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Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars

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Abstract

Improvements in water use efficiency of crops are essential under the scenarios of water scarcity predicted by global change models for the Mediterranean region. In recent years, deficit irrigation, including partial root drying (PRD), has been proposed as an irrigation technique to improve water use efficiency and standardize grapevine yield and quality. The objective of this study was to evaluate the impact of deficit irrigation on photosynthetic responses of field grown grapevines of cv. Moscatel and Castelão. The treatments were: full irrigation (FI), corresponding to 100% ET_c; non-irrigated, but rain fed (NI) and partial root zone drying (PRD) and deficit irrigation (DI), both corresponding to an irrigation of 50% ET_c. While in the DI treatment water was applied to both sides of the root system, in the PRD treatment, water was supplied to only one side of the root system, alternating sides periodically. In both cultivars, PRD and DI vines showed intermediate pre-dawn leaf water potential (ψ_{pd}) values (around -0.4 MPa) while FI vines, showed ψ_{pd} around -0.2 MPa during the growing season. NI showed the lowest ψ_{pd} , reaching -0.6 MPa in Moscatel and -0.8 MPa in Castelão, at the end of growing season. Water status of PRD vines remained closer to FI than DI, especially at midday. Photosynthetic rates and fluorescence parameters of the deficit irrigation treatments (PRD and DI) did not show significant differences from FI vines, for most of the season. In Moscatel, although PRD generally showed g_s , relative stomatal limitation (RSL) and intrinsic water use efficiency (A/g_s) values closer to NI than DI, most of the differences between PRD and DI were not statistically significant. Our results showed a stability of the photosynthetic machinery in grapevines under low-to-moderate water availabilities, as demonstrated by the maintenance of the activity of three Calvin Cycle enzymes and of the V_{cmax} values. However, a decline in J_{max} was observed in NI vines, that can be a result either of a decrease in ATP production or, alternatively, of decreased mesophyll conductance to CO₂ diffusion. In general terms, stomatal limitation of photosynthesis is likely to be dominant in non-irrigated plants. Deficit irrigation had no negative impact on CO₂ assimilation, despite less water application than in full-irrigation. Differences among varieties may be related to differences in sensitivity of stomata, shoot growth and/or the interaction between rootstock and cultivar to soil water availability.

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Keywords: Fluorescence; Gas exchange; Irrigation; Partial rootzone drying; *Vitis vinifera*; Water stress

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1. Introduction

Water deficit is one of the most important environmental factors inhibiting photosynthesis, growth and production under field conditions in the Mediterranean (Chaves et al., 2002). Many horticultural crops, including grapevines, rely on irrigation water during drought periods because rainfall occurs primarily during the dormant season, with subsequent vine growth being dependent upon stored soil water. However, the use of irrigation has been considered a controversial practice in traditional viticulture, since excessive soil water availability can cause significant reductions in berry colour and composition (Jackson and Lombard, 1993).

A key to improve winegrape quality in irrigated vineyards is to achieve an appropriate balance between vegetative and reproductive development, as an excess of shoot vigour may have undesirable consequences for fruit composition (McCarthy, 1997). A mild water stress maintained through deficit irrigation may reduce vine vigour and competition for carbohydrates by growing tips, as well as promoting a shift in the partition of photoassimilates and secondary metabolites towards reproductive tissues. These changes in plant metabolism by mild water stress may increase the quality of the fruit and wine produced (Matthews and Anderson, 1988, 1989). The deliberate restriction of irrigation water may, therefore, be a legitimate management strategy to manipulate crop water use and this is embodied in the technique known as regulated deficit irrigation (RDI) (Boland et al., 1993; Alegre et al., 1999; Dry et al., 2001). However, accurate monitoring of both vine and soil moisture status is required, and RDI may also result in changes to specific characteristics such as the size of grape berries (McCarthy, 1997).

A new irrigation technique called partial rootzone drying (PRD) has been used in commercial vineyards in Australia. PRD irrigation in grapevines has been shown to increase water use efficiency and decrease vegetative vigour without significantly reducing crop yield and berry sizes (Loveys et al., 2000). In PRD, a percentage of crop evapotranspiration is applied in alternate sides of the vine, allowing part of the root system to be always in contact with drying soil, while the other half is in contact with wet soil. The beneficial effects of PRD are hypothesised to be due to the

reduction of stomatal conductance and growth by chemical signals, possibly abscisic acid (ABA) synthesized by the roots in the drying soil zone and transported to the leaves in the transpiration stream (Loveys, 1984; Zhang and Davies, 1989a, 1989b).

Several studies have reported the effects of PRD on stomatal conductance, ABA in leaves and xylem, shoot and root growth rate (Loveys, 1984; Loveys et al., 2000; Stoll et al., 2000; Dry et al., 2000a, 2000b; Liu et al., 2001; dos Santos et al., 2003). Detailed physiological responses of grapevine to PRD and other deficit irrigation under natural field conditions have not received much attention. In previous studies, we have shown in cultivar Moscatel that leaf stomatal conductance, when measured under field conditions, but controlling CO₂, light and temperature in the leaf cuvette, was more reduced under PRD than in an irrigation treatment where the same amount of water was given to both sides of the root system (DI). However, in seasonal and diurnal courses of instantaneous measurements no large differences could be detected (de Souza et al., 2003), implying that the regulation is very subtle.

The knowledge of the mechanisms that regulate grapevine photosynthesis under different water regimes is of great interest to understand the key factors that regulate plant water use efficiency and ultimately grape yield and quality while saving water. In the present work we did a detailed characterization of the photosynthetic carbon assimilation, stomatal conductance and plant water status under field conditions in two cultivars with distinct vigour and berry characteristics. Plants were subjected to PRD and DI irrigation systems and compared with full irrigation and the traditional rainfed conditions in the area.

2. Materials and methods

2.1. Plant material and location

The experiment was conducted in a commercial vineyard located 70 km south-east of Lisbon, at the Centro Experimental de Pegões. The climate is Mediterranean, with hot, dry summers and mild winters. The soil is derived from podzols, with a sandy surface layer (0.6–1.0 m) and clay at 1 m depth. The

two varieties of *Vitis vinifera* L. studied were Moscatel (syn. Muscat of Alexandria), a vigorous and productive white variety used for wine and table grapes, and Castelão, an early season red wine variety (dos Santos et al., 2003). Both were grafted on 1103 Paulsen rootstock in 1997 and 1996, respectively. The grapevines were spaced 2.5 m between rows and 1.0 m within rows and trained on a vertical trellis with a pair of movable foliage wires for upwards shoot positioning. The vines were spur pruned on a bilateral Royat Cordon (~16 buds per vine).

2.2. Irrigation and experimental design

Irrigation water was applied with drip emitters (4 L h⁻¹ for FI and PRD and 2 L h⁻¹ for DI), two per vine, positioned 30 cm from the vine trunk (out to both sides of the rows), and distributed in both sides of root system. The water was supplied according to the crop evapotranspiration (ET_c) calculated from the evaporation of a Class A pan and corrected with the crop coefficients (K_c) proposed by Prichard (1992). The irrigation treatments were: rain fed non-irrigated (NI); partial rootzone drying (PRD, 50% of the ET_c was supplied to only one side of the root system, alternating sides each 15 days, approximately) deficit irrigated (DI, 50% of the ET_c was supplied to both sides of the vine); full irrigated (FI, 100% of the ET_c was supplied to both the sides of the root system). Irrigation was applied twice per week since the beginning of berry development (June) until harvest (August and September). Cumulative rainfall for the experimental period was 110 mm in 2002.

2.3. Water relations

Pre-dawn (ψ_{pd}) and midday (ψ_{md}) leaf water potential of six sun exposed and fully expanded leaves per treatment were measured with a Scholander-type pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA), from the beginning of berry development until harvest.

2.4. Gas exchange and chlorophyll fluorescence

Daily net CO₂ assimilation (A), stomatal conductance (g_s), and A–C_i response curves were measured on sun-exposed and recently fully mature

leaves, using a portable Li-6400 IRGA (Licor, Lincoln, Nebraska, USA) with four to eight replications per treatment for instantaneous measurements and four replications per treatment for A–C_i response curves. For A/C_i response curves the leaf chamber temperature was kept at 25 °C, photosynthetic photon flux density (PPFD) of 1200 μmol photons m⁻² s⁻¹ and relative humidity of 60–70%. The maximum rate of Rubisco carboxylation (V_{cmax}), maximum electron transport capacity at saturating light (J_{max}) and the rate of triose-P utilization (TPU) were obtained by fitting the model of Farquhar et al. (1980) with modifications by Sharkey (1985) to A/C_i response curves as described by Maroco et al. (2002). The net CO₂ assimilation biochemical model for C₃ plants of Farquhar et al. (1980) can be written as:

$$A = V_c - 0.5V_o - R_d = V_c \left(1 - \frac{0.5O}{\tau C_i} \right) - R_d \quad (1)$$

where V_c and V_o are the rates of carboxylation and oxygenation of Rubisco, R_d is the mitochondrial respiration, O and C_i are the oxygen and CO₂ partial pressures in intercellular spaces, respectively and τ is the Rubisco specificity factor for CO₂/O₂ factor. According to Farquhar et al. (1980) with modifications by Sharkey (1985), V_c is a minimum function of the carboxylation rates limited by either (i) kinetic properties and amount of Rubisco (W_c); (ii) the rate of RuBP regeneration (W_j); or (iii) the availability of inorganic phosphate (W_p). That is V_c = minimum{W_c, W_j, W_p}.

The rate of carboxylation limited by the amount and kinetic properties of Rubisco is given by:

$$W_c = \frac{V_{cmax} C_i}{C_i + K_c(1 + O/K_o)}$$

where V_{cmax} is the maximum rate of carboxylation, while K_c and K_o are the Michaelis constants for the carboxylation and oxygenation processes, respectively.

The rate of carboxylation limited by the regeneration of RuBP is a function of the rate of electron transport given by:

$$W_j = \frac{JC_i}{4(C_i + O/\tau)}$$

where it is assumed that four electrons are enough to generate the three ATP and two NADPH required in

the Calvin cycle to regenerate RuBP, and J is the rate of electron transport through PSII given by:

$$J = \frac{\alpha I}{\sqrt{1 + \alpha^2 I^2 / J_{\max}^2}}$$

where α is the fraction of incident light that is converted into electrons and J_{\max} is the maximum, light saturated, rate of electron transport.

Finally, the rate of carboxylation limited by inorganic phosphate availability inside the chloroplast is given by:

$$W_p = 3 \text{ TPU} + \frac{V_c O}{2C_i \tau}$$

where TPU is the rate of triose-P utilization (for sucrose and starch synthesis) (Sharkey, 1985). Michaelis–Menten constants (K_c and K_o), α , τ and temperature dependence of the model parameters were corrected as described by Harley et al. (1992). According to these authors, the temperature dependence of K_c , K_o , α , τ is described by an exponential function of the type parameter = $\exp[c - \Delta H_a/RT]$, where c is a scaling constant characteristic for each parameter, ΔH_a is the activation energy for the parameter, R is the ideal gas-constant, and T is the leaf temperature in degrees Kelvin. For details of constants used to parameterise the photosynthetic model, see Table 1. $V_{c\max}$ and R_d were estimated by fitting the model to measured $A-C_i$ data for C_i less than 20 Pa because, in this C_i range V_c is limited only by W_c , if light is saturating, as it was in this case. Finally, J_{\max} and TPU were estimated by fitting the model, with

previously estimated $V_{c\max}$ and R_d , to the complete $A-C_i$ data using a non-linear curve fitting routine from SAS (SAS Institute, Cary, North Carolina, USA).

The limitation to photosynthesis imposed by closed stomata was calculated from A/C_i response curves using the equation $\text{RSL} = ((A_{C_i=35 \text{ Pa}} - A_{C_a=35 \text{ Pa}}) / A_{C_i=35 \text{ Pa}}) \times 100\%$, where RSL is the relative stomatal limitation, $A_{C_i=35 \text{ Pa}}$ is the net CO_2 assimilation at intercellular CO_2 of 35 Pa, and $A_{C_a=35 \text{ Pa}}$ is the net CO_2 assimilation at ambient CO_2 of 35 Pa. Estimates of $A_{C_i=35 \text{ Pa}}$ were obtained from previously fitted Eq. (1) to A/C_i (or C_a) data.

Chlorophyll a fluorescence was measured from berry set to harvest using a Mini Pam fluorometer (H. Walz, Effeltrich, Germany), in the same leaves used for the daily courses of gas exchange. The PSII photochemical efficiency (Φ_{PSII}) was calculated as $\Phi_{\text{PSII}} = (F'_m - F_s) / F'_m$ and non-photochemical quenching (NPQ) calculated as $\text{NPQ} = (F_m - F'_m) / F'_m$. The electron transport rate (ETR) was calculated as $\text{ETR} = \Delta F / F'_m \times \text{PPFD} \times 0.5 \times 0.84$ (Björkman and Demmig, 1987). Measurements were done at two weeks intervals from beginning of berry development until harvest, during the 2002 growing season.

2.5. Enzyme assays

For determination of enzyme activity six discs (3.56 cm^2) per plant (four plants per treatment) were harvested in the light, frozen in liquid N_2 and stored -80°C until assay. The frozen leaf discs were ground to a fine slurry in 1.5 ml of an extraction solution

Table 1
List of constant model parameters used to estimate $V_{c\max}$, J_{\max} , TPU and R_d ^a

| Parameter | Units | Temperature parameters | Values | Units |
|-----------|---|------------------------|---------|----------------------|
| K_c | Pa CO_2 | $c(K_c)$ | 35.79 | – |
| | | $\Delta H_a(K_c)$ | 80.47 | kJ mol^{-1} |
| K_o | kPa O_2 | $c(K_o)$ | 9.59 | – |
| | | $\Delta H_a(K_o)$ | 14.51 | kJ mol^{-1} |
| τ | – | $c(\tau)$ | –3.9489 | – |
| | | $\Delta H_a(\tau)$ | –28.99 | kJ mol^{-1} |
| T | K | – | 298 | – |
| R | $\text{kJ K}^{-1} \text{ mol}^{-1}$ | – | 0.00831 | – |
| α | mol electrons per mol photons | – | 0.18 | – |
| PPFD | $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ | – | 1200 | – |
| O | Pa | – | 20580 | – |

K_c and K_o , Michaelis–Menten constants for CO_2 and O_2 , respectively; τ , Rubisco specificity factor for CO_2/O_2 ; T , leaf temperature; R , universal gas constant; α , efficiency of light energy conversion on an incident light basis; PPFD, incident light; O , oxygen concentration.

^a Data from Harley et al. (1992).

containing 200 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 10 mM β -mercaptoethanol, 2 mM dithiothreitol (DTT), 2% triton X-100, 4%(v/v) ‘Complete-protease inhibitor cocktail with EDTA’, 10% polyvinylpyrrolidone, and 10% glycerol. The enzymatic activities of glyceraldehyde-3-phosphate dehydrogenase (G3PDH; EC 1.2.1.13), ribulose-5-phosphate kinase (Ru5PK; EC 2.7.1.19) and stromal fructose-1,6-biphospahte phosphatase (FruBPase; EC 3.1.3.11) were measured spectrophotometrically by following the oxidation of NADH at 340 nm. The activity of G3PDH was measured by adding 20 μ L of leaf extract in 1 mL of reaction mixture containing 100 mM Tris–HCl pH 8.0; 2 mM $MgCl_2$; 1 mM EDTA; 0.4 mM NADH; 6 mM 3-phosphoglyceric acid (PGA); 20 units 3-phosphoclyceric phosphokinase (PGK). The reaction was started with 5 mM ATP. The activity of Ru5PK was measured, after preincubation for 15 min, by adding 20 μ L of leaf extract in 1 mL of reaction mixture containing 100 mM Tris–HCl pH 8.0; 8 mM $MgCl_2$; 40 mM KCl; 1 mM PEP; 10 mM ATP; 1 mM NADH; 20 mM DTT; 8 units pyruvate kinase; 10 units lactate dehydrogenase; and 5 units phosphoriboisomerase. The reaction was started adding 5 mM Ribose-5-Phosphate. For the FruBPase assay, 50 μ L of leaf extract was added into 1 ml reaction mixture containing 200 mM Tris–HCl pH 8.0; 10 mM $MgCl_2$; 10 mM DTT, 1 mM EDTA, 0.4 NADP; 4 units glucose-6-phosphate isomerase; 2 units glucose-6-phosphate dehydrogenase. The reaction was started with 1 mM fructose-1,6-bisphosphate. The enzymatic activities were expressed in μ mol of NADH per unit leaf area.

2.6. Leaf nitrogen content and vine vigour

Similar leaves to those used for photosynthesis measurements were harvested for the analysis of nitrogen. The leaves (four leaves per treatment) were harvested in different dates throughout the growing season. Total nitrogen content of the leaves were measured by the Kjeldahl method, using leaf material that had been dried at 80 °C and powdered.

In this study the vine vigour was estimated by the pruning weight. At winter pruning, shoot number and weight were taken to determinate the shoot weight per vine.

2.7. Statistical data analyses

Two-way factorial analyses of variance (ANOVA), with sampling time and treatments as main factors, was used to test the main effects and factor interactions on ψ_{pd} , ψ_{md} , gas exchange and fluorescence parameters. One-way ANOVA was used to test the treatment effects on enzymes, nitrogen contents and pruning weight. Significant differences between treatments means were evaluated with Tukey’s HSD tests with $\alpha = 0.05$ using the ‘‘Statistica’’ software (Version 5.0 StatSoft, Tulsa, OK, USA). All measurements shown are the mean \pm S.E.

3. Results

3.1. Environmental conditions and plant water status

Fig. 1 shows the rainfall and average temperature during 2002 and the 30-year average for the region. The amount of rainfall during spring and at the end of experimental period (September) was higher in 2002 as compared to the 30-year average. On the other hand, the average temperature followed the same pattern as the 30-year average, ranging between 20 and 22.5 °C during the summer. During the measurements period, the maximal temperature (around 35 °C) was recorded at the middle of June and the end of July (Fig. 2).

The vine water status was influenced by the irrigation treatments. In both cultivars, PRD and DI showed intermediate pre-dawn leaf water potential

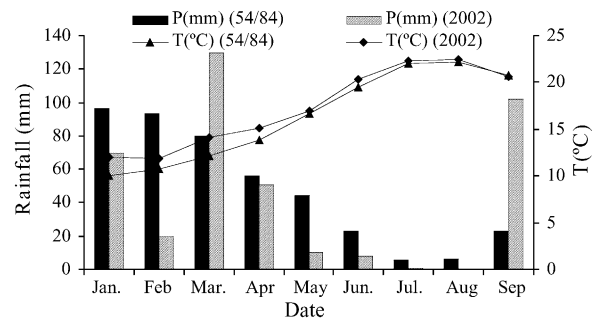


Fig. 1. Total rainfall (bars) and monthly mean air temperature (lines) at the experimental site during the 2002 season and average values of 30 years (1954–1984).

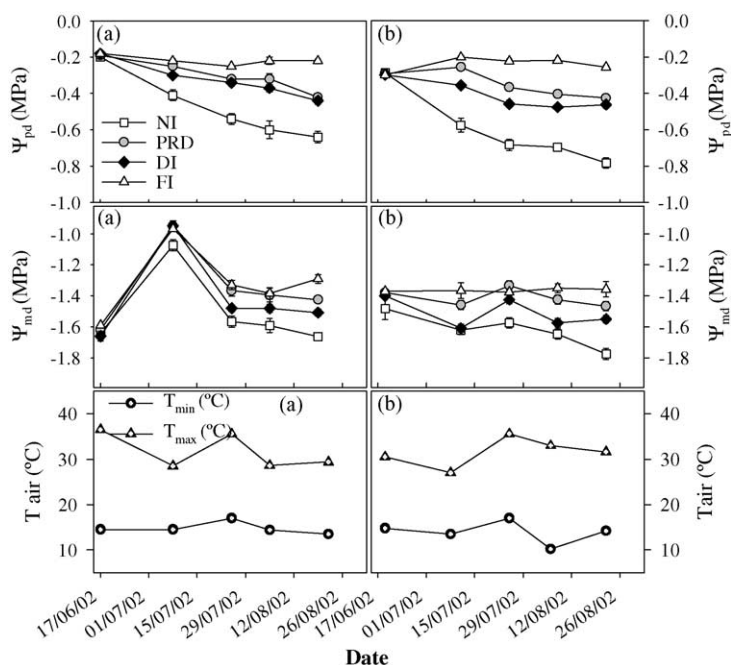


Fig. 2. Seasonal evolution of leaf water potential at pre-dawn (ψ_{pd}) and at midday (ψ_{md}) and minimal (T_{min}) and maximum (T_{max}) temperatures in Moscatel (a) and Castelão (b) recorded during the days of measurements. Values of leaf water potential are mean \pm S.E.

(ψ_{pd}) values (around -0.4 MPa) as compared to NI and FI vines. However, in Castelão, PRD vines showed significantly higher values than DI vines during July. The NI vines showed increasing differences in ψ_{pd} from July onwards as compared with the other treatments. At the end of the growing season, the ψ_{pd} in NI vines reached -0.6 MPa in Moscatel and -0.8 MPa in Castelão. FI vines showed an almost constant ψ_{pd} (around -0.2 MPa) throughout the growing season in both cultivars. The midday leaf water potential (ψ_{md}) followed a pattern similar to the ψ_{pd} . The ψ_{md} in PRD vines was in general closer to FI vines than DI vines during growing season. However, there were no statistically differences between PRD and DI in most of the sampled dates in both cultivars.

3.2. Seasonal evolution of gas exchange and chlorophyll fluorescence derived parameters

The ANOVA test revealed a significant effect ($P < 0.01$) of irrigation treatments in gas exchange and fluorescence parameters recorded in both cultivars (Fig. 3). During the growing season, the highest reductions in photosynthesis (A) and stomatal con-

ductance (g_s) occurred in NI vines. The effect of PRD and DI was more pronounced in g_s than A . Although there were no statistically differences between PRD and DI, the values of g_s in PRD were closer to NI than DI vines at the middle of growing season, especially in Moscatel. In Castelão, FI vines also showed a reduction in gas exchange at the end of growing season, whereas in Moscatel the values of A and g_s were maintained almost constant (around $12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $0.25 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively).

The quantum yield of PSII reaction centres (Φ_{PSII}) in Moscatel leaves were not affected the irrigation treatments during the growing season. However, in Castelão, NI vines showed a decrease in Φ_{PSII} of 47% as compared to FI vines at the middle of the growing season. In both cultivars, there was no indication of permanent photoinhibition, as demonstrated by pre-dawn F_v/F_m values always close to 0.8.

In Moscatel, there was an increase (around 35%) of intrinsic water use efficiency (A/g_s) in PRD and NI vines as compared to FI vines. Although the A/g_s in PRD was closer to NI values, it was not statistically significant different from DI. In Castelão, there were

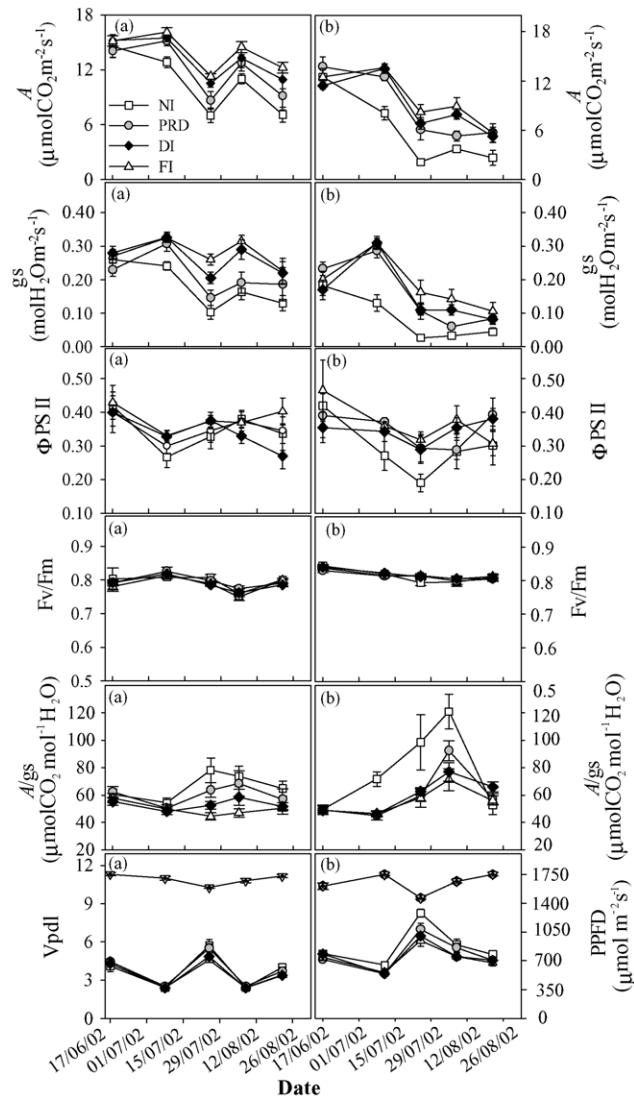


Fig. 3. Seasonal trends at midday of photosynthesis (A), stomatal conductance (g_s), quantum yield of PSII (Φ_{PSII}), efficiency of excitation capture by open PSII in dark-adapted leaves (F_v/F_m), intrinsic water use efficiency (A/g_s), leaf-to-air vapour pressure deficit (V_{pdl}) and photosynthetic photon flux density (PPFD) in cultivars Moscatel (a) and Castelão (b). Values are the mean \pm S.E.

statistically significant differences only between irrigated (FI, DI, PRD) treatments and the non-irrigated treatment (NI).

During the growing season, the highest values of leaf-to-air vapour pressure deficit (V_{pdl}) occurred on 25 July for both cultivars. In Moscatel, NI and PRD vines showed higher values of V_{pdl} (around 5.5–5.7) than DI and FI vines (around 4.6–4.8). In Castelão, NI vines reached a value near to 8, PRD and DI vines

showed the intermediate values (around 6.4–6.9) and FI, the lowest values (6). However, there were no significant differences between DI and PRD vines in both cultivars.

There was a significant correlation of A and g_s with pre-dawn water potential (Fig. 4). However, the best correlations were obtained using midday values of A and g_s . The dependence of A and g_s on ψ_{pd} was slightly higher in Castelão than in Moscatel as shown by the

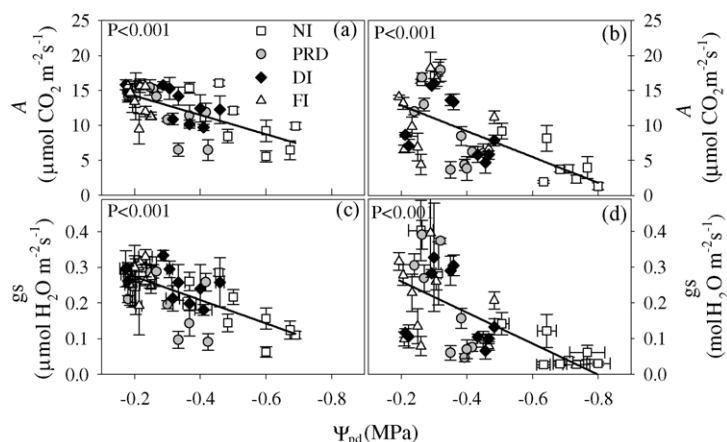


Fig. 4. Dependence of photosynthesis of Moscatel (a) ($y = 16.97 + 13.74x$, $R^2 = 0.41$) and Castelão (b) ($y = 16.55 + 18.45x$, $R^2 = 0.37$) and of stomatal conductance of Moscatel (c) ($y = 0.34 + 0.32x$, $R^2 = 0.44$) and Castelão (d) ($y = 0.34 + 0.41x$, $R^2 = 0.38$) on pre-dawn leaf water potential (ψ_{pd}). Values are mean \pm S.E. Measurements were taken throughout the growing season at midday.

slope of the equation regression (although the differences were not statistically significant). For ψ_{pd} around -0.6 MPa, A was reduced by 48% and g_s by 56% in Moscatel, whereas in Castelão, A was reduced by 67% and g_s by 74%. Despite the higher reductions in Castelão, there were no significant differences between the two varieties due to the large variability observed. According to the regression equation, the maximum A and g_s (extrapolating at $\psi_{pd} = 0.0$) were similar for both cultivars.

The coefficients of determination (R^2 , indicating the proportion of the variability in A that is explained by g_s) between A and g_s were high in both cultivars (0.81 and 0.91) (Fig. 5), which suggest a strong regulation of photosynthesis by stomatal aperture, or at least a co-regulation. Both cultivars showed also significant correlation of A/g_s and ETR/A with g_s ($P < 0.001$). However in A/g_s , Moscatel showed a higher coefficient of determination (0.70) as compared to Castelão (0.48). The response of ETR/A to g_s showed that the increase of this ratio is higher in Castelão than Moscatel when stomatal conductance is close to zero.

3.3. $A-C_i$ response curves

In Fig. 6 are illustrated the maximum rate of Rubisco carboxylation (V_{cmax}), maximum electron transport capacity at saturating light (J_{max}), the rate of

triose-P utilization (TPU) and the relative stomatal limitation (RSL) estimated from A/C_i response curve. In both cultivars, there was no effect of the water treatments on V_{cmax} . In Moscatel, J_{max} decreased by 46% in NI vines as compared to FI vines that showed the highest values ($233 \mu\text{mol m}^{-2} \text{s}^{-1}$). PRD and DI vines showed the intermediate values around 203 – $219 \mu\text{mol m}^{-2} \text{s}^{-1}$. In Castelão, there were no significant differences among water treatment in J_{max} .

In Moscatel, the TPU was reduced by 26% in NI and PRD vines as compared to FI vines, whereas the decrease of DI by 14% was not statistically significant. In Castelão, NI vines showed reductions in TPU of 36% in NI vines, whereas DI and PRD showed reductions by 18% as compared to FI vines.

There was an increase in RSL in NI and PRD vines as compared to DI and FI vines in Moscatel, whereas in Castelão, only in NI vines an increase in RSL was observed. In both cultivars, there were no significant differences between PRD and DI vines regarding the parameters estimated from $A-C_i$ curves (J_{max} , TPU and RSL).

3.4. Enzymatic activities and leaf nitrogen content

The activity of some photosynthetic enzymes was also measured in Castelão and Moscatel in August during the ripening period (Fig. 7). The activities of G3PDH (a key enzyme in the reductive phase of the

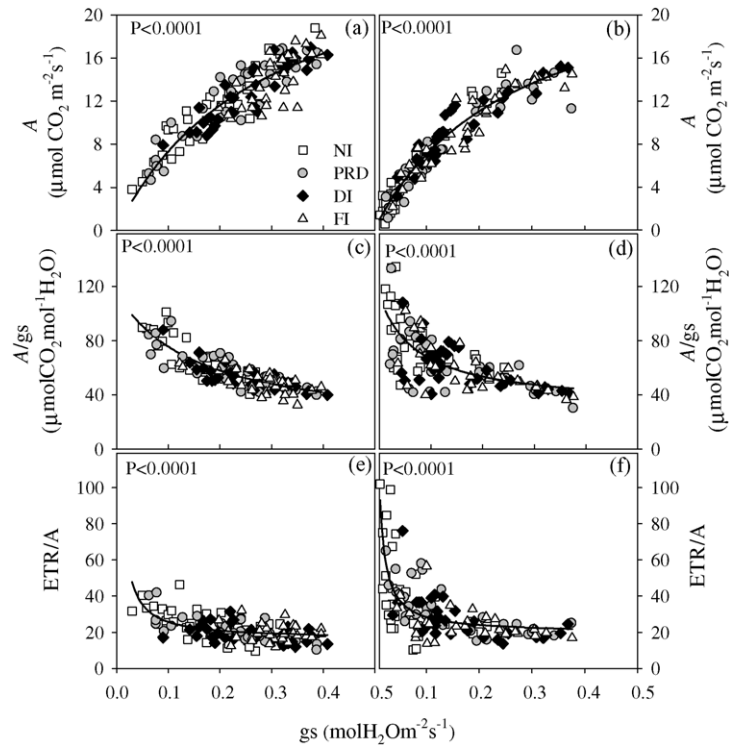


Fig. 5. Dependence on stomatal conductance of photosynthesis in Moscatel (a) ($y = 28.02x/(0.28 + x)$, $R^2 = 0.81$) and Castelão (b) ($y = 25.62x/(0.26 + x)$, $R^2 = 0.91$); of intrinsic water use efficiency (A/g_s) in Moscatel (c) ($y = 12.80 + 16.45/(0.156 + x)$, $R^2 = 0.70$), and Castelão (d) ($y = 30.86 + 6.25/(0.0759 + x)$, $R^2 = 0.48$); and of the ratio ETR/A in Moscatel (e) ($y = 16.17 + 0.96/x$, $R^2 = 0.32$) and Castelão (f) ($y = 19.65 + 0.879/x$, $R^2 = 0.48$). Measurements were taken throughout the growing season at midday.

Calvin Cycle), FruBPase (a key enzyme in the utilization of triose-P) and Ru5PK (a key enzyme in the regenerative phase of the Calvin Cycle) were not affected significantly by the different water availability in both cultivars. No significant differences ($P > 0.05$) were observed in leaf N among treatments. These results suggest that there were no effects of the irrigation treatments on the biochemical processes associated with CO_2 assimilation and triose-P utilization.

3.5. Vine vigour

In both cultivars, DI and FI vines showed the highest values of shoot biomass collected at pruning (Fig. 8). However, the statistically significant differences among irrigations treatments were only observed in Castelão. In this cultivar, there was a significant reduction (36%) in pruning weight in NI and PRD as compared to DI and FI vines.

4. Discussion

The different water availability in the soil resulted in different vine water status and consequently had effects on carbon uptake during the growing season (Fig. 2). It is noteworthy that the water status at midday in PRD of both cultivars was much closer to FI as compared to DI vines, although the latter treatment received the same amount of water as PRD. This did not happen at predawn, where PRD and DI were much closer to each other. These results support the hypothesis underlying the PRD technique that partial root dehydration caused by alternating the sides of the irrigation, is controlling plant water status, possibly by a chemical signal travelling from the roots to shoots, leading to stomatal closure and therefore water conservation (Loveys et al., 2000; Stoll et al., 2000).

The values of stomatal conductance and midday water potential do agree with the hypothesis of more

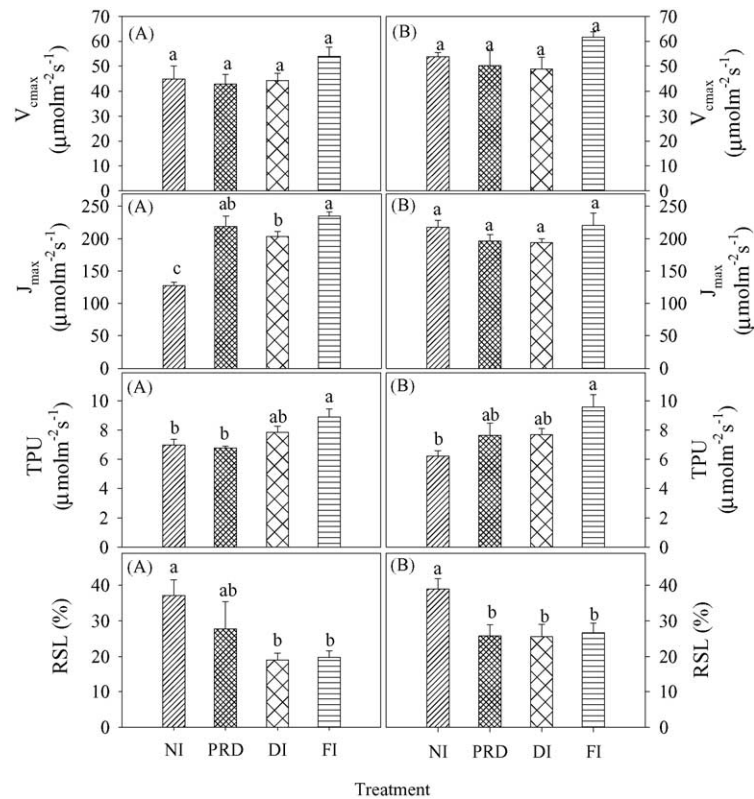


Fig. 6. Estimated model parameters (V_{cmax} , J_{max} , TPU) and relative stomatal limitation (RSL) for the irrigation treatments in Moscatel (A) and Castelão (B) measured in August. Values are mean \pm S.E. Different letter suffixes show statistically significant differences ($P < 0.05$).

intense closure of stomata at midday in PRD vines as compared with DI, as observed in the seasonal course of A and g_s (Fig. 3), mainly for the cultivar Moscatel. We observed in a previous work (de Souza et al., 2003) that under field conditions point measurements such as those given by leaf porometers, may not reveal stomatal control as effectively as other more integrated measurements such as stem sap flow or leaf $\delta^{13}\text{C}$ discrimination. In addition, the decrease in leaf elongation without the detection of any reduction in stomatal conductance can occur, as showed by Saab and Sharp (1989). In our study the vine vigour was significantly reduced in NI and PRD as compared to DI and FI as showed by pruning weight in cv. Castelão (Fig. 8). In the same experiment, but in previous years (2000 and 2001), the inhibition of vegetative growth in PRD vines as compared with DI was also observed in both cultivars (dos Santos et al., 2003).

Increased stomatal limitation in NI and PRD as compared to DI and FI is also confirmed by RSL estimations from A/C_i curves (Fig. 6) and by the higher intrinsic water use efficiency, given by A/g_s in Moscatel (Fig. 3). Increased xylem sap ABA concentration was considered as a potential cause of the observed decrease in stomatal conductance as shown in several studies (Davies et al., 2000; Stoll et al., 2000; Liu et al., 2001). However, this response can vary among species as demonstrated by Fort et al. (1997, 1998) working with seedlings of young oak and *Betula pendula*, who did not find an increase of ABA concentration in xylem sap originated in the roots under PRD conditions. A chemical signal from dehydrating roots leading to a change in apoplastic ABA content in leaves may be responsible for increased stomatal closure under PRD (Holbrook et al., 2002).

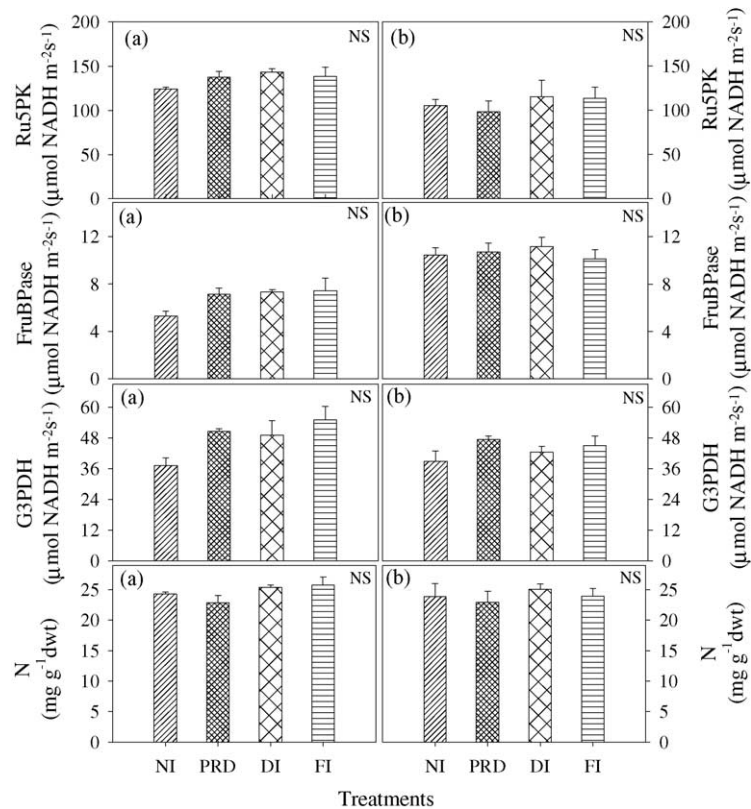


Fig. 7. In vitro activities of glyceraldehyde-3-phosphate dehydrogenase (G3PDH), ribulose-5-phosphate kinase (Ru5PK) and fructose-1,6-biphosphate phosphatase (FruBPase) and leaf N contents (N) on different water treatments in cultivars Moscatel (a) and Castelão (b) measured in August. Values are mean \pm S.E. NS: non-significant.

Although there was a decrease in stomatal conductance, the photosynthetic performance of PRD and DI was similar to FI vines as demonstrated by the seasonal evolution of A (Fig. 3). The lowest photosynthetic rates obtained in NI vines of cv.

Castelão were accompanied by reductions in Φ_{PSII} suggesting a down-regulation of photochemistry to match the decreased CO_2 assimilation capacity under water stress conditions. In this cultivar, the reductions of photosynthesis observed in FI vines during the

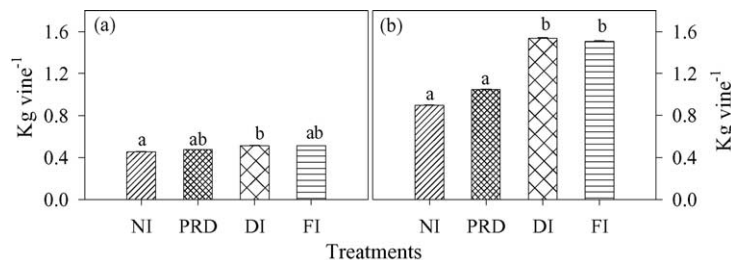


Fig. 8. Effects of different water treatments on pruning weight in Moscatel (a) and Castelão (b). Values are mean \pm S.E. Different letter suffixes show statistically significant differences ($P < 0.05$).

growing season was probably due to the aging of leaves, since precocity of vegetation in this cultivar is higher as compared to Moscatel.

Water stress had no effects on the maximal primary photochemical efficiency of PSII, determined in dark-adapted leaves in both cultivars. Permanent photo-inhibition in grapevines was shown to be rare under drought (Flexas et al., 1999; Maroco et al., 2002; de Souza et al., 2003).

It is also interesting to note the large scattering of A and g_s when related to ψ_{pd} (Fig. 4), especially in cultivar Moscatel, indicates a higher resilience of carbon assimilation and stomatal conductance to dehydration, so that high values can be maintained for a large range of leaf water potentials (from -0.2 down to -0.4 MPa). Furthermore, the cv. Moscatel has been considered well adapted to water stress conditions, showing high values of g_s when others cultivars had already reached values near to zero (Regina and Carbonneau, 1996). On the other hand, ψ_{pd} may not be the best indicator of the intensity of water stress, because deep roots may be influencing ψ_{pd} , whereas stomata may be responding to signals resulting from dehydration of superficial roots, before any change in leaf water potential is detectable. Mid morning g_s has been suggested as an integrative parameter to reflect water stress experienced by plants, namely in grapevines (Flexas et al., 2002; Medrano et al., 2002). In our study, a good correlation between several physiological parameters and midday g_s was observed in both cultivars (Fig. 5) indicating that g_s rather than ψ_{pd} is a better indicator of water stress intensity.

Some studies have shown that the decreased photosynthesis under water deficit can be also associated with perturbations of the biochemical process (Correia et al., 1990; Tezara et al., 1999, 2002; Lawlor and Cornic, 2002; Parry et al., 2002). In the present study, the relative importance of stomatal versus metabolic limitations was assessed from A/C_i curves (Fig. 6) and key enzymes of the Calvin Cycle (Fig. 7). Although we are aware of the occurrence of patchy stomatal conductance, which can induce artefacts in C_i calculations (for review see Mott and Buckley, 1998), it has been acknowledged that under field conditions the effects of patchiness may be minimized (Guanasekera and Berkowitz, 1992), because the plants are subjected to a relatively gradual rate of stress imposition. Indeed, in a study with a

different grapevine cultivar, cv. Tempranillo, Maroco et al. (2002) showed a good agreement between stomatal versus non-stomatal effects estimated both from biochemical measurements and A/C_i estimates.

We observed that J_{max} (related to RuBP regeneration) and TPU were more affected by water deficits than V_{cmax} (dependent on Rubisco activity). In fact, we observed no statistically significant differences in V_{cmax} among treatments in accordance with the reported higher resistance of Rubisco activity to water stress than photophosphorylation (Giménez et al., 1992; Tezara et al., 1999). Under mild stress, Escalona et al. (1999) did not observe a significant reduction in Rubisco carboxylation efficiency for ψ_{pd} of -0.5 ± 0.04 MPa, in the cultivar Tempranillo of grapevines. However, under severe drought (ψ_{pd} around -1.0 MPa) a significant decrease in V_{cmax} was reported by Maroco et al. (2002) in field-grown grapevines.

The biochemical limitations of photosynthesis were also evaluated by measurements of in vitro activities of key enzymes of Calvin Cycle, namely G3PDH, RuP5PK and FruBPase (Fig. 7), which are involved in RuBP regeneration and triose-P utilization. In our study, even in NI vines that reached ψ_{pd} values of -0.7 Mpa, no statistically significant reductions on the activities of those enzymes were observed. Because leaf N content also did not decrease under lower water availability, our results support previous studies indicating a high stability of the biochemical photosynthetic machinery under water deficit.

Although there were no significant reductions in RuP5PK, one of the enzymes involved in RuBP regeneration, there was a decrease of J_{max} in stressed vines. The reduced J_{max} could be related to inhibition of ATP synthesis as reported by some authors (Tezara et al., 1999; Lawlor, 2002). Furthermore, the reductions in the mesophyll conductance to CO_2 (g_{mes}) between the intercellular spaces and the chloroplasts that will restrict the CO_2 availability to the chloroplast, may also explain the reduced J_{max} observed (Centritto et al., 2003; Ethier and Livingston, 2004). The reductions in some parameters from $A-C_i$ curve analyses (as J_{max} and TPU) observed in stressed plants might be underestimated by a reduction in g_{mes} , not considered in the parameterization. In grapevines, the apparent carboxylation efficiency and the compensation point for CO_2 remained unchanged under severe

drought when analysed on a chloroplastic CO₂ concentration (C_c), rather than C_i, basis, suggesting that previously reported metabolic impairment was probably due to decreased g_{mes} (Flexas et al., 2002). Therefore, our results contribute to confirm that reduction in grapevines photosynthesis under mild to moderate drought is mainly due to an increase in the resistance of CO₂ diffusion mainly by stomatal closure, but probably also by reduced mesophyll conductance.

5. Conclusion

In conclusion, our results showed that water status of PRD vines remained closer to FI than DI, especially at midday, suggesting that PRD may restrict stomatal conductance, as compared to equivalent water availability for the plants, as in the case of the DI treatment. Depressive effects of PRD on shoot growth were present in Castelão, the most drought sensitive cultivar. Differences among varieties may be related to differences in sensitivity of stomata, shoot growth and/or the interaction between rootstock and cultivar to soil water availability. We also observed a high resistance of the photosynthetic apparatus of grapevine leaves to restricted water availability under field conditions. In fact, the decreased stomatal conductance observed in some cases in PRD vines had no major negative impact on carbon assimilation. The benefits of PRD and DI relative to FI were the restricted water consumption with similar CO₂ assimilation leading to improved water use efficiency. Although the differences between the PRD and DI treatments in our study were subtle, they may become more marked in years or regions with lower precipitation, as well as with more drought responsive varieties.

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