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doi:10.1093/jxb/eri216

Journal of Experimental Botany

RESEARCH PAPER

Impact of deficit irrigation on water use efficiency and carbon isotope composition (δ^{13}C) of field-grown grapevines under Mediterranean climate

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Received 15 July 2004; Accepted 29 April 2005

Abstract

The objective of this study was to evaluate the effect of deficit irrigation on intrinsic water use efficiency (A/g_s) and carbon isotope composition (δ^{13}C) of two grapevine cultivars (Moscatel and Castelão), growing in a commercial vineyard in SW Portugal. The study was done in two consecutive years (2001 and 2002). The treatments were full irrigation (FI), corresponding to 100% of crop evapotranspiration (ETc), rain-fed (no irrigation, NI), and two types of deficit irrigation (50% ETc): (i) by supplying the water either to one side of the root system or to the other, which is partial rootzone drying (PRD), or (ii) dividing the same amount of water by the two sides of the root system, the normal deficit irrigation (DI). The water supplied to the PRD treatment alternated sides approximately every 15 d. The values of predawn leaf water potential (Ψ_{pd}) and the cumulative integral of Ψ_{pd} (S_Ψ) during the season were lower in 2001 than in the 2002 growing season. Whereas differences in Ψ_{pd} and S_Ψ between PRD and DI were not significantly different in 2001, in 2002 (a dryer year) both cultivars showed lower values of S_Ψ in the PRD treatment as compared with the DI treatment. This suggests that partial rootzone drying may have a positive effect on water use under dryer conditions, either as a result of better stomatal control and/or reduced vigour. The effects of the water treatments on δ^{13}C were more pronounced in whole grape berries and pulp than in leaves. The δ^{13}C of pulp showed the best correlation with intrinsic water use efficiency (A/g_s) as well as with S_Ψ. In spite of the better water status observed in PRD compared with DI in the two cultivars in 2002, no statistical differences between the two treatments were observed in A/g_s and δ^{13}C. On the other hand, they showed a higher δ^{13}C compared with FI. In conclusion, it is apparent that the response to deficit irrigation varies with the environmental conditions of the particular year, the driest conditions exacerbating the differences among treatments. The highest values of δ^{13}C found in the pulp of NI vines in Castelão compared with Moscatel suggest different sensitivities to water deficits in the two cultivars, as was empirically observed.

Key words: Carbon isotope composition, deficit irrigation, grapevines, partial rootzone drying, water use efficiency.

Introduction

Drought is one of the most important factors limiting crop yield and quality worldwide, especially in regions with a climate of the Mediterranean type. In many regions, viticulturists rely on irrigation water during drought periods. However, there is considerable controversy concerning the positive and negative effects of grapevine irrigation on growth as well as on must and wine quality. The amount of irrigation water has to be regulated since excess irrigation may contribute to increased vegetative growth leading to excessive water loss, fungal diseases, and shading of grape

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clusters. By contrast, a mild water stress imposed through deficit irrigation may reduce vine vigour and competition for carbohydrates by the growing tips, and may increase the berry and wine quality (Matthews and Anderson, 1988, 1989). Furthermore, the lack of available water resources in some areas is leading to a significant pressure on viticulturists to bring about improvements in the efficiency of water use as traditional irrigation practices are unsustainable.

Deliberate withholding of irrigation water may, therefore, be a useful 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). In RDI, the control of growth is made by imposing water stress at key stages of fruit development. However, this technique requires the precise control of water application, which is difficult to achieve in practice, and the reduction in irrigation has often been at the expense of yield. An alternative approach to RDI is deficit irrigation (DI) that gives a constant, but partial, amount of transpired water throughout the growing season. A special kind of deficit irrigation is the so-called partial rootzone drying (PRD) where the same amount of water is only given to one side of the root system, while the remainder is left to dry; this was proposed as a way to standardize grapevine yield and quality (Loveys *et al.*, 2000). PRD functioning relies on hormonal signals, possibly ABA, originated from the roots in response to the low soil water potential within the dry zone and transported to the leaves in the transpiration stream, leading to the reduction of growth and stomatal conductance (Loveys, 1984; Davies and Zhang, 1991; Loveys *et al.*, 2000; Souza *et al.*, 2003). Partial stomatal closure caused by roots signalling may lead to a decrease in transpiration and, possibly, to an increase in water use efficiency (WUE) (During *et al.*, 1997; Turner, 1997; Loveys *et al.*, 2000).

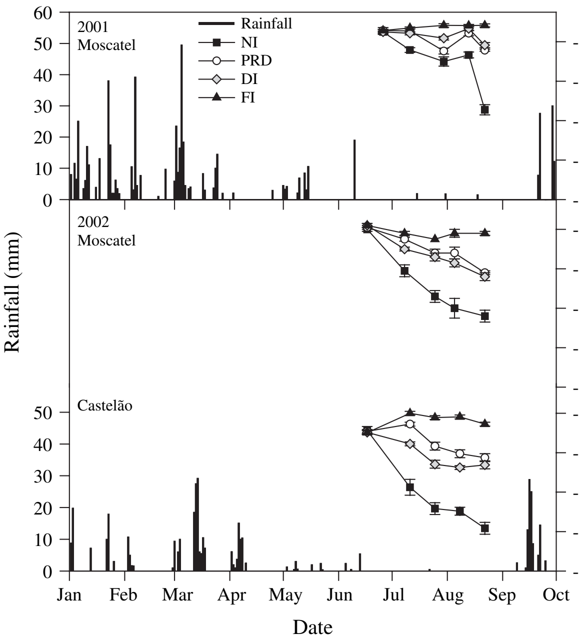

Fig. 1. Seasonal distribution of rainfall and predawn water potential (Ψ_{pd}) measured in different water treatments in Moscatel (2001 and 2002) and in Castelão (2002). The water treatments are NI (squares), PRD (circles), DI (diamonds), and FI (triangles). Values of Ψ_{pd} are means ±SE.

In previous work by this laboratory, when the amount of irrigation was halved in both PRD and DI, as compared to full irrigation (FI), differences between the two deficit irrigation treatments were more pronounced in growth than in gas exchange, implying that the regulation of stomatal conductance in PRD was very subtle (dos Santos *et al.*, 2003; Souza *et al.*, 2005). In contrast to gas exchange techniques that provide measurements of photosynthesis rates at a single point in time, leaf carbon isotopic composition ($\delta^{13}C$) integrates the ratio of intercellular (p_i) to air CO_2 concentration (p_a) for longer periods. The basis of the biochemical discrimination against ^{13}C in C_3 plants lies with the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) which discriminates against ^{13}C because of the intrinsically lower reactivity of ^{13}C compared with ^{12}C (Farquhar *et al.*, 1982; Brugnoli and Farquhar, 2000). Thus the isotopic composition reflects the effect of the plant water status on photosynthesis throughout the growing season (Farquhar and Richards, 1984). In the present study it was used to evaluate the intrinsic plant water use efficiency as it was for many plant species in response to different environmental conditions (Zhang *et al.*, 1994; Le Roux *et al.*, 1996; Osório *et al.*, 1998; Martin *et al.*, 1999; Robinson *et al.*, 2000; Araus *et al.*, 2003), including grapevines (Gaudillère *et al.*, 2002).

Although the whole-plant $\delta^{13}C$ is dominated by CO_2 assimilation and diffusion into leaves, internal partitioning and metabolism of primary assimilates may produce differences in $\delta^{13}C$ among plant organs (Leavitt and Long, 1985; Gleixner *et al.*, 1993; Le Roux-Swarthout *et al.*, 2001; Ghashghaie *et al.,* 2001; Brugnoli and Farquhar, 2000). This influence is particularly important in deciduous woody species, such as grapevine, where stored organic compounds are the dominant carbon sources for leaf growth in the early spring. In such instances, $\delta^{13}C$ of leaf tissue not only represents p_i/p_a and water use efficiency of the growing season, but also reflects the previous year's carbon assimilation and allocation. For grapevines, sugar $\delta^{13}C$ in the grape berries can be used to characterize soil water availability in the vineyard, since the sugars of mature berries integrate leaf photosynthetic isotopic discrimination of carbon during berry ripening. (Gaudillère *et al.*, 2002). However, the effect of grapevine water status on fractionation of $\delta^{13}C$ in different organs/tissues of the plant has not been studied.

The aim of this study was, therefore, to evaluate grapevine water use efficiency when subjected to different water regimes, namely the two deficit irrigations treatments with and without partial rootzone drying, compared with fully irrigated and rain-fed grapevines. Water use efficiency, evaluated through the measurements of $\delta^{13}C$, provides an estimate of stomatal closure integrated over time that will help to understand differences in stomatal conductance and/or sink/source balance between treatments that are not easily perceived with point measurements. Furthermore, the effects of watering treatments on the fractionation of ^{13}C were explored in different tissues (leaves and in different parts of the berries) and in two grapevine varieties. The inter-annual variability was evaluated in one of the varieties.

Materials and methods

Location and irrigation treatments

The experiments were conducted in a commercial vineyard located 70 km east of Lisbon, at the Centro Experimental de Pegões, during 2001 and 2002. The climate is of the Mediterranean type with hot, dry summers and mild winters. The two years were quite different regarding the amount of precipitation, especially from January to May, but similar in respect to the near absence of rain in July and August (Fig. 1). 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).

Table 1. *Integral water stress (S_{Ψ}) of different water treatments in Moscatel during the 2001 and 2002 growing seasons and in Castelão during 2002*

Values are means ±SE. Differences that are statistically significant at $P < 0.05$ among water treatments are indicated by different small letters, and between years by different capital letters.

Treatment	S_{Ψ} (MPa d^{-1})		
	Moscatel		Castelão
	2001	2002	2002
NI	8.36±0.65 aA	20.98±1.59 aB	29.98±0.92 a
PRD	3.11±0.37 bA	9.92±0.71 bB	13.26±0.61 b
DI	1.91±0.15 bcA	11.78±0.52 cB	17.63±0.27 c
FI	0.55±0.12 cA	5.56±0.36 dB	6.50±0.35 d

Irrigation water was applied with drip emitters (4.0 l h^{-1} for FI and PRD and 2.0 l h^{-1} for DI), two per vine, positioned 30 cm from the vine trunk (out to both sides of the rows), and distributed to 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 (*Kc*) proposed by Prichard (1992). The irrigation treatments were rain-fed, non-irrigated (NI); partial rootzone drying (PRD, 50% of the ETc was supplied to one side of the root system only, alternating sides approximately each 15 d); deficit irrigation (DI, 50% of the ETc was supplied to both sides of the vine, 25% to each side); full irrigation (FI, 100% of the ETc was supplied to both the sides of the root system, 50% in each side). Water was supplied twice per week from the beginning of berry development (June) until harvest (September). Cumulative rainfall during the experimental period (mid-June until the end of August) was 5.2 mm in 2001 and 0.5 mm in the 2002 growing season. The total amount of water supplied to FI plants was 168 mm (420 l per vine) and 197 mm (493 l per vine) for 2001 and 2002, respectively. The PRD and DI vines received half of that quantity.

Water relations and gas exchange

Predawn (Ψ_{pd}) leaf water potential was measured weekly with a Scholander-type pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA), from the beginning of berry development until harvest. The measurements were taken on six fully expanded leaves per treatment on five dates from June to August (Fig. 1), just prior to irrigation. The Ψ_{pd} was used to calculate a water stress integral (S_{Ψ}) as proposed by Meyers (1988). The S_{Ψ} expresses the

Table 2. *Growth parameters of different water treatments measured at veraison (leaf area, leaf layer number) or at pruning time (the others) in Moscatel during 2001 and 2002 and in Castelão during 2002*

Differences statistically significant at $P < 0.05$ among water treatments are indicated by different small letters

		Treatment	Pruning weight (g vine^{-1})	Shoot weight (g)	Water shoots (no. vine^{-1})	Leaf layer number (no. at veraison)	Main leaf area (m^2 vine^{-1})	Lateral leaf area (m^2 vine^{-1})	Total leaf area (m^2 vine^{-1})
2001	Moscatel	NI	455.9 b	36.4 b	8.0 b	2.4 b	na	na	na
		PRD	509.8 ab	41.2 b	9.4 b	2.7 b	na	na	na
		DI	520.5 ab	42.6 b	12.7 a	3.6 a	na	na	na
		FI	579.6 a	50.8 a	12.9 a	3.8 a	na	na	na
2002	Moscatel	NI	455.1 c	29.7 b	1.5 c	2.1 c	2.8 b	1.9 b	4.7 b
		PRD	476.1 bc	28.8 b	2.0 b	2.2 c	3.3 ab	1.7 b	4.9 b
		DI	528.9 ab	31.8 ab	2.9 a	3.2 b	4.0 ab	2.1 ab	6.0 ab
		FI	512.0 a	33.4 a	2.9 a	3.6 b	4.5 a	3.6 a	8.1 a
2002	Castelão	NI	897.3 b	47.9 b	2.7 b	1.6 d	4.4 a	0.8 b	5.2 c
		PRD	1052.3 b	56.1 b	2.9 b	2.3 c	4.6 a	1.0 ab	5.6 bc
		DI	1531.7 a	76.2 a	5.5 a	3.3 b	5.5 a	1.5 b	7.0 ab
		FI	1505.5 a	74.9 a	4.7 a	3.7 a	6.2 a	1.5 a	7.7 a

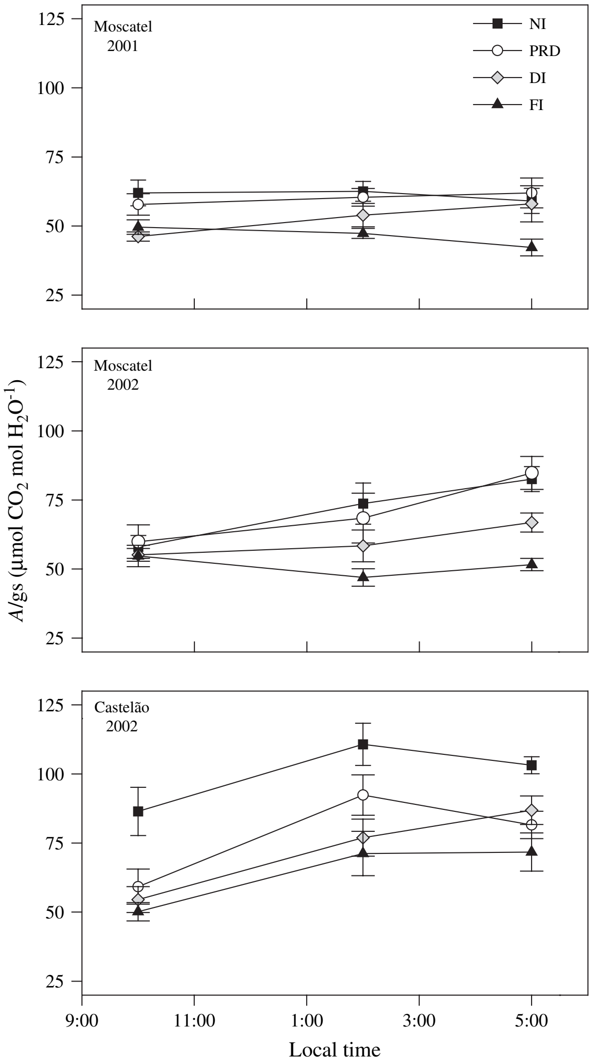

Fig. 2. Diurnal course of intrinsic water use efficiency (A/g_s) in Moscatel in 2001 and 2002 and Castelão in 2002. The water treatments are NI (squares), PRD (circles), DI (diamonds), and FI (triangles). The measurements were done in August. Values are means ±SE.

stress intensity experienced by each treatment by integrating the duration of water status below a maximum value of Ψ_{pd}. It was estimated from t measurements of Ψ_{pd} at intervals of n days by means the formula:

$$S_\Psi = \left| \sum_{i=0}^{i=t} (\Psi_{\mathrm{pd}\,i+1} - c) x n \right|$$

where $\Psi_{\mathrm{pd}\ i,\ i+1}$ is the mean Ψ_{pd} for any interval i, i+1, and c is the maximum Ψ_{pd} measured during the study. In this case, for the c value −0.13 MPa was used as the maximum Ψ_{pd} value measured in the FI treatment.

Net CO_2 assimilation rate (A) and stomatal conductance (g_s) were measured at midday on sun-exposed fully mature leaves (from primary shoots) using a portable Li-6400 IRGA (Li-Cor, Lincoln, Nebraska, USA). All measurements were replicated 4–8 times. Measurements were taken at 2 week intervals from the beginning of berry development until harvest. The A and g_s values were used to calculate the instantaneous intrinsic water use efficiency (A/g_s). The seasonally integrated values of A/g_s were obtained by calculating the area of the curve of A/g_s seasonal evolution over time for each year and cultivar.

Carbon isotope composition

Samples for the determination of carbon isotope composition from mature leaves were collected from six plants per treatment at harvest. The leaves were harvested from primary and lateral shoots (primary and lateral leaves, respectively). The lateral leaves were previously selected at the beginning of their growth during the veraison period and only collected at harvest time. The objective was to investigate if the $\delta^{13}C$ of new leaves formed during the highest water stress period could be more affected by water treatments than primary leaves formed at the beginning of the season. Berry samples consisted of 30 berries per replicate (six replicates per treatment) taken randomly from exposed clusters. Berry samples were divided in whole berries, pulp, skin, and seeds. The skins were peeled from frozen berries to avoid mixing with pulp. The dried leaves and berry samples 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 Ltd. Crewe UK). Carbon isotopic composition was expressed as

$$\delta^{13}C = ((R_s - R_b)/R_b) \times 1000$$

where R_s is the ratio $^{13}C/^{12}C$ of the sample and R_b is the $^{13}C/^{12}C$ of the PDB (Pee Dee Belemnite) standard.

Vegetative growth

Leaf area per shoot (8 shoots per treatment) was assessed periodically in count shoots from bud break onwards in a non-destructive way, using the methodologies proposed by Lopes and Pinto (2000). Primary leaf area was estimated using a mathematical model with four variables: shoot length, leaf number, and area of the largest and the smallest leaf. Lateral leaf area estimation was done by another model that uses the same variables with the exception of lateral shoot length. The area of single leaves was estimated using an empirical model based on the relationship between the length of the two main lateral leaf veins and leaf area on 1645 leaves of all sizes, using a leaf area meter (LI-3000; Li-Cor, Lincoln, Nebraska, USA). Leaf area per plant was calculated by multiplying the leaf area average by the mean shoot number.

Statistical analyses

Three-factorial analyses of variance (ANOVA), with year, sampling time, and treatments as the main factors, were used to test the main effects and factor interactions on Ψ_{pd} and A/g_s. Two-factorial ANOVA with year and treatments was used to test the main effects and factor interaction on S_Ψ, SA/g_s, and $\delta^{13}C$. Statistically significant differences between factor groups were evaluated with Tukey's HSD for α=0.05 using the 'Statistica' software (version 5.0 StatSoft, Tulsa, OK, USA). All measurements shown are the means ±SE. Linear regressions were obtained using Sigma Plot software (version 7.0, SPSS Science, Chicago).

Results

Environmental conditions, plant water status, and vegetative growth

There were large differences in rainfall distribution during the growing seasons of 2001 and 2002 (Fig. 1). The higher rainfall in spring and summer (248 mm) in 2001 led to

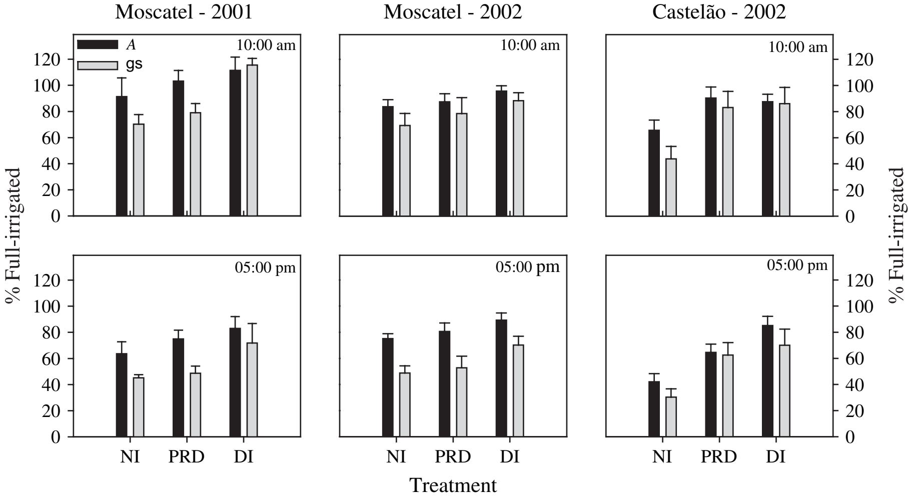

Fig. 3. The effects of different water treatments on photosynthesis (A) and stomatal conductance (g_s) in Moscatel during 2001 and 2002 and Castelão, during 2002. The values are means ±SE and the data are expressed as% of Full-irrigated treatment (FI). The measurements were made in August at the beginning and at the end of the day.

higher values of predawn water potential (Ψ_{pd}) for all watering regimes, compared with 2002 (Fig. 1). In Moscatel, a significant interaction was observed for Ψ_{pd} between years, sampling date, and treatments (P <0.05). In both years, FI vines showed almost constant Ψ_{pd} throughout the growing season, *c.* −0.13 MPa in 2001 and slightly lower in 2002 (−0.2 MPa). By contrast, NI vines showed a progressive decline in Ψ_{pd} from July onwards, with lower values observed in 2002 compared with 2001. The two deficit irrigation treatments (PRD and DI) had Ψ_{pd} values intermediate between FI and NI, but generally closer to FI. In Castelão, measured only in 2002, a similar pattern among treatments was observed. However, in this cultivar, Ψ_{pd} of PRD was significantly higher than DI vines (Fig. 1) and the Ψ_{pd} of NI vines at the harvest time reached values (*c.* −0.8 MPa) which were lower than those of NI in Moscatel (*c.* −0.6 MPa).

The intensity and duration of water stress, as indicated by the cumulative integral of predawn leaf water potential (S_Ψ, MPa d^{-1}), was significantly different among watering treatments in cv. Moscatel (Table 1). In both years, NI vines showed the highest values of S_Ψ compared with other treatments. The values of S_Ψ in all treatments were lower in 2001 than in 2002, and there was a significant interaction between year and treatment (Table 1). However, only in the driest year, 2002, was there a significant difference between PRD and DI, with PRD showing a lower S_Ψ. A similar response of S_Ψ to the treatments was also observed in Castelão.

NI and PRD vines presented the lowest pruning weight per vine, being significantly different from the FI and DI ones in Castelão, in 2002 (Table 2). The number of water shoots was significantly lower in NI and PRD compared with FI and DI, in both the two years and the two cultivars. Total leaf area at veraison presented significantly higher values (P <0.05) in FI than in NI, whereas PRD and DI vines showed values not significantly different from those of NI and FI, respectively (Table 2). The differences of total leaf area observed between treatments were mainly due to differences in the lateral shoot leaf area, as primary shoot leaf area was similar in the different watering treatments.

Intrinsic water use efficiency

The diurnal courses of intrinsic water use efficiency (A/g_s) measured in August are shown in Fig. 2. The ANOVA did not reveal a significant interaction between treatment, years and sampling time in A/g_s. The statistical analysis showed significant effects of water treatments in 2002, in both cultivars. In Moscatel, there was a trend for higher A/g_s in NI and PRD compared with FI vines. However, there were no significant differences compared with DI vines. In Castelão, NI showed the highest values in A/g_s throughout the day. At midday, PRD showed values closer to NI vines compared with DI and FI vines. The higher A/g_s ratio in NI, and in some cases in PRD and DI, compared with FI, is attributed to a larger reduction in stomatal conductance (g_s) than in photosynthesis (A), mainly at the end of the day, as shown in Fig. 3.

The integrated A/g_s (SA/g_s) at midday throughout the growing period, estimated by the area of the graphic of the seasonal evolution of A/g_s measured at midday, showed a similar pattern in both years and cultivars (Fig. 4), with a decline from NI to FI. In both cultivars, the SA/g_s values of PRD and DI exhibited intermediate values between NI and FI vines. However, there were no statistically significant differences between the irrigated treatments (PRD, DI, and FI).

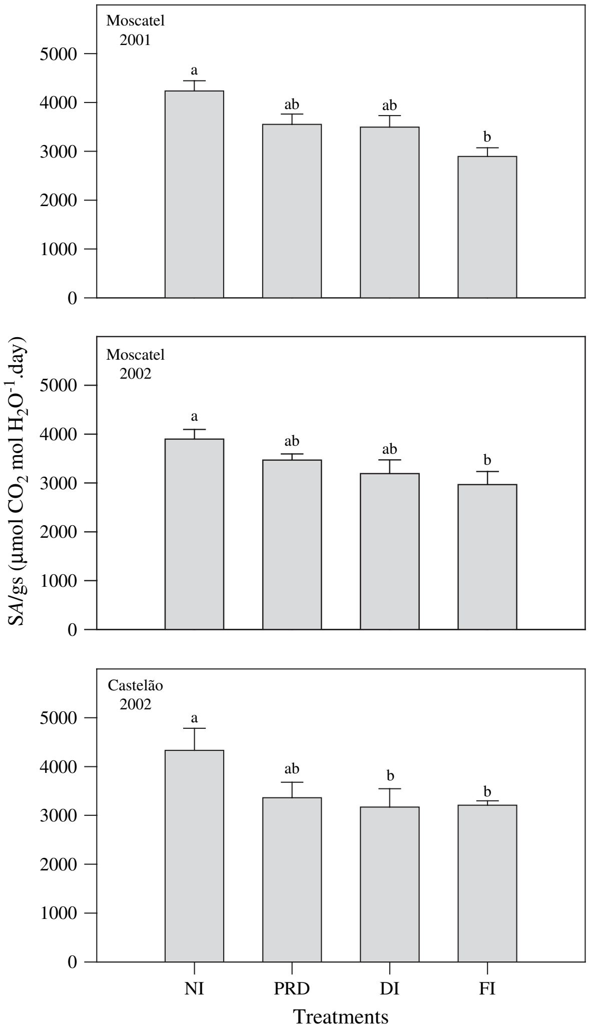

Fig. 4. Integrated intrinsic water use efficiency (SA/g_S) in Moscatel during 2001 and 2002, and Castelão during 2002 under different water treatments (NI, PRD, DI, and FI). Values are means ±SE. Different letters suffixes show statistically significant differences for $P < 0.05$.

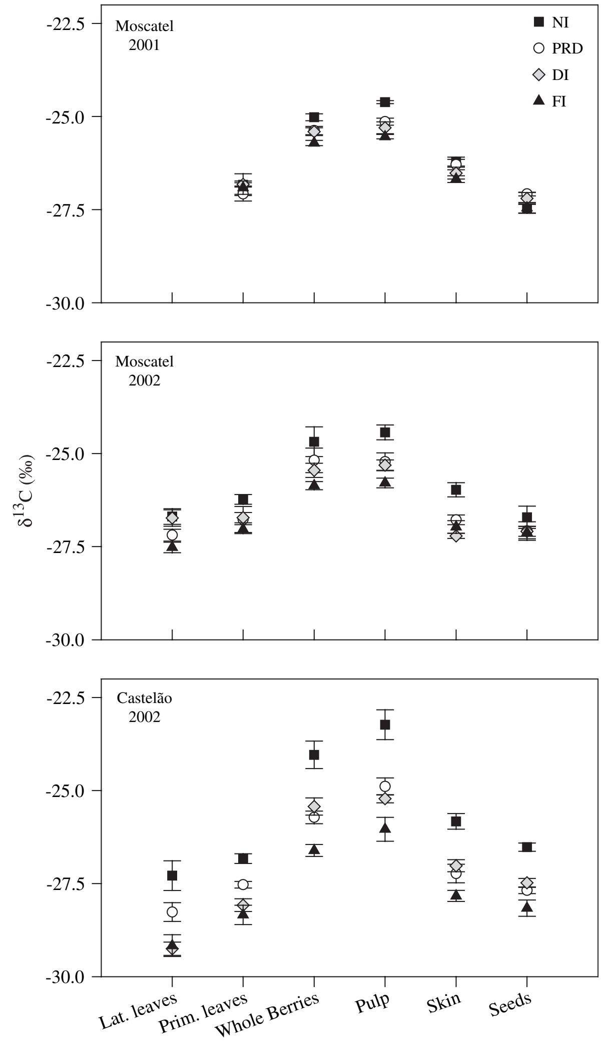

Fig. 5. Carbon isotope composition in different parts of the vines subjected to different water treatments (NI, PRD, DI, and FI). The water treatments are NI (squares), PRD (circles), DI (diamonds), and FI (triangles). Value are means ±SE.

Carbon isotopic composition ($\delta^{13}C$)

Figure 5 shows the effects of the watering treatments on δ^{13}C values in leaves (primary and lateral leaves), whole grape, pulp, skin, and seeds in Moscatel and Castelão varieties. The tissues of NI plants were less depleted in ^{13}C (higher δ^{13}C, lowest discrimination against ^{13}C) than the other treatments, and FI vines showed the lowest δ^{13}C (higher discrimination against ^{13}C).

In the leaves of Moscatel, the only significant differences in δ^{13}C were observed in lateral leaves between the NI and FI treatments. In whole berries and pulp, there were also significant differences in δ^{13}C as revealed by ANOVA after accounting for the effects of year. In general, NI vines showed the highest values of δ^{13}C while FI had the lowest, with PRD and DI providing intermediate values. However, the differences between DI and FI in δ^{13}C of whole berries were not statistically significant. There was a significant interaction between treatment and year for δ^{13}C in skin berries, with a significant difference between NI and irrigated treatments only observed in 2002. There was no significant difference among water treatments in δ^{13}C of seeds in cv. Moscatel.

The effect of watering treatments in δ^{13}C was more pronounced in Castelão than in Moscatel. In Castelão, the δ^{13}C of pulp (the lowest discrimination) ranged from $-23‰$ to $-26‰$ in NI and FI, respectively, whereas in Moscatel ranged from $-24‰$ to $-26‰$ in the same treatments. The differences among treatments were similar in different parts of the plant. NI vines showed the highest δ^{13}C values, PRD and DI, the intermediate, and FI the lowest values. However, discrimination values of lateral leaves in the PRD treatment were similar to the ones measured on primary leaves of the NI treatment.

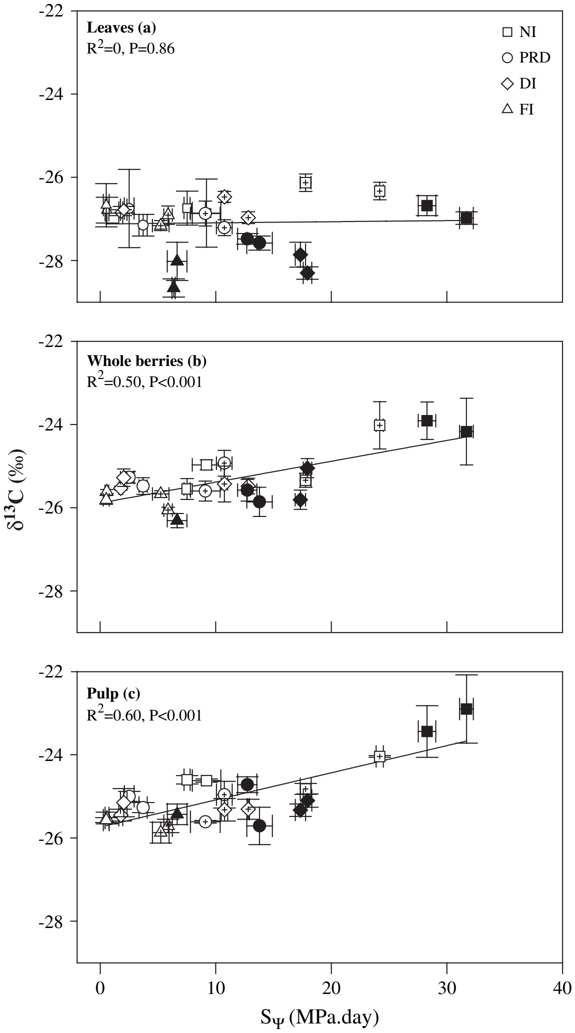

Fig. 6. Relationship of δ^{13}C in leaves (a), whole berries (b) ($y= -25.89+0.05x$), and pulp (c) ($y= -27.73+0.06x$) with water-stress integral (S_{Ψ}). Values are means ±SE. Both years and cultivars are mixed. The water treatments are NI (squares), PRD (circles), DI (diamonds), and FI (triangles). Moscatel is represented by open symbols in 2001, symbols with crosses inside in 2002 and Castelão in 2002 is represented by closed symbols.

In general, the water treatments showed a similar pattern of δ^{13}C throughout the different parts of the plants, in both years and cultivars. There was a substantial enrichment of ^{13}C in whole berries and pulp relative to leaves, skin, and seeds. The lowest ^{13}C occurred in lateral leaves, mainly in Castelão, where values reached −30‰ in FI vines. The highest values of δ^{13}C were shown in pulp tissues of NI vines in Castelão compared with those of Moscatel.

Significant correlations were found between δ^{13}C of the whole grape berries and of the pulp and S_{Ψ} considering all years and cultivars (Fig. 6). However, the δ^{13}C and S_{Ψ} association were clearly higher in the pulp as shown by the determination coefficient (R^2=0.60).

The water use efficiency was also better correlated using δ^{13}C of the pulp than with whole berries or leaves. There was an enrichment of ^{13}C in pulp tissues with an increase in the A/g_s ratio measured in both cultivars during the 2002 growing season (Fig. 7). The same behaviour was observed when SA/g_s was used as an integrated parameter to correlate with δ^{13}C. The δ^{13}C of the pulp showed the best determination coefficient with SA/g_s (R^2=0.74) compared with δ^{13}C of whole grapes (R^2=0.62) or leaves (R^2=0.17) when both cultivars and years are considered (Fig. 8).

Discussion

Vines subjected to deficit irrigation practices such as PRD and DI, revealed an intermediate degree of water stress between NI and FI vines, as shown by S_{Ψ} calculated from Ψ_{pd} values throughout the growing season (Table 1). The cumulative integral of predawn leaf water potential was shown to be a useful parameter to estimate the degree of water stress in different crops (Meyers, 1988) including grapevine (Ginestar, 1998*a*, *b*). In 2002, the driest year, PRD vines were significantly less stressed than DI in both varieties, suggesting a higher stomatal closure in PRD vines. The tendency for a lower leaf area and vigour of PRD compared with DI (Table 2) could also contribute to the explanation of the highest values of predawn leaf water potential observed in PRD vines (dos Santos *et al.*, 2003).

Soil water availability affected carbon isotopic composition of the tissues, which reflects the seasonal transpiration efficiency, i.e. the ratio of net photosynthesis to water transpired over the growing season (Farquhar and Richards, 1984). For both deficit irrigation treatments (PRD and DI), there was an increase in water use efficiency, as estimated by δ^{13}C, in both cultivars and all tissues studied, when compared with full-irrigated treatment FI. However, in most instances, the intrinsic water use efficiency (A/g_s) was not significantly different between PRD and DI. In addition, in the same experiment, these irrigation treatments did not show statistically significant differences in water use efficiency estimated as the ratio between yield and the amount of water supplied (data not shown). This suggests that a lower vigour/leaf area, rather than differences in g_s, was the key factor for a better plant water status in PRD than in DI.

The carbon isotope composition was different in leaves, berries, and seeds, as shown in Fig. 5. There was a relative enrichment of ^{13}C in berries compared with leaves and the δ^{13}C of pulp was better correlated with S_{Ψ} (Fig. 6) and intrinsic water use efficiency (both A/g_s and SA/g_s) (Figs 7, 8) than was the δ^{13}C of the whole grape, seeds, or leaves. These data indicate that ^{13}C composition of the berry pulp can be as good an indicator as berry sugar δ^{13}C in integrating water use by grapevines during the period of berry growth and development, as previously described by Gaudillère *et al.* (2002).

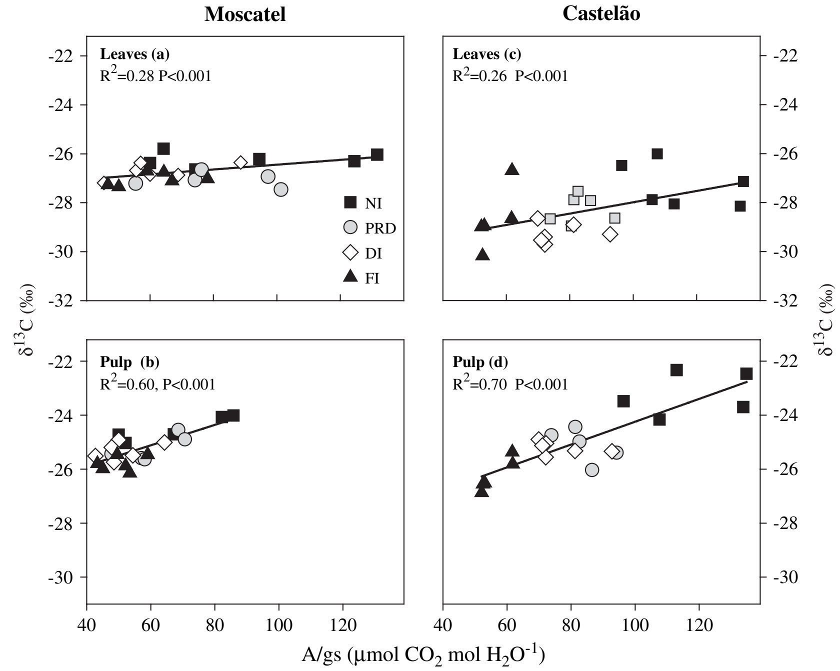

Fig. 7. Relationship of δ^{13}C in leaves (a) ($y= -27.81+0.02x$) and pulp (b) ($y= -27.42+0.03x$) of Moscatel and leaves (c) ($y= -30.30+0.02x$) and pulp (d) ($y= -28.47+0.04x$) of Castelão with intrinsic water use efficiency (A/g_s). Each point represents one replicate of the water treatments. The measurements of A/g_s were made in August 2002.

The highest δ^{13}C values in berries were probably due to the lower discrimination of ^{13}C in sugar and organic acids present in the pulp. These were imported from leaves after veraison (Di Marco *et al.*, 1977), when stress was more severe and stomata were more closed, thus providing photosynthates with higher ^{13}C. Differences among plant parts can also be attributed to differences in lipid composition, fractionation processes during transport, and/or synthesis of metabolites, contributing to changes in the ^{13}C signature of different metabolites and organs (Brugnoli and Farquhar, 2000). On the other hand, fractionation during respiration could contribute to the impoverishment of the leaf tissue in ^{13}C. Several authors have shown that discrimination during dark respiration processes can occur, releasing CO_2 enriched in ^{13}C compared with several major leaf reserves and whole leaf organic matter (Duranceau *et al.*, 1999, 2001; Ghashghaie *et al.*, 2001; Tcherkez *et al.*, 2003).

The tissues of lateral leaves, formed during the period of high water stress, were slightly more depleted in ^{13}C than primary leaves, in both cultivars. Another explanation is that the higher photosynthetic activity in lateral leaves compared with primary shoots during the later stages of the growing season (Shultz *et al.*, 1996) might have contributed to the highest discrimination in lateral leaves.

Although there is a linear association between δ^{13}C of berries and S_Ψ, other factors, including cultivar sensitivity to water stress, could contribute to the low determination coefficient (Fig. 6). The cultivar Moscatel has been considered to be well adapted to water-stress conditions as shown by Regina and Carbonneau (1996). In this study, it was observed that carbon assimilation and stomatal conductance in Moscatel can be maintained for a large range of predawn leaf water potentials, whereas in Castelão the sensitivity of stomata to water deficits was higher (Souza *et al.*, 2005). The highest values of δ^{13}C found in the pulp of NI vines confirm that water stress was higher in Castelão as compared to Moscatel (Fig. 5).

Conclusions

The results show that deficit irrigation treatments promoted an increase in water use efficiency as compared with full irrigation, either in the short-term, as expressed by the A/g_s ratio or in the long-term shown by the increase in ^{13}C in the plant tissues, especially the berries. It was also apparent that the response to deficit irrigation varied with the grapevine variety and with the environmental conditions of the particular year, differences between treatments being more marked under drier conditions. In a drier year (2002), PRD induced higher leaf water potentials than DI. This resulted from reduced leaf area and higher midday stomatal closure in PRD than in DI. Nevertheless, no statistically significant differences in WUE between the two treatments were found, as shown by gas exchange and the isotope carbon composition of grape berries. This suggests that stomatal closure in PRD plants had only a marginal effect on plant

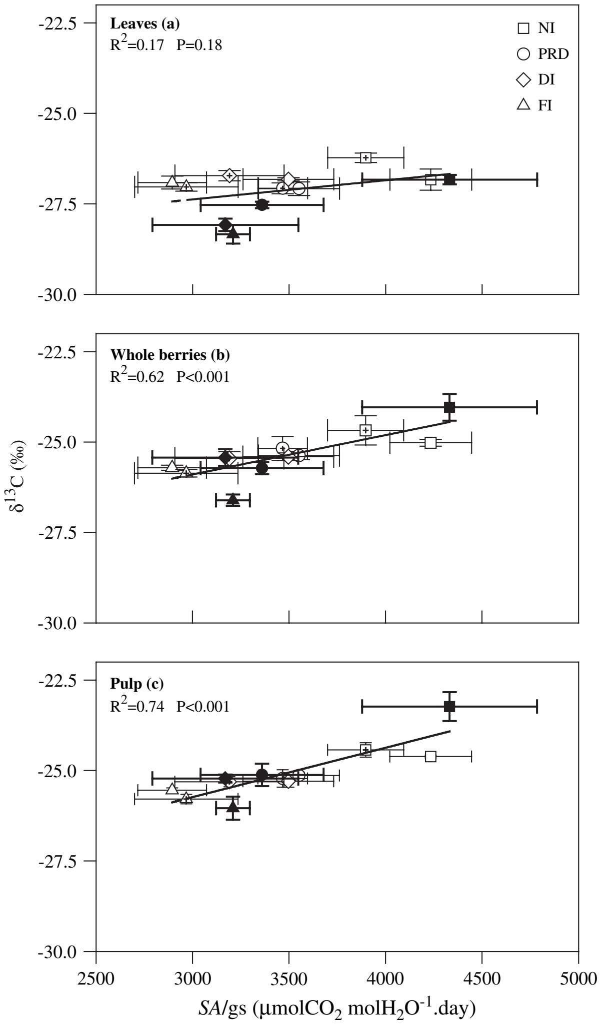

Fig. 8. Relationship of $\delta^{13}C$ in leaves (a) ($y= -28.96+0.001x$), whole berries (b) ($y= -29.16+0.001x$), and pulp (c) ($y= -29.83+0.001x$) with integral of intrinsic water use efficiency (SA/g_s). Values are means ±SE. Both years and cultivars are mixed. The water treatments are NI (squares), PRD (circles), DI (diamonds), and FI (triangles). Moscatel is represented by opened symbols in 2001, symbols with crosses inside in 2002 and Castelão in 2002 is represented by closed symbols.

water status compared with growth reduction. The good correlation observed between $\delta^{13}C$ in berry pulp and S_Ψ or SA/g_s indicates that carbon isotope composition of this particular tissue may be a valuable index for the evaluation of plant water availability during the growing season. Under Mediterranean climates, where severe water deficits are likely to occur by the end of the growing season due to restricted summer rainfall, tissue $\delta^{13}C$ may be highly correlated with the amount of water given by irrigation.

Acknowledgements

The financial support from EU project IRRISPLIT (IAC3-CT-1999-00008) is acknowledged. CRS was a recipient of a doctoral fellowship granted by Fundação para Ciência e Tecnologia (PRAXIS XXI/BD/21856/99). We also thank A Rodrigues and E Breia for technical support in the laboratory and field experiments.

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