# Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines (*Vitis vinifera* cv. Moscatel)

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*Abstract.* The effects of 'partial rootzone drying' (PRD) irrigation compared with other irrigation systems, namely non-irrigated (NI), full irrigation (FI) and deficit irrigation (DI), on stomatal conductance and carbon assimilation were evaluated in field-grown grapevines (*Vitis vinifera* L. cv. Moscatel). At the end of the growing season, pre-dawn leaf water potential was highest in FI ($-0.18 \pm 0.01$ MPa; mean $\pm$ s.e.), intermediate in PRD ($-0.30 \pm 0.01$ MPa) and DI ($-0.36 \pm 0.02$ MPa), and lowest in NI vines ($-0.64 \pm 0.03$ MPa). Stomatal conductance measured under controlled conditions of light and temperature was reduced in NI (*ca* 60%) and PRD (*ca* 30%) vines compared with DI and FI vines. Under ambient conditions, NI vines had lower rates of stomatal conductance (*ca* 26%), net $CO_2$ assimilation (*ca* 28%) and light-adapted PSII quantum yields (*ca* 47%) than PRD, DI and FI vines. No significant differences were found among the three irrigated treatments. Both maximum electron transport rate ($J_{max}$; *ca* 30%) and triose-phosphate utilization rates (TPU; *ca* 20%) were significantly lower in NI and PRD vines than in DI and FI vines. Carbon isotope composition ($\delta^{13}C$) of grape berries was highest in NI vines ($-24.3‰$), followed by PRD ($-25.4‰$) and DI ($-25.8‰$) and lowest in FI ($-26.4‰$) vines, suggesting a long-term increase in the efficiency of leaf gas exchange in NI compared with PRD, DI and FI vines. Sap-flow data and estimates of relative stomatal limitation are in accordance with the observed stomatal closure in PRD vines. In this study, we show that PRD irrigation was able to maintain a vine water status closed to FI, but with double water use efficiency, which was due to a reduction of stomatal conductance with no significant decrease in carbon assimilation.

*Keywords*: carbon isotope composition, fluorescence, gas exchange, partial rootzone drying, *Vitis vinifera*, water stress.

## Introduction

For many regions with a Mediterranean climate, grapevine production depends upon water stored in soil during winter and spring. In summer, grapevines are often subjected to soil water deficits combined with high solar irradiance, high temperature and high vapour pressure deficit, which reduces leaf gas exchange and negatively affects grape yield and quality (Kliewer *et al.* 1983; Chaves and Rodrigues 1987; Schultz 1996). Stomatal closure appears to be the main

cause for the decrease in the photosynthetic rate under mild drought conditions (Chaves 1991; Cornic 2000, Chaves *et al.* 2002). However, different reports also suggest the occurrence of non-stomatal effects on the reduction of photosynthesis, associated with the decrease in PSII efficiency and mesophyll metabolism, particularly when drought is associated with high light and temperature (Correia *et al.* 1990; Lu and Zhang 1999; Lawlor and Cornic 2002). In grapevines, non-stomatal effects on photosynthesis

occur when vines are subjected to drought under field conditions (Escalona *et al.* 1999; Maroco *et al.* 2002).

Roots of plants in a drying soil have the capacity to influence stomatal conductance in the absence of any change in water status owing to stomatal sensitivity to chemical signals produced in the drying roots (Zhang and Davies 1989*a*, 1989*b*; Tardieu *et al.* 1992; Croker *et al.* 1998; Davies *et al.* 2000). Increased water stress results in the release of ABA from root tips into the transpiration stream to the leaves, where it triggers stomatal closure (Davies and Zhang 1991; Davies *et al.* 1994).

Irrigation is currently used as a cultural practice to improve and standardize grapevine yield and quality (Battilani and Mannini 2000). However, irrigation has been considered a controversial practice in traditional viticulture because one of its major effects on grapevines is increased plant vigour. The use of assimilates for leaf growth restricts fruit set and development, whereas excessive leaf area can lead to excessive water loss, fungal diseases and shading of fruit, causing a reduction in fruit and wine quality (Smart *et al.* 1985; Dry *et al.* 1996; Dry and Loveys 1998). Dry *et al.* (1996) showed that watering only half the root system whilst keeping the other half dry can significantly restrict vegetative growth without affecting yield and grape quality. This irrigation technique is called 'partial rootzone drying' (PRD) (Loveys *et al.* 2000).

The hypothesis associated with PRD is based on the reduction of the stomatal conductance and growth caused by chemical signals, possibly ABA, synthesized by the roots in the drying zone and transported to the leaves in the transpiration stream (Loveys 1984; Gowing *et al.* 1990). Partial stomatal closure can lead to a decrease in transpiration and, possibly, an increase in water use efficiency (Bergmann *et al.* 1982; Düring *et al.* 1997; Turner 1997). However, the physiological response of grapevine, other than stomatal conductance, to PRD irrigation under natural field conditions has not received much attention. Also, in most cases PRD was compared with full irrigation treatments (absence of water deficits) and not with a system giving the same amount of water as PRD, as done in the present experiment.

We hypothesize that by triggering stomatal closure, PRD irrigation may prevent excessive water losses and lead to a better water balance in the long term. This effect may prevent the metabolic inhibition of $CO_2$ assimilation that otherwise would occur if stress under field conditions was allowed to develop extensively (e.g. Escalona *et al.* 1999; Maroco *et al.* 2002). The aim of the present work was to analyse the effects of PRD on photosynthesis, stomatal conductance and water use efficiency (both in the short and long term) in field-grown grapevines. The contributions of stomatal and non-stomatal limitations to photosynthesis were studied by combined measurements of gas exchange and chlorophyll fluorescence. The physiological responses observed under PRD irrigation were compared with the ones observed under traditional drip irrigation systems, and in particular with the deficit irrigation (DI) treatment, where the same amount of water as in PRD was given to the whole root system.

## Materials and methods

### Plant material and location

The experiment was conducted during 2000 in a commercial vineyard located 70 km east of Lisbon, Portugal, 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 deep. We used *Vitis vinifera* L. cv. Moscatel (syn. Muscat of Alexandria) grafted in 1997 to 1103 Paulsen rootstock. 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).

### Irrigation and experimental design

Irrigation water was applied with drip emitters, two per vine, operating at 4 L h$^{-1}$ and positioned 30 cm from the vine trunk (out to both sides of the rows), distributed on both sides of the root system. The water was supplied according to the crop evapotranspiration (ETc) 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); PRD (50% of the ETc was supplied to only one side of the root system, alternating sides each 15 d); DI (50% of the ETc was supplied to both sides of the vine); and full irrigation (FI; 100% of the ETc was supplied to both the sides of the root system). Irrigation was done twice per week from the beginning of berry development (June) until harvest (September). Cumulative rainfall for the experimental period was 33 mm and the amount of water applied in the irrigation treatments was 144 mm for FI and 72 mm for DI and PRD.

Fully developed leaves from adult vines were randomly chosen from a Latin square experimental design with the four irrigation treatments.

### Water relations

Pre-dawn leaf water potential ($\psi_L$) was measured with a Scholander-type pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA), at weekly intervals from the beginning of berry development until harvest. The measurements were done on six sun-exposed and fully expanded leaves per treatments. Sap-flow measurements were done with a sap-flow system from Dynamax (Houston, TX, USA). Sap-flow sensors (SGA10 TYPE) were installed in main branches of representative vines, according to the manufacturer's instructions. Data were collected every 30 min throughout the growing season and recorded with a Campbell logger using the Flow 32 software supplied by the manufacturer. Sap-flow data were expressed on a leaf-area basis by dividing the sap flux density by the branch leaf area calculated in a non-destructive way, using a model with four variables — shoot length, leaf number, area of the largest and area of the smallest leaf — as described by Lopes and Pinto (2000).

### Gas exchange and chlorophyll fluorescence

Daily net $CO_2$ assimilation ($A$), stomatal conductance ($g_s$), and $A$–$C_i$ response curves (where $C_i$ is the internal $CO_2$ partial pressure) were measured on sun-exposed (at saturating light for photosynthesis) and recently fully mature leaves, with a portable Li-6400 infrared gas

analyser (Li-Cor, Lincoln, NE, USA) with 4 replications per treatment for instantaneous measurements and 6–8 replications per treatment for $A$–$C_i$ response curves. Response curves of net photosynthesis to internal $CO_2$ ($A$–$C_i$) were obtained with the same equipment with the leaf chamber temperature kept at 25°C, photosynthetic photon flux density (PPFD) of 1200 µmol photons m$^{-2}$ s$^{-1}$ and relative humidity at 60–70%. The maximum rate of Rubisco carboxylation ($V_{Cmax}$), mitochondrial respiration ($R_d$), 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) using the NONLIN procedure of SAS (version 8.0; SAS Institute, Cary, NC, USA). The limitation to photosynthesis imposed by closed stomatal conductance in treatments was estimated through the relative stomatal limitation (RSL, in percent) calculated from $A$–$C_i$ response curves using the equation:

$$\text{RSL} = [(A_{Ci=35Pa} - A_{Ca=35Pa})/A_{Ci=35Pa}] \times 100,$$

where $A_{Ci=35Pa}$ is the net $CO_2$ assimilation at an intercellular $CO_2$ pressure of 35 Pa, and $A_{Ca=35Pa}$ is the net $CO_2$ assimilation at an ambient $CO_2$ partial pressure ($C_a$) of 35 Pa. Estimates of $A_{Ci=35Pa}$ were obtained from previously fitted $A$–$C_i$ data to the equation:

$$A = V_C - 0.5V_O - R_d = V_C(1 - 0.5O/\tau C_i) - R_d,$$

where $V_C$ and $V_O$ are the rates of carboxylation and oxygenation of Rubisco, respectively, $R_d$ is the mitochondrial respiration, $O$ and $C_i$ are the oxygen and $CO_2$ partial pressures in intercellular spaces, respectively, and $\tau$ is the specificity of Rubisco for $CO_2/O_2$ (Farquhar *et al.* 1980).

Chlorophyll *a* fluorescence was measured weekly from berry set to harvest with a Mini Pam fluorometer (H. Walz, Effeltrich, Germany), in the same leaves used for the daily courses of gas exchange. The photochemical efficiency of PSII ($\Phi_{PSII}$) was calculated as:

$$\Phi_{PSII} = (F_m' - F_s)/F_m',$$

where $F_m'$ is the maximum chlorophyll fluorescence of light-adapted leaves, and $F_s$ is steady-state chlorophyll fluorescence. Non-photochemical quenching (NPQ) was calculated as:

$$\text{NPQ} = (F_m - F_m')/F_m',$$

where $F_m$ is the maximum chlorophyll fluorescence of dark-adapted leaves. The electron transport rate (ETR) was calculated as:

$$\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.5 \times 0.84$$

where $\Delta F$ is the variable fluorescence yield of light-adapted leaves ($F_m' - F_s$) (Björkman and Demmig 1987).

### Carbon isotope composition

Samples for the determination of carbon isotope composition from mature leaves and grape berries were collected from four plants per treatment at harvest. The dried leaf (two mature leaves per plant) and berry samples (10 berries randomly taken from 200 berries sampled) were ground into a fine homogeneous powder and 1-mg subsamples were analysed for $\delta^{13}C$ using a Europa Scientific ANCA-SL Stable Isotope Analysis System (Europa Scientific, Crewe, UK). Carbon isotope composition was expressed as $\delta^{13}C = [(R_s - R_b)/R_b] \times 1000$, where $R_s$ is the $^{13}C/^{12}C$ ratio of the sample and $R_b$ is the $^{13}C/^{12}C$ of the PDB (Pee Dee Belemnite) standard.

### Statistical data analysis

Statistically significant effects were evaluated by one-way analysis of variance, and significant differences between treatments means were evaluated with Tukey's Honestly Significant Difference (HSD) tests with α=0.05 by Statistica software (version 5.0, StatSoft, Tulsa, OK, USA). All measurements shown are the means ± s.e.

## Results

### *Seasonal evolution of water potential, gas exchange and maximum quantum yield of PSII*

As illustrated in Fig. 1, pre-dawn leaf water potential ($\psi_L$) decreased in NI vines from July onwards, being significantly lower than the $\psi_L$ of PRD, DI and FI vines. FI vines showed an almost constant $\psi_L$, higher than –0.2 MPa throughout the growing season except for 9 and 16 August. During the season, PRD vines maintained water potential values close to those of FI vines. At the end of the season $\psi_L$ was significantly lower in DI (–0.30 MPa) than in PRD vines (–0.21 MPa).

Non-irrigated vines showed the largest reductions in stomatal conductance and photosynthesis as the season progressed, reaching values below 0.10 mol $H_2O$ m$^{-2}$ s$^{-1}$ and 5 µmol $CO_2$ m$^{-2}$ s$^{-1}$, respectively, in the middle of August. FI vines had the highest values, with $A$ between 12 and 14 µmol $CO_2$ m$^{-2}$ s$^{-1}$ and $g_s$ between 0.3 and 0.5 mol $H_2O$ m$^{-2}$ s$^{-1}$. Statistically significant differences in photosynthesis and stomatal conductance were only observed between the irrigated treatments (FI, PRD and DI) and the non-irrigated treatment. Predawn values of $\Phi_{PSII}$ ($F_v/F_m$, measured under dark-adapted conditions) remained close to 0.8 in all treatments throughout the season (Fig. 1).

### *Diurnal course of gas exchange, chlorophyll fluorescence and sap flow*

Diurnal time courses of gas exchange and chlorophyll fluorescence parameters in a typical day in August are shown in Fig. 2. NI vines showed the lowest $A$ (*ca* 28%) and $g_s$ (*ca* 17%) rates during the whole day. Differences between FI, PRD and DI vines were only observed at late afternoon, with FI vines exhibiting higher values of $A$ (around 13 µmol $CO_2$ m$^{-2}$ s$^{-1}$) than PRD and DI vines (around 9 µmol $CO_2$ m$^{-2}$ s$^{-1}$). However, marked differences (around 0.10 mol $H_2O$ m$^{-2}$ s$^{-1}$) in stomatal conductance between the PRD and DI vines were apparent when measurements were taken under controlled conditions of light, temperature and $CO_2$ (Fig. 3). Under those conditions, PRD and NI vines kept stomata more closed than DI and FI vines. The stomatal conductance was about 0.3 mol $H_2O$ m$^{-2}$ s$^{-1}$ in DI and FI vines, 0.24 mol $H_2O$ m$^{-2}$ s$^{-1}$ in PRD and 0.14 mol $H_2O$ m$^{-2}$ s$^{-1}$ in NI vines. Nevertheless, net photosynthesis was not lower in PRD vines than in FI and DI vines, being reduced only in NI vines (*ca* 27%).

The decrease (*ca* 70%) of net photosynthesis rates observed in NI vines during the course of day was accompanied by a decrease (*ca* 50%) in quantum yield of PSII reaction centres and an increase of NPQ and ETR/$A$ (Figs 2*b*, *d*, *f*). The NPQ reached values of 4.3 and the ETR/$A$ increased to 46.5 in NI vines. Statistically significant differences between treatments were only observed by 1600 hours in the relative intrinsic water use efficiency

($A/g_s$). The $A/g_s$ in PRD and NI vines was higher than 45 µmol $CO_2$ mol $H_2O^{-1}$, which contrasts with DI and FI vines where $A/g_s$ was lower than 35 µmol $CO_2$ mol $H_2O^{-1}$.

The daily transpiration measured with sap flow sensors on some days in August is shown in Fig. 4. The diurnal pattern of sap flow showed differences between all irrigation treatments on clear days. On sunny days, the FI vines showed greatest daily transpiration with sap flow rates higher than 300 g $h^{-1}$ $m^{-2}$. DI vines showed higher values of sap flow than PRD vines, whereas NI vines showed the lowest values (less than 100 g $h^{-1}$ $m^{-2}$).

### Modelled biochemical parameters from A–C_i curves

Table 1 shows $V_{Cmax}$, $R_d$, $J_{max}$, TPU and RSL estimated from $A$–$C_i$ response curves. Although $V_{Cmax}$ and $R_d$ were lower in NI and PRD vines than in FI and DI vines, the differences were not statistically significant ($P<0.05$) owing to the high variability observed in these estimates. $J_{max}$ was significantly lower in NI and PRD vines (125–136 µmol $m^{-2}$ $s^{-1}$) than in FI and DI vines (170–180 µmol $m^{-2}$ $s^{-1}$). TPU estimated from the $A$–$C_i$ curves was decreased (*ca* 25%) in NI and PRD vines compared with DI and FI vines. However, the difference between PRD and DI was not statistically

significant. Finally, the relative stomatal limitation of photosynthesis increased from 24% in FI and DI vines to 29% in PRD and 38% in NI vines. However, no significant differences were observed in RSL estimates for the irrigated treatments.

### δ¹³C in leaves and grape berries

The $\delta^{13}C$ measured in the leaves and berries collected at harvest was correlated ($r^2=0.68$, $P<0.05$) with $\Psi_L$ measured at the end of the dry season (August 2000), which was used as a water stress indicator (Figs 5a, b). For leaves, statistically significant differences for $\delta^{13}C$ were observed only between the irrigated (average for the three irrigated treatments, –27‰) and the non-irrigated treatments (–25‰) (Fig. 6a). However, in grape berries, the $\delta^{13}C$ was significantly lower in PRD (–25‰) than FI (–26‰) vines, whereas there were no differences between DI and FI vines (Fig. 6b).

### Discussion

Irrigation using the partial rootzone drying technique allowed vines to maintain a higher water status than the conventional deficit irrigation. In PRD, $\Psi_L$ remained closer
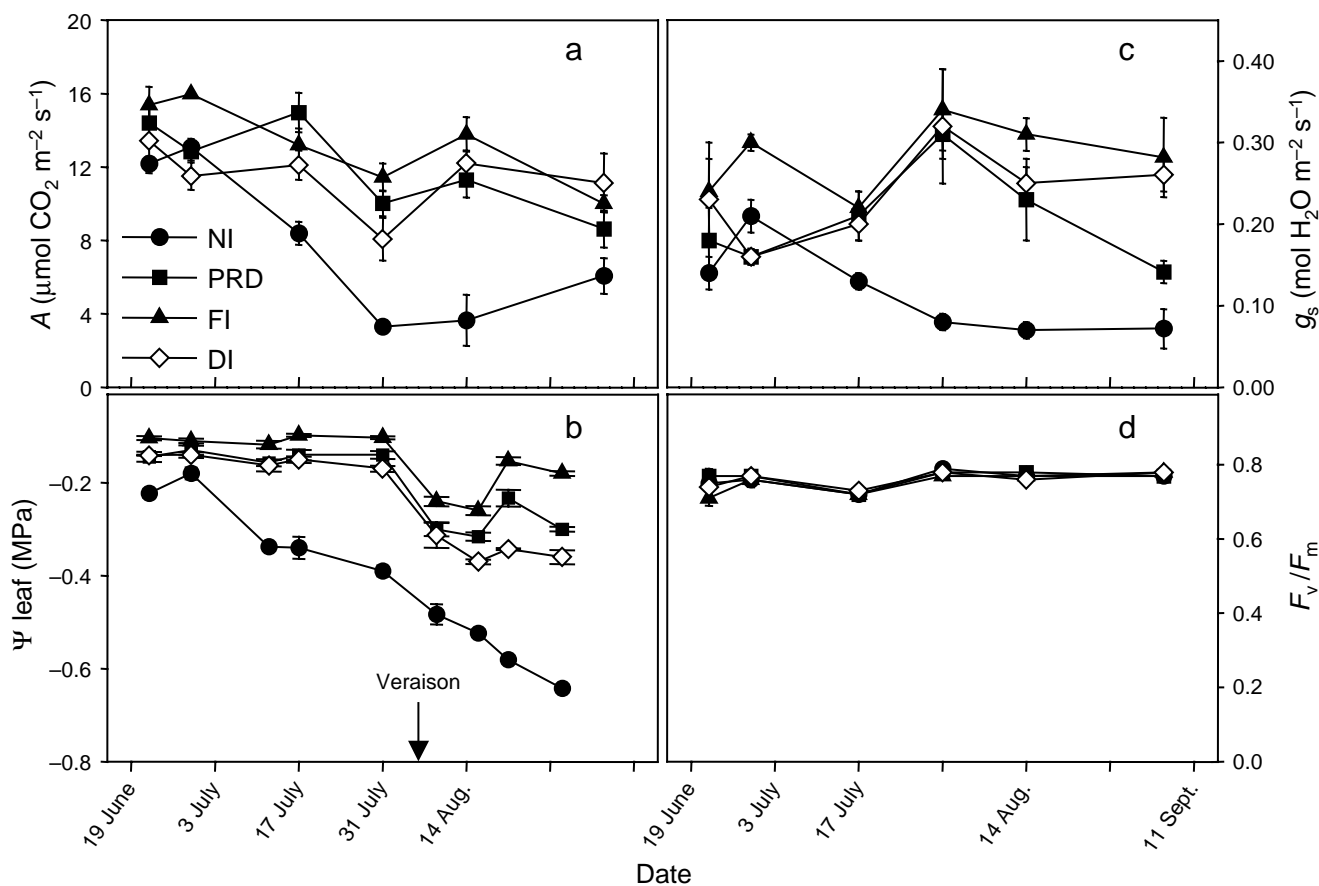


**Fig. 1.** Seasonal trends of (*a*) photosynthesis (*A*), (*b*) pre-dawn water potential ($\Psi_L$), (*c*) stomatal conductance ($g_s$) and (*d*) efficiency of excitation capture by open PSII in dark-adapted leaves ($F_v/F_m$). *A* and $g_s$ are maximal values measured at 1400 hours. Values are means ± s.e.

to the FI vines than to the DI vines (Fig. 1). These results indicate that PRD vines reduced transpiration and stomatal conductance compared with DI vines, in spite of the same amount of water supplied. This was observed in the late afternoon in the diurnal course, as well as in the measurements done under controlled conditions of $CO_2$, light and temperature (Figs 2, 3). Furthermore, these results are supported by sap flow measurements, which reflect water use over a longer term. The lower sap-flow rates in PRD vines than in DI vines suggest a stronger reduction of stomatal conductance in PRD vines (Fig. 4). These results support the hypothesis postulated by Loveys *et al.* (2000) that PRD decreases stomatal conductance, presumably as a

result of the production of chemical signals in dehydrating roots. Stoll *et al.* (2000) observed an increase of ABA concentration in xylem sap originated by roots in contact with dry soil and also an increase of xylem sap pH, which are known to promote stomatal closure under water stress conditions (Davies *et al.* 2000). As expected, the differences that we found between PRD and DI are much more subtle than those observed between PRD and FI. Indeed, large differences have been reported between PRD and FI-type treatments by Stoll *et al.* (2000) and Dry *et al.* (2000).

Statistically significant reductions in photosynthesis as the season progressed were observed only in NI vines. This decrease in *A* was accompanied by reductions in stomatal
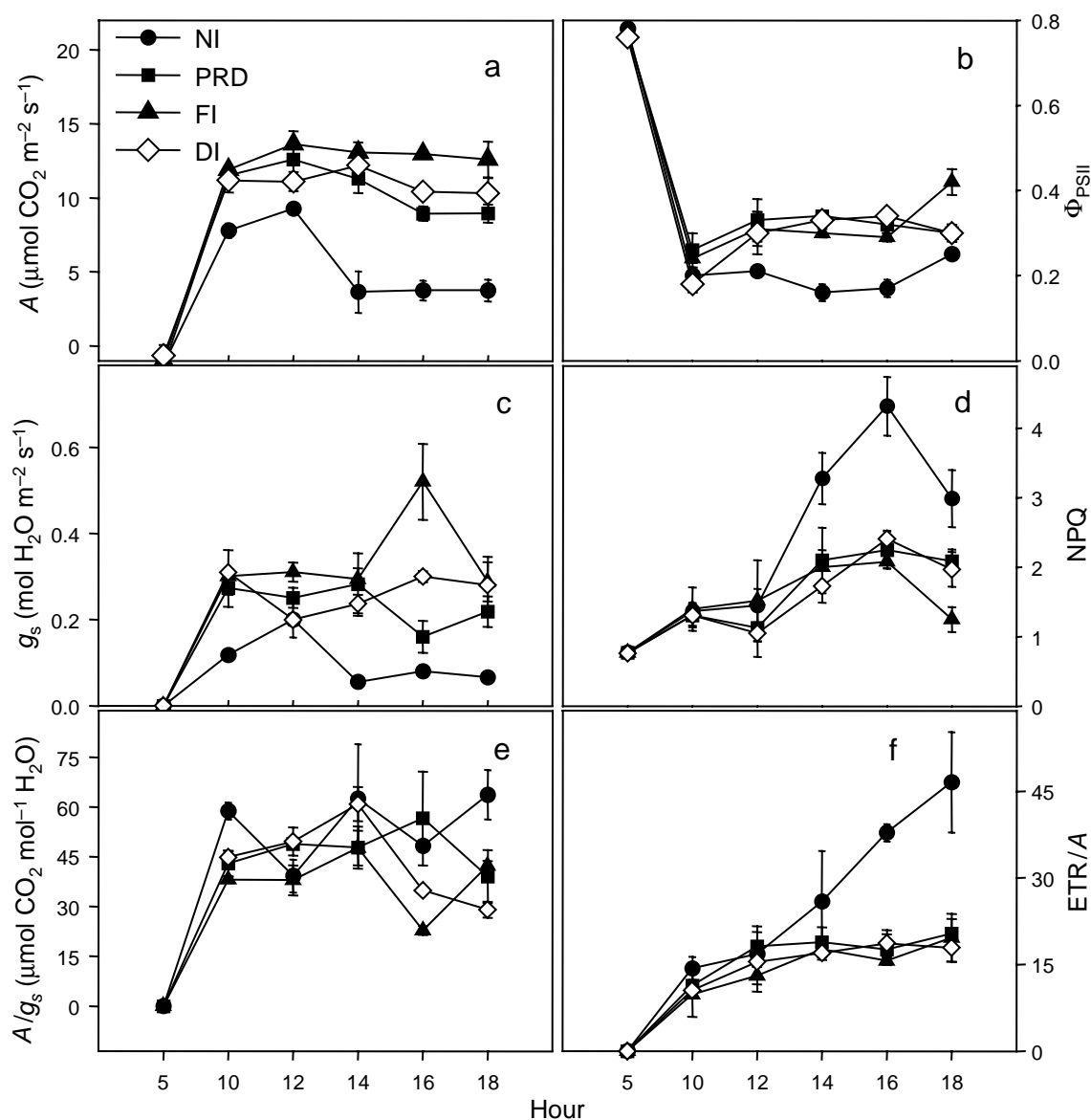


**Fig. 2.** Daily courses of (*a*) photosynthesis (*A*), (*b*) quantum yield of PSII ($\Phi_{PSII}$), (*c*) stomatal conductance ($g_s$), (*d*) NPQ, (*e*) intrinsic water use efficiency ($A/g_s$) and (*f*) the ratio ETR/*A*. The measurements were done on 14 August (one week after veraison). Values are means ± s.e.
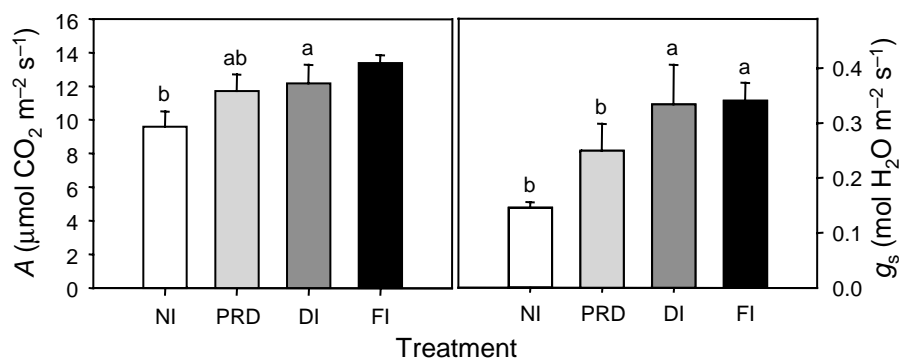
**Fig. 3.** Photosynthesis (*A*) and stomatal conductance (*g*$_s$) in cv. Moscatel under controlled conditions: 350 μmol mol$^{-1}$ $CO_2$, 1200 μmol m$^{-2}$ s$^{-1}$ PPFD and 25°C leaf temperature. Measurements were done at the end of August. Values are means ± s.e. Different letter suffixes show statistically significant differences at α=0.05.

conductance and in $\Phi_{PSII}$, suggesting a downregulation of photosynthesis to match the decreased $CO_2$-assimilation capacity under water stress conditions. However, no photo-inhibition (as estimated by pre-dawn $F_v/F_m$) was observed (Fig. 1). These results are in accordance with reports of high tolerance of the photochemical apparatus of grapevine leaves under environmental stress (Gamon and Pearcy 1990; Chaumont *et al.* 1995), including drought (Flexas *et al.* 1999*a*, 1990*b*; Maroco *et al.* 2002). The major process involved in protection against photodamage during water stress is probably the increase of non-photochemical energy dissipation, as we observed in NI vines (Fig. 2). This results in the reduction of the quantum yield of PSII photo-chemistry but allows the maintenance of a balance with electron flow requirements for carbon metabolism (Weis and

Berry 1987; Krause and Weis 1991). Furthermore, the increased ETR/*A* ratio also observed in NI vines indicates that at least some of the electrons are not used directly in $CO_2$ assimilation but rather in other processes such as photorespiration, which has been reported to increase under drought (Leegood 1995; Flexas *et al.* 1999*b*). These responses in grapevines play an important role in the regulation between the photochemical and the carbon assimilation reactions under water stress, as in other species adapted to Mediterranean climates (Quick *et al.* 1992).

Rubisco activity, RuBP regeneration and metabolism of triose-P are three major metabolic steps identified as important for optimal photosynthetic performance. It is noteworthy that the maximum Rubisco activity ($V_{Cmax}$) was not statistically significantly different among treatments
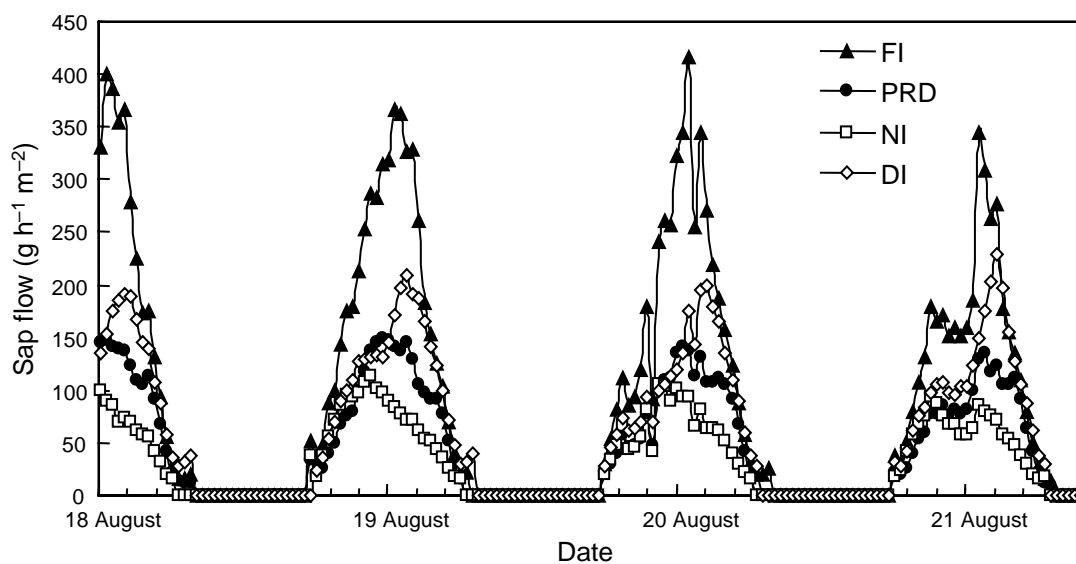


**Fig. 4.** Diurnal course of sap flow per unit leaf area on different water treatments. Measurements were done during a week in August 2000.

**Table 1. Estimated model parameters ($V_{Cmax}$, $J_{max}$, TPU and $R_d$) and RSL for the irrigation treatments**
Values are means ± s.e. Different superscript letters show statistically significant differences at α=0.05

| Treatment | $V_{Cmax}$ (μmol m$^{-2}$ s$^{-1}$) | $J_{max}$ (μmol m$^{-2}$ s$^{-1}$) | TPU (μmol m$^{-2}$ s$^{-1}$) | $R_d$ (μmol m$^{-2}$ s$^{-1}$) | RSL (%) |
|---|---|---|---|---|---|
| NI | 44.92 ± 4.82[a] | 125.18 ± 5.03[a] | 7.48 ± 0.24[a] | 1.94 ± 0.99[a] | 38 ± 0.03[a] |
| PRD | 46.09 ± 3.67[a] | 136.62 ± 6.03[a] | 8.24 ± 0.40[ab] | 2.76 ± 0.80[a] | 29 ± 0.02[ab] |
| DI | 53.63 ± 6.69[a] | 171.09 ± 13.34[b] | 9.79 ± 0.61[bc] | 4.12 ± 1.52[a] | 23 ± 0.03[b] |
| FI | 54.91 ± 3.38[a] | 180.35 ± 15.65[b] | 9.99 ± 0.52[c] | 3.89 ± 0.75[a] | 24 ± 0.01[b] |

(although there was a trend for lower $V_{Cmax}$ in NI vines than in FI vines), whereas RuBP regeneration (which is proportional to $J_{max}$) was significantly lower in NI and PRD than in DI and FI vines. This is in accordance with the decrease in the quantum yield of PSII photochemistry observed in NI, allowing the adjustment of electron flow to the limited carbon uptake as a result of stomatal closure. This could also be related to the high sensitivity of photophosphorylation to leaf water deficit as reported by Giménez *et al.* (1992). However, Rubisco activity seems to be more resistant to water stress than photophosphorylation, with significant effects occurring only for severe drought. In studies with

grapevine, Escalona *et al.* (1999) did not observe a significant reduction in Rubisco carboxylation efficiency for $\Psi_L$ of –0.5 ± 0.04 MPa, while Maroco *et al.* (2002) observed a significant reduction of $V_{Cmax}$ for $\Psi_L$ of –0.95 ± 0.01 MPa in the same cultivar. In contrast, TPU capacity was significantly reduced in both NI and PRD vines compared with FI vines. Drought can cause a decline of TPU due to diminished supply of inorganic phosphate to the Calvin cycle, consistent with a slow-down in growth generally observed in drought-stressed plants (Chaves 1991). Statistically significant growth inhibition was indeed observed in NI and PRD vines (see dos Santos *et al.* 2003). Although no
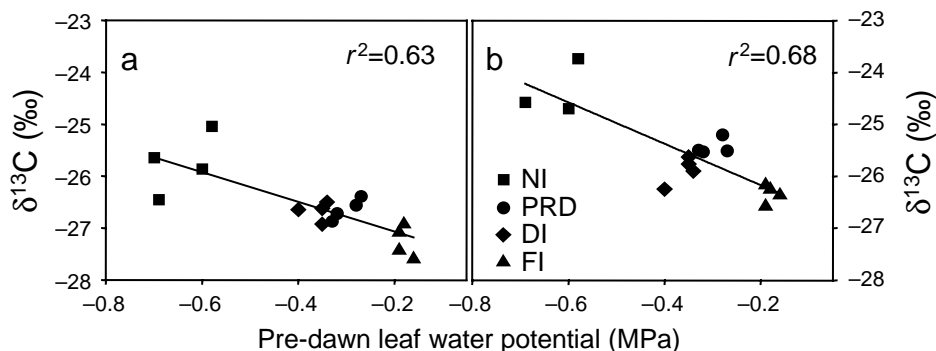


**Fig. 5.** Relationship between pre-dawn leaf water potential recorded at the end of August and δ$^{13}$C measured in leaves (*a*) and grape berries (*b*) at harvest in September. ($r^2$ is significant at α=0.05.)
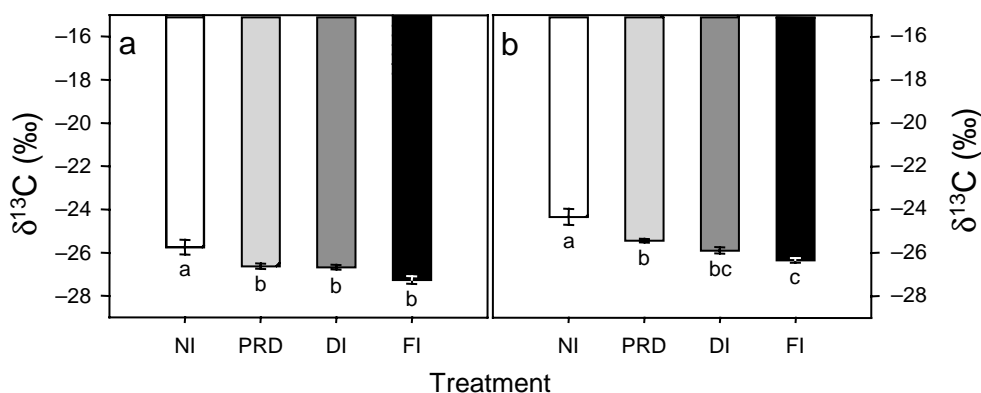


**Fig. 6.** Effects of different water treatments on isotope composition (δ$^{13}$C) of leaves (*a*) and grape berries (*b*) sampled at harvest in September. Values are means ± s.e. Different letter suffixes show statistically significant differences at α=0.05.

significant differences were found between irrigated treatments, PRD and NI vines showed an increase of RSL of photosynthesis, indicating that stomatal conductance was more reduced than in DI and FI vines. The increased RSL of photosynthesis in NI and PRD vines confirms the results of $A$ and $g_s$ measured under controlled conditions.

One of the main effects of cultivating grapevines under PRD was reported to be an improvement of water use efficiency (Dry *et al.* 1996; Loveys *et al.* 2000). In the present study we measured the carbon isotope composition ($\delta^{13}C$) of leaves and grape berries to evaluate the long-term water use efficiency in the different treatments. The $\delta^{13}C$ is an integrative indicator of the relative efficiency of $CO_2$ assimilation to water loss and reflects the long-term effect of the plant water status throughout the growth season. It is related to the ratio of intercellular and atmospheric $CO_2$ concentrations ($C_i/C_a$) and is therefore dependent on both $A$ and $g_s$ (Farquhar *et al.* 1982). It is well documented that water stress changes water use efficiency and carbon isotope composition in different species (Farquhar and Richards 1984; Osório *et al.* 1998). In grapevines, Gibberd *et al.* (2001) observed a negative linear relationship between transpiration efficiency and $^{13}C$ discrimination. The decrease in stomatal conductance is reflected in an increase of $\delta^{13}C$ due to the reduced discrimination by Rubisco (Farquhar *et al.* 1982). Our results have shown that $\delta^{13}C$ of tissue samples were affected by plant water deficit (Fig. 5). The carbon isotope composition of grape berries suggests increased stomatal control and therefore increased water use efficiency at the leaf level in NI and PRD vines as compared with FI and DI vines (Fig. 6). The higher $\delta^{13}C$ in grape berries than in leaves may be due to the fact that the carbon laid down in the leaves occurs early in the season when stress is mild and differences in stomatal conductance between treatments are not so marked or are even absent. In contrast, carbon in the berries was derived from photosynthesis occurring after veraison when the stress effects were more accentuated. That is, berry tissue may have originated from photoassimilates with a less negative $\delta^{13}C$. In addition, the high accumulation of malic acid in the immature berries probably occurs through β-carboxylation of phospho*enol*pyruvate by PEP carboxylase (Di Marco *et al.* 1977), which favours $^{13}C$ (Farquhar and Richards 1984; Brugnoli and Farquhar 2000).

In conclusion, PRD irrigation was able to maintain a vine water status closer to that of fully irrigated vines. This was the result of decreased stomatal conductance with no depressive impact on carbon assimilation. This led to increased intrinsic water use efficiency and increased long-term water use efficiency. While the benefits of PRD relative to NI (increased $CO_2$ assimilation) and to FI (increased water use efficiency) were obvious, from a strictly physiological point of view differences between the PRD and DI treatments were subtle. Nevertheless, vegetative growth, as measured by leaf area and pruning weight, was significantly affected as described in a companion study (dos Santos *et al.* 2003). Whether this is sufficient to recommend this practice at the commercial level, at least under conditions of moderate drought as those prevailing in this experiment, will depend on the integrated effects on vegetative growth (with impact on microclimate) and fruit quality. However, this is an area where much research is still needed to evaluate the relative PRD benefits compared with traditional irrigation techniques under different environmental conditions: over several years and with different levels of soil and atmosphere water stress.

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