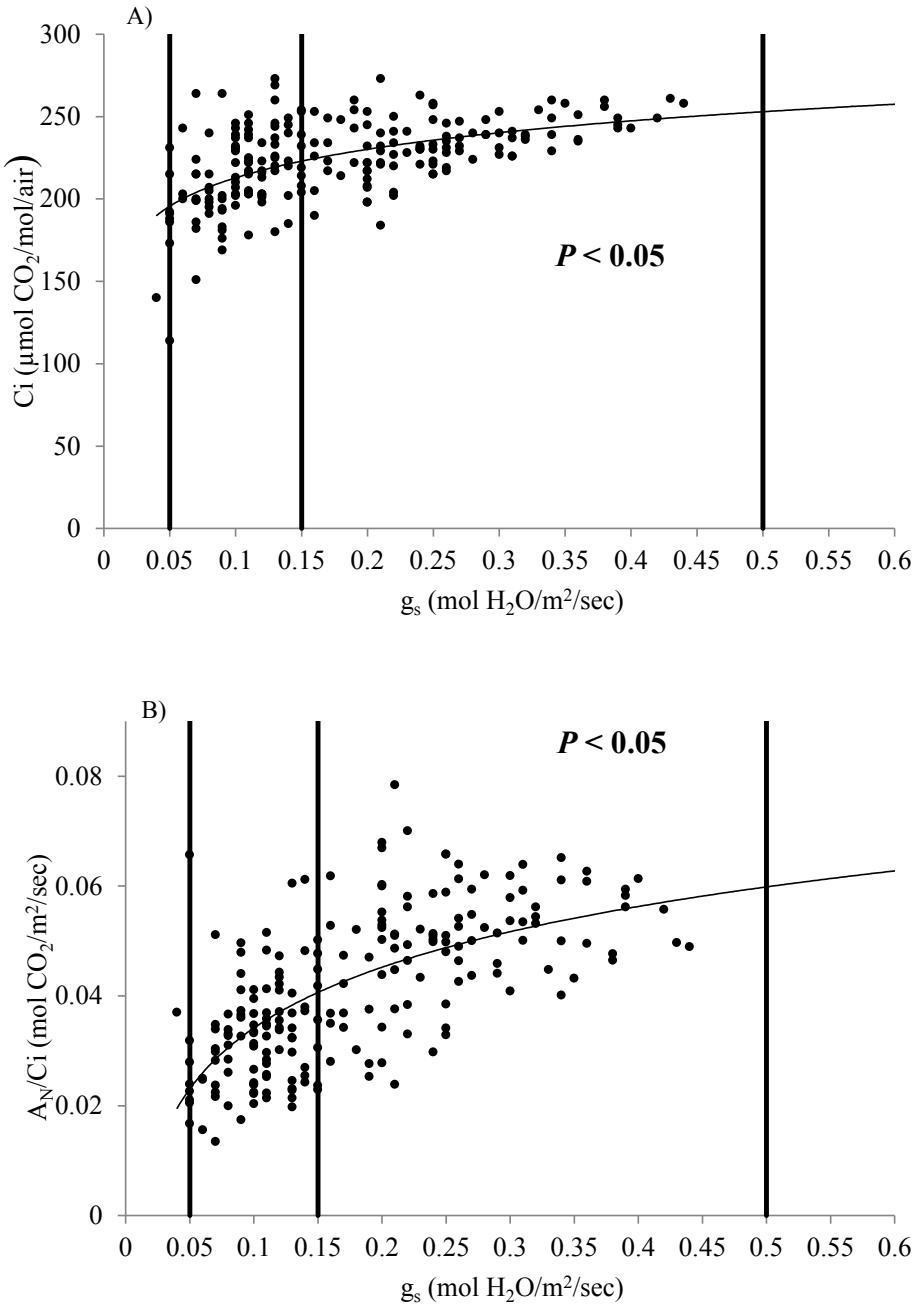


FIGURE 3

Relationships between internal CO_2 concentration (Ci) and g_s and between A_N/Ci ratio and g_s of grapevines (cv. Carménère) growing at field conditions. Solid vertical lines represent the threshold values proposed by Cifre *et al*. (2005) to define water stress using stomatal conductance as an index: g_s values from 0.5 to 0.15, 0.15 to 0.05 and < 0.05 mol H_2O/m^2/sec correspond to mild, moderate and severe water stress phases,respectively.

0.5 mol/m^2/sec.

Figs 4A and 4B show the intrinsic (WUEi = A_N/g_s) and instantaneous (WUE = A_N/E) water-use efficiencies of the cv. Carménère for the different values of stomatal conductance. The values of WUEi and WUE increased from mild to moderate water stress, but the pattern of WUEi was better than that of WUE. The mean values of WUEi and WUE for mild water stress (g_s values from 0.5 to 0.15 mol/m^2/sec) ranged between 28.7 and -73.4 µmol/mol and 0.8 to 2.9 mmol/mol, respectively, while those for moderate water stress (g_s values from 0.15 to 0.05 mol/m^2/sec) ranged between 41.5 and 110.3 µmol/mol and 0.9 and 4.6 mmol/mol, respectively.

Vine water status and stomatal sensitivity factor

A significant linear relationship was observed between Ψs and g_s (r^2 = 0.39) for measurements taken before and after véraison, with an increasing scattering from -1.6 to -0.4 MPa (Fig. 5). In addition, a very large scattering of g_s was observed for Ψs ranging between

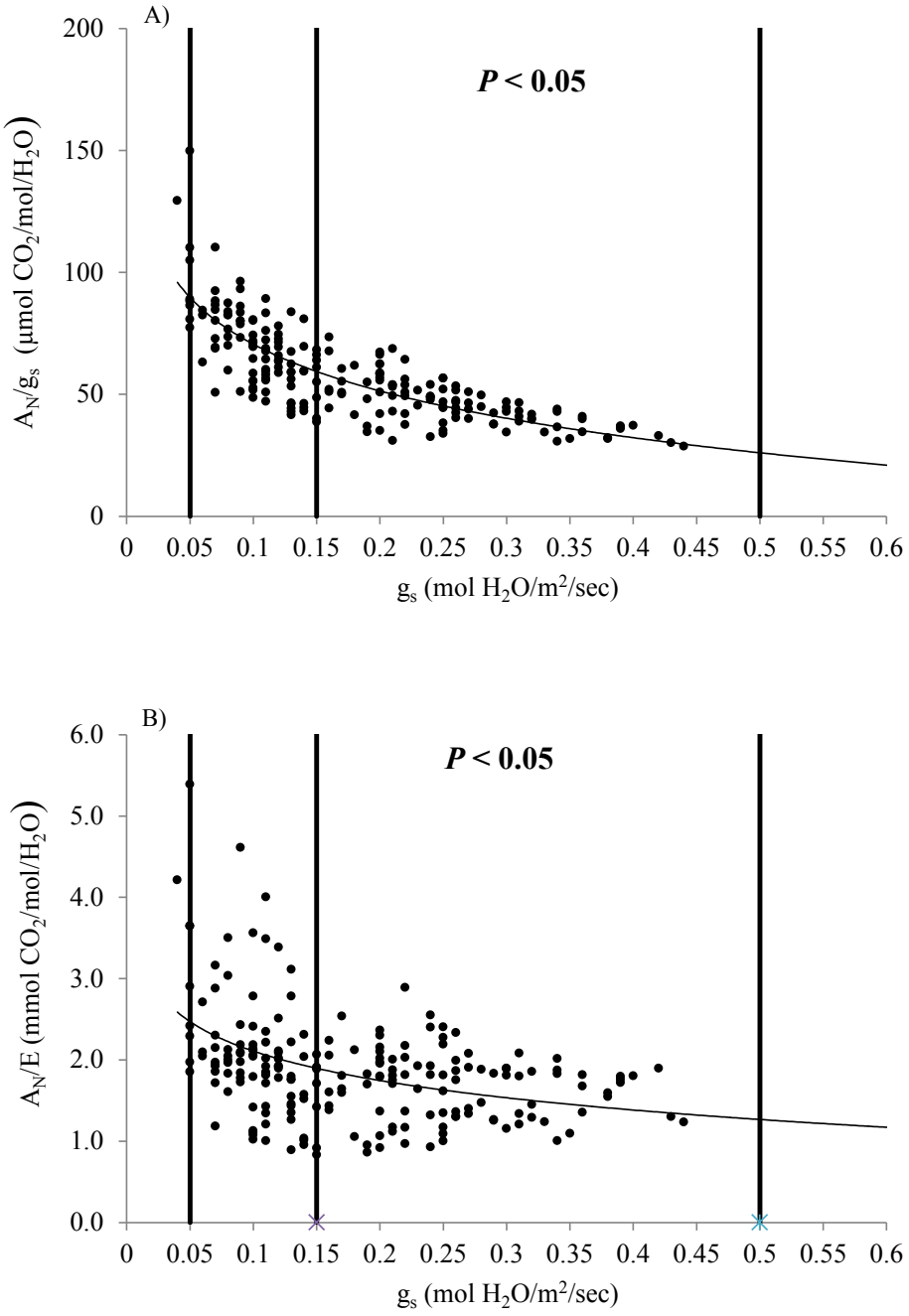


FIGURE 4

Relationships between intrinsic water-use efficiency [ratio of net CO_2 assimilation (A_N) to stomatal conductance (g_s)] and g_s, and water-use efficiency [ratio of A_N to leaf transpiration (E)] and g_s of grapevine (cv. Carménère) growing at field conditions. Solid vertical lines represent the threshold values proposed by Cifre *et al*. (2005) to define water stress using stomatal conductance as an index: g_s values from 0.5 to 0.15, 0.15 to 0.05 and < 0.05 mol H_2O/m^2/sec correspond to mild, moderate and severe water stress phases, respectively.

-0.4 and -0.9 MPa. For this range, the cv. Carménère maintained values of stomatal conductance > 0.1 mol/m^2/sec. Also, Fig. 5 shows that stressed and non-stressed leaves were observed both before (black circles) and after (white circles) véraison. Finally, leaves under severe water stress (Ψs < -1.4 MPa) were only observed in the véraison-pre-harvest period in the 2005/2006 season (Fig. 1).

The stomatal sensitivity (*k*) calculated for the water deficit ranges suggested by Van Leeuwen *et al*. (2009) is indicated in Fig. 6. This figure shows that *k* decreased as water stress increased, with values of 234 and 120 for no and severe water stress respectively. Moreover, Fig. 7 indicates that the values of *k* ranged between 264 and 480 and 255 and 297 for the measurements taken before and after véraison respectively. In this case, values of r^2 for the linear regressions between g_s and the 'Ball factor' ranged between 0.23 and 0.78.

DISCUSSION

Gas exchange behaviour under open field conditions

The response of plants to drought is generally categorised as being typical for desiccation or drought tolerance mechanisms instead of drought avoidance, since drought is a form of stress that all plants can tolerate but none can avoid. In this regard, we can distinguish between desiccation postponement (the ability to maintain tissue hydration) and desiccation tolerance (the ability to function while dehydrated), which sometimes are referred to as drought tolerance at high and low water potentials respectively. For grapevines, the range of observed responses exhibited by the different cultivars is very large (Chaves *et al*., 2010) and several classifications seem possible. Based on all the parameters measured in the present study, the cultivar Carménère could be classified as drought tolerant at low water potentials, with a large range of physiological parameters changing in response to water stress. The stomatal aperture reflects the differential turgor between guard cells and neighbouring cells. The epidermal turgor can be affected by humidity and drought, so that appropriate stomatal responses could occur passively (i.e. hydraulically) without any active (i.e. metabolic) involvement (Schultz, 2000). In the case of the cultivar Carménère, the results show that its stomatal closure cannot be purely hydraulic, since the stomata remained partially open even at Ψs between -1.6 and -1.4 MPa (severe water stress) (Fig. 5). This result suggests that, for complete stomatal closure to occur, an extra signal from the roots may be needed (i.e. ABA). In this regard, Sauter *et al*. (2001) suggested that the stomatal sensitivity to the root signal could vary in different cultivars.

In this study of field-grown grapevines, a good hyperbolic relationship was observed between A_N and g_s, with a large scattering of the data. The A_N-g_s relationship was observed for different cultivars under non-irrigated and irrigated conditions (both field- and pot-grown vines), which suggests

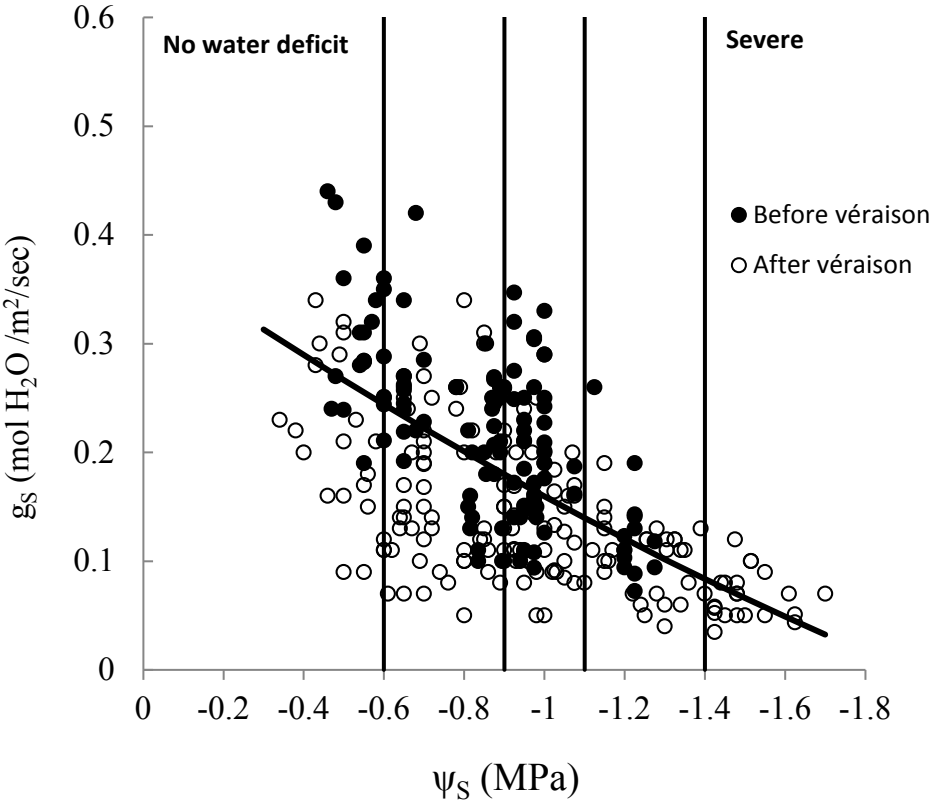


FIGURE 5

Relationship between stomatal conductance (g_s) and midday stem water potential (Ψs) of cv. Carménère under different water stress conditions. The dataset was divided into measurements taken before (black circles) and after véraison (white circles). Lines represent the thresholds to define water stress levels according to Van Leeuwen *et al*. (2009).

that this association can be used as an integrative tool to evaluate the response of different photosynthetic processes to drought (Flexas *et al*., 2002; Medrano *et al*., 2002; Cifre *et al*., 2005). In this regard, it should be mentioned that the maximum value of A_N observed in this study was close to 15 µmol/m^2/sec, which is lower than that (19 µmol/m^2/sec) reported by Medrano *et al*. (2002). The large scattering of gas exchange measurements reported in this study have also been observed in field-grown and potted grapevines under different growing conditions (Flexas & Medrano, 2002; Flexas *et al*., 2002; Medrano *et al*., 2002). The factors that can explain this scattering are related to the constantly changing light, temperature and vapour pressure conditions present across the canopy (Flexas & Medrano, 2002; Flexas *et al*., 2002; Medrano *et al.*, 2002). The values of A_N and g_s observed in this study are within the ranges indicated in the literature for isohydric (Mourvèdre, Manto Negro, Tempranillo, Grenache and Ekigaïna) and anisohydric (Syrah and Marselan) cultivars growing under different levels of water stress. For instance, Romero *et al*. (2010) indicated that A_N and g_s ranged between 4 and 16 µmol/m^2/sec and 0.03 and 0.3 mol/m^2/sec, respectively for cv. Mourvèdre. Flexas and Medrano (2002) observed that A_N was between 0 and 18 µmol/m^2/sec and g_s was between 0 and 0.5 mol/m^2/sec for Manto Negro and Tempranillo, while Prieto *et al*. (2010) indicated that A_N was between 0 and 21 µmol/m^2/sec and that g_s ranged between 0 and 0.4 mol/m^2/sec for the cvs Syrah, Marselan, Grenache, Mourvèdre and Ekigaïna. Finally, Chaves *et al*. (2007) observed that A_N and g_s ranged between 4 and 15 µmol/m^2/sec and 0.05 and 0.3 mol/m^2/sec for the cvs Moscatel and Castelão, respectively.

With regard to water-use efficiencies, Cifre *et al.* (2005) and Flexas *et al.* (2010) indicated that a decreasing range of g_s, from 0.5 to 0.05 mol/m^2/sec produces a decline in A_N, which results in the progressive increase of WUEi. This pattern was also found in the ungrafted Carménère grapevines growing under field conditions. According to Romero *et al*. (2010) and Prieto *et al*. (2010), WUEi ranged between 10 and 230 µmol/mol in isohydric and anisohydric cultivars. These values of WUEi are similar to those reported in this study for the cv. Carménère under midday stem water potentials, ranging from -0.3 to -1.7 MPa. From a plant physiological point of view, WUEi should be understood as a cultivar-specific way to express water-use efficiency, since it avoids the large fluctuations observed in the values of WUE (A_N/E) that are caused by differences in evaporative demand without changes in stomatal conductance (Schultz, 2000).

Stomatal conductance response under changing plant water status

Similar distributions of Ψs for the g_s values ranging from 0.45 to about 0.05 mol/m^2/sec were observed regardless of whether the data was collected before or after véraison (Fig. 5). The lowest values of Ψs and g_s observed after véraison can be explained through the water restriction observed in the 2005/2006 season. These results are similar to the ones reported by Prieto *et al*. (2010) in their work with anisohydric grapevine cultivars (Syrah and Marselan).

The stomatal sensitivity values reported in this study (Figs 6 and 7) are similar to those found by Schultz *et al*. (1999), who reported that the *k* values changed over the growing season, going from high (poorly sensitive) in the early season to lower

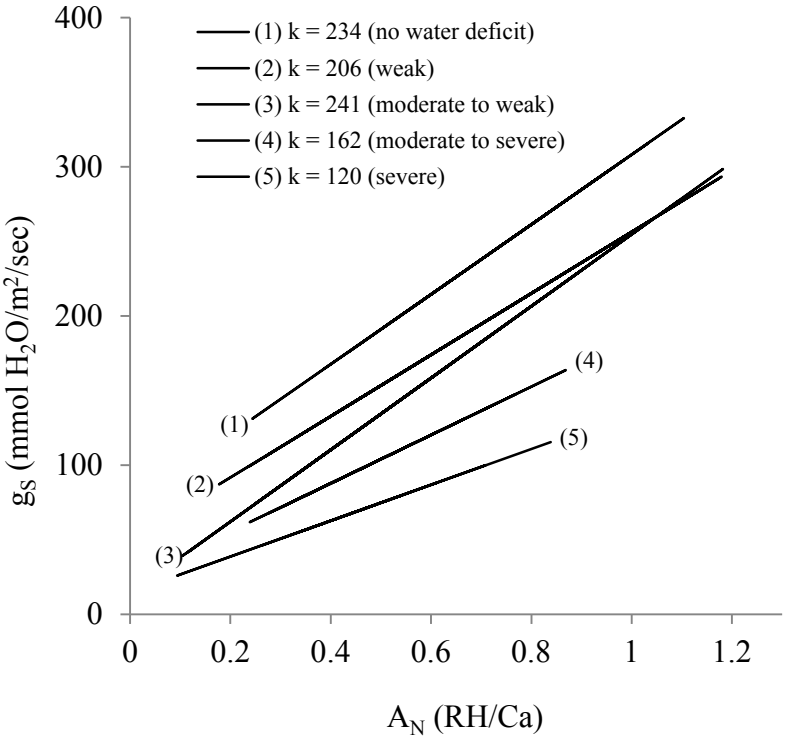


FIGURE 6

Relationship between stomatal conductance (g_s) and the 'Ball factor' [A_N(RH/Ca)] of cv. Carménère under different water stress conditions. Lines represent thresholds to define water stress levels according to Van Leeuwen *et al*. (2009).

values as véraison and harvest approached. In addition, these authors indicated that high values of *k* (inverse relationship with stomatal closure control) are associated with cultivars with low stomatal sensitivity to stressful environmental conditions. Furthermore, these authors also indicated that *k* ranged between 73 and 159 in anisohydric cultivars (Riesling, Chardonnay, Cabernet Sauvignon, Syrah, Touriga Nacional and Semillon) and had a value of 83 in an isohydric cultivar (Grenache). Prieto *et al*. (2010) observed *k* values of 150 and 120 in anisohydric (cv. Syrah) and isohydric (cv. Ekigaïna) grapevines respectively. The decrease in *k* may reflect the effect of shoot lignification late in the season, which alters the hydraulic capabilities of plant organs such as shoots and leaves. In the present study, véraison and shoot lignification usually started in the second half of January, a period that coincides with the shift in stomatal sensitivity (Schultz *et al*., 1999; Schultz, 2000). Fig. 7 indicates that the values of *k* were higher before than after véraison for the Carménère grapevines. In this regard, Speirs *et al*. (2013) indicated that the stomatal sensitivity increased (lower values) as the level of water stress increased, possibly due to an earlier or accelerated shoot lignification induced as a response of stress-hormone feedback. Similarly, Schultz (2000) observed that the stomatal sensitivity increased as the pre-dawn water potential decreased.

In Carménère, the transpiration could be sustained, despite presenting a large leaf area (GDC training system supports large canopies) through less sensitive stomata. In this case, the system needs a high water transport capacity in the soil-plant-atmosphere continuum and might be less sensitive to xylem embolisms. In this regard, Zufferey *et al*. (2011) described diurnal cycles of embolism formation and repair in petioles of stressed grapevines (cv. Chasselas). Nevertheless, the results showed that once véraison was reached, the stomatal sensitivity factor shifted to a more responsive state (Fig. 7), possibly because of the natural lignification of shoots, which resulted in a more conservative water behaviour after this period (Domec & Johnson, 2012; Rogiers *et al*., 2012; Zhang *et al*., 2012).

CONCLUSIONS

In this study, significant non-linear relationships were observed between A_N and g_s ($r^2 = 0.82$), Ci and g_s ($r^2 = 0.54$) and A_N/Ci and g_s ($r^2 = 0.49$) for ungrafted Carménère grapevines

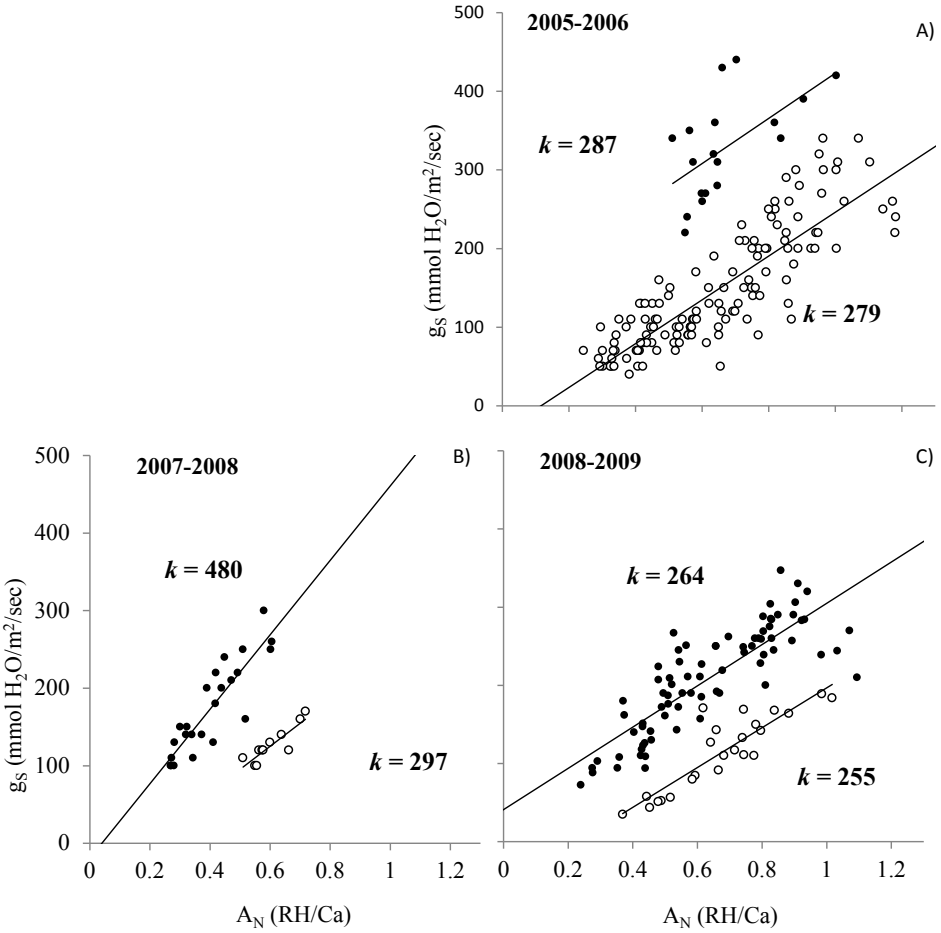


FIGURE 7

Stomatal sensitivity factor (*k*) of field-grown grapevines (cv. Carménère). The linear regressions between g_s and the 'Ball factor' were: A) **2005/2006**: y = 287.49(x) + 135.78 ($r^2 = 0.35$) and y = 278.61(x) - 32.076 ($r^2 = 0.72$) for black and white circles respectively; B) **2007/2008**: y = 480.05(x) -18.951 ($r^2 = 0.78$) and y = 105.44(x) + 58.706 ($r^2 = 0.23$) for black and white circles respectively; C) **2008/2009**: y = 263.89(x) + 41.05 ($r^2 = 0.70$) and y = 254.85(x) - 57.907 ($r^2 = 0.79$) for black and white circles respectively. The black and white circles indicate measurements taking before and after véraison, respectively.

growing in a drip-irrigated commercial vineyard. The values of A_N diminished from 14.9 to 3.5 µmol/m²/sec as g_s decreased from 0.5 to 0.05 mol/m²/sec, which resulted in a progressive increase in WUEi. In this cases, the mean values of WUEi for mild water stress (g_s values from 0.5 to 0.15 mol/m²/sec) ranged between 28.7 and 73.4 µmol/mol, while those for moderate water stress (g_s values from 0.15 to 0.05 mol/m²/sec) ranged between 41.5 and 110.3 µmol/mol. Also, a significant linear relationship was observed between Ψs and g_s ($r^2 = 0.39$) for measurements taken before and after véraison, with an increasing scattering from -1.6 to -0.4 MPa. Finally, *k* decreased as water stress increased, with values of 234 and 120 for no and severe water stress respectively, while *k* ranged between 264 and 480 and 255 and 297 for the measurements taken before and after véraison respectively. Based on all the parameters measured in the present study, the cultivar Carménère could be classified as drought tolerant at low water potentials, with a large range of physiological parameters changing in response to water stress.

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