

A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality

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Abstract. The effects of moderate irrigation, compared with non-irrigation, on leaf photosynthesis and transpiration, grape yield, and quality parameters, were studied over ten years in two Spanish cultivars (Tempranillo and Manto Negro) of field-grown grapevines (*Vitis vinifera* L.). The aim was to increase our knowledge of the relationships between water availability, canopy water losses, photosynthesis, and fruit yield and quality. A second aim was to analyse some of the mechanisms of photosynthetic down-regulation under drought, such as the capacity for RuBP regeneration and Rubisco activity.

Moderate irrigation improved plant water status, leaf photosynthesis and transpiration. Considering the results over ten years, soil water availability (estimated as pre-dawn leaf water potential, Ψ_{PD}) largely determined leaf photosynthesis and leaf transpiration. Decreased photosynthesis was due to both stomatal and non-stomatal factors. The latter were related to decayed electron transport rate and reduced RuBP regeneration capacity, but not to decreased Rubisco activity.

Moderate irrigation also improved grape yield, although this effect was much larger in Tempranillo than in Manto Negro. Moreover, the correlation between photosynthesis and grape yield was significant in Tempranillo, but not in Manto Negro. In contrast, the correlation between Ψ_{PD} and several parameters reflecting fruit quality (such as soluble solids and total polyphenol content) was significant only in Manto Negro. These results suggest that there is a close link between water availability and grape yield, mostly through water stress effects on photosynthesis. Drought effects on grape quality are linked to water availability but not to photosynthesis or yield.

Keywords: drought, fruit quality, grapevines, photosynthesis, *Vitis vinifera*, water availability, yield.

Introduction

In Mediterranean climates water stress typically occurs during summer, when light levels and temperature are high. Grapevine (*Vitis vinifera* L.) is an excellent plant for studying physiological responses and acclimation to drought under realistic crop conditions, because it develops most of its phenologic cycle during summer. The recent widespread use of vine irrigation in countries with a dry summer has amplified this interest, especially owing to the poorly understood relationships between soil water availability, water loss through canopy transpiration, grapevine photosynthesis, and fruit yield and quality.

The response of grapevine photosynthesis to water stress has attracted the interest of plant ecophysiologicalists since the early development of portable gas exchange systems (Kriedemann and Smart 1969; Liu *et al.* 1978). Like many other species, there is a tight curvilinear correlation between stomatal conductance (g_s) and net photosynthesis (A_N) in grapevines (Düring 1987; Escalona *et al.* 1999a; Flexas *et al.* 2002a). This correlation, combined with the fact that stomatal closure is among the first responses to drought, introduced the concept that the drought-induced decrease of photosynthesis was caused by stomatal closure (Kriedemann and Smart 1969). This is likely to occur under mild water stress, when transpiration (E), g_s and A_N decrease, and

Abbreviations used: A_N , net CO₂ assimilation; A_D , net CO₂ assimilation integrated over a day; A_{SAT} , light- and CO₂-saturated photosynthesis; E , leaf transpiration; E_D , leaf transpiration integrated over a day; ETP, evapotranspiration; ETR, electron transport rate; F_v/F_m , efficiency of excitation capture by open PSII in dark-adapted leaves; g_s , stomatal conductance; NPQ, non-photochemical quenching; RuBP, ribulose-1,5-bisphosphate; SWA, estimated soil water availability; SS, juice-soluble solids content; TA, titratable acidity; TPC, total polyphenol content; TAC, total anthocyanin content; Ψ_{PD} and Ψ_{MD} , pre-dawn and midday leaf water potential, respectively.

water-use efficiency (i.e. A_N/E and A_N/g_s) increases (Düring 1987; Escalona *et al.* 1999a; Flexas *et al.* 2002a). When water stress is more severe, non-stomatal regulation of photosynthesis is observed, including decreased light- and CO_2 -saturated photosynthesis (A_{SAT}) (Quick *et al.* 1992; Escalona *et al.* 1999a), reduced photochemistry (Flexas *et al.* 1999a, 2002a), depressed activity of photosynthetic enzymes (Maroco *et al.* 2002), decreased mesophyll conductance (Flexas *et al.* 2002a) and, under extremely severe stress, photoinhibition (Flexas *et al.* 1998).

The expected relationship between photosynthesis and the accumulation of sugars and other compounds in the fruit has stimulated an interest in determining environmental, cultivar and breeding effects on the relationships between photosynthesis, fruit yield and quality (Williams 1996). As photosynthesis decreases under drought, the accumulation of sugars is reduced, so grapevine growth and grape yield are expected to decrease as soil water availability decreases. In fact, irrigation has been shown to increase grape yield from 1.5- to 4-fold, depending on the irrigation schedule, the amount of water applied, cultivar, environmental conditions and other cultural practices (Bravdo *et al.* 1985; Hepner *et al.* 1985; Matthews *et al.* 1987; Schultz 1996; Williams 1996). In contrast, it is generally assumed that the highest fruit quality is achieved under suboptimal crop conditions, including suboptimal water availability. However, the precise dependence of fruit quality on soil and environmental conditions, as well as on breeding practices, is largely variable and cultivar-dependent (Mullins *et al.* 1998). Such interactions cause important variations in quality parameters depending on the conditions in a particular year, and are finally reflected in vintage variation in wine quality.

Variation in grape quality is largely dependent on irrigation dosage and schedule, environmental conditions and cultivar. Therefore, moderate irrigation was reported not to affect or even improve grape quality (Hepner *et al.* 1985), and was proposed as an effective technique to regulate final fruit and wine quality (Matthews *et al.* 1990). On the contrary, excessive soil water availability causes significant reductions in fruit colour, sugar content and acidity imbalances (Bravdo *et al.* 1985; Hepner *et al.* 1985; Matthews *et al.* 1990; Esteban *et al.* 1999). However, the important year-to-year variations in grape quality parameters reduce the scope of these results, and vintage variations remain, regardless of irrigation. A long-term evaluation of irrigation effects on grape quality parameters would provide a wider experimental basis on which to evaluate the possibilities of using controlled irrigation for producing high quality grapes.

Therefore, a deeper knowledge of the mechanisms that regulate grapevine carbon assimilation and partitioning under different water regimes is of great interest in the framework of precision agriculture, since these mechanisms

play an important role in the regulation of the fragile balance between grape yield and quality. Eventually, this will lead to the description of physiological criteria for irrigation scheduling and dosage. This is especially important in a crop such as grapevine, where applying irrigation to improve yield may compromise fruit quality, which is essential to produce premium-quality wines. The main objective of this study was to improve our knowledge about the inter-relationships between water availability, canopy water losses, photosynthesis, and fruit yield and quality. Such correlations are obtained from studies of two different Spanish cultivars (Tempranillo and Manto Negro) growing under field conditions in a commercial vineyard, trained either in bilateral cordon (Tempranillo) or goblet (Manto Negro), and subjected to either irrigation or no irrigation for ten consecutive years. A second objective was to improve our understanding of the mechanisms of photosynthetic down-regulation under drought.

Materials and methods

Growth conditions and treatments

The study was carried out in a commercial vineyard (Herederos de Ribas S.A. Mallorca, Spain, 40° N, 0° E) from 1992 to 2001. The environmental conditions were typical of a Mediterranean summer, with high temperature (average maximum temperature from June to August between 28°C and 33°C, depending on the year), high irradiance (between 250 and 350 sunshine hours per month, with midday peak photosynthetic active radiation usually above 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), high potential evapotranspiration (ETP, summer average between 120 and 170 $\text{L m}^{-2} \text{month}^{-1}$) and little or no precipitation during summer (see data from particular years, described by Delgado *et al.* 1995; Escalona *et al.* 1999a; Flexas *et al.* 2002a).

Two cultivars of grapevine (*Vitis vinifera* L.) were studied: Tempranillo, a common Spanish cultivar, and Manto Negro, a local cultivar from Mallorca, which is recognised as more drought-resistant. All plants were ten years old at the beginning of the experiment (1992), grafted on R-110 rootstock, spaced at 1.4 × 2.6 m and either trained on a bilateral cordon (Tempranillo) or on the traditional goblet (Manto Negro). Soil was a typical stony Mediterranean 'terra rossa' and was more than 2 m deep. Before 1992, this particular vineyard was traditionally cultured without irrigation. A moderate irrigation treatment was started in 1992 in five rows (70 vines per row) per cultivar. The irrigation dosage was adjusted to about 30% ETP, as measured with an evaporimeter pan, and applied by a drip system (one dripper per plant, at 3.6 L h^{-1}) twice a week from June until the end of August. This dosage and schedule were chosen from previous experience by García-Escudero (1991), when trying to improve grape yield while keeping grape quality high. The precipitation from occasional rainfall was subtracted from the calculated irrigation dose. The only significant rain throughout the ten-year period occurred in 1996 (30 L m^{-2} in the second week of July). In 2000 and 2001 a third treatment, irrigation at 100% ETP, was added.

Soil water availability (SWA) during the growing season was estimated. The soil depth explored by the roots was estimated to be 2 m (Mullins *et al.* 1998). The percentage of stones in the soil averaged 25%, and the difference between soil water content at field capacity and wilting point was determined by a soil moisture retention curve, resulting in a field capacity of 26% v/v and a wilting point of 13% v/v. From these data, the maximum water storage capacity was calculated to be 195 L m^{-2} . Winter ETP was considered negligible (no leaves were

present, the soil was completely covered with white stones and standard tilling practices were maintained). SWA in early April (at the beginning of the growing season) was calculated by considering the rainfall between October and April, and was usually at the maximum storage capacity. In 0% ETP plots, total SWA during the growing season (April–September) was calculated by adding the rainfall of that period to SWA in early spring. For irrigated plots, the irrigation dosage was added to the summer rainfall.

Parameters studied

The performance of 0% ETP vines was compared with that of irrigated vines. Sampling was always made in the central row of five consecutive rows in the same treatment plot, to avoid border effects. Three sampling times per summer were established: June, July and August, corresponding to three different states of grape maturation: green berries, veraison and ripening, respectively.

At each of these three sampling times, the following parameters were measured on young, fully-exposed, totally-expanded leaves (usually the sixth to eighth from the apex), as already described (Flexas *et al.* 1998): (i) pre-dawn and midday leaf water potential (Ψ_{PD} and Ψ_{MD} , respectively), (ii) gas exchange (A_N , g_s and E), and (iii) chlorophyll fluorescence parameters. Of these, the efficiency of excitation capture by open PSII in dark-adapted leaves (F_v/F_m) was determined at pre-dawn, whereas the electron transport rate (ETR) and non-photochemical quenching (NPQ) were measured at midday. Given the close inverse relationship observed between ETR and NPQ (Flexas *et al.* 2002a), only ETR was included in the statistical analysis. Gas exchange and Ψ_{PD} were determined throughout the ten years analysed, although Ψ_{MD} was not determined in 1992, and chlorophyll fluorescence was not measured in 1992 and 1993. Owing to different configurations of the gas-exchange analysers used in different years, E data from 1993 and 1994 were missing.

In addition, all these parameters were determined on a daily basis (six times per day, from 6 am to 9 pm, local time) in half of the years (1997–2001). From these measurements, daily-integrated A_N (A_D), E (E_D) and daily water use efficiency (A_D/E_D) were calculated as described by Escalona *et al.* (1999b). Occasionally (i.e. in Tempranillo and a few sampling times during 2000 and 2001), leaves similar to those used for gas-exchange analysis were freeze-clamped into liquid nitrogen around midday. From extracts of these samples, RuBP content and Rubisco activity were analysed. RuBP content was assayed by determining the amount of $^{14}CO_2$ incorporated into acid-stable products by partially purified Rubisco (Seeman and Sharkey 1986). Total and initial Rubisco activities were determined according to Parry *et al.* (1997).

Grapes were harvested at the end of August. To determine the harvest date, the evolution of soluble solid content was followed

weekly during maturation until a plateau was reached. Grape yield (kg plant⁻¹) was determined in six plants per cultivar and treatment (only during the period 1994–2001). At this time, several fruit quality parameters were determined as well. Berries were carefully separated from six to eight randomly chosen clusters and three samples of 100 berries were randomly taken from the total pool. Thereafter the samples were placed inside a dry-freezer (Virtis 12 LS) at $-56^\circ C$ for 48 h, and the dried samples stored for chemical analysis. Must was obtained from the remaining berries, and pH, titratable acidity (TA) and soluble solids (SS) were determined in triplicate.

The phenolic fraction was obtained from 3 g of dry sample by maceration in methanol acidulated with 2% formic acid, in an ultrasonic water bath. This step was repeated several times until total colour extraction was achieved. The extracted solutions were centrifuged at 8000 g for 10 min at $10^\circ C$ (5221 Beckman centrifuge). Supernatants were combined and concentrated to 10 mL in a rotary evaporator (Heidolph WB 2001) at $35^\circ C$, and the solution was filtered through a membrane filter (0.45 μm). The final extract was stored at $-20^\circ C$ for analysis and the total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Singleton and Rossi 1965). Total anthocyanin content (TAC) was obtained as the sum of free and acylated anthocyanidin-3-glucosides, determined by HPLC following the method of González-San José *et al.* (1990). Finally, since all yield and quality parameters were measured only once per year, the other parameters (water relations, photosynthesis and transpiration) were averaged over each year in order to compare them with quality parameters.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) with irrigation treatment and cultivar as main effects (Anon 1998). Year effect was not considered because previous analysis showed it was not significant, even if only data from non-irrigated plants were analysed.

A Pearson correlation analysis including all the parameters determined was also performed. Possible differences between cultivars in the correlation between two parameters were analysed by comparison of regression lines (Snedecor and Cochran 1994).

Results and discussion

Grapevine responses to drought depend more strongly on soil water availability than on leaf water status

On average, and including data for irrigated and non-irrigated plants, an ANOVA analysis showed that Manto Negro maintained a higher Ψ_{PD} than Tempranillo (-0.18 as compared with -0.26 MPa, $P < 0.05$). The inter-annual

Table 1. Pearson correlation coefficients among three different water status parameters (pre-dawn leaf water potential, Ψ_{PD} ; midday leaf water potential, Ψ_{MD} ; and soil water availability, SWA) and several studied variables: midday stomatal conductance (g_s), daily-integrated CO_2 assimilation (A_D) and transpiration (E_D), unitary grape yield, soluble solids (SS) and total polyphenol content (TPC)

* denotes significant correlation ($P < 0.05$)

	Ψ_{PD} (MPa)	Ψ_{MD} (MPa)	SWA (L m ⁻²)
g_s (mol H ₂ O m ⁻² s ⁻¹)	0.67*	0.51*	0.73*
A_D (mmol CO ₂ m ⁻² d ⁻¹)	0.82*	0.76*	0.84*
E_D (mmol H ₂ O m ⁻² d ⁻¹)	0.68*	0.65*	0.68*
Unitary grape yield (kg plant ⁻¹)	0.48*	0.27	0.71*
SS (°Brix)	0.40*	0.19	-0.10
TPC (mg g ⁻¹ DM)	-0.50*	-0.48*	-0.38*

variations of climate also caused different intensities of drought. For instance, in 1992–1994 and 2000–2001 drought was especially intense, leading to summer-averaged Ψ_{PD} between -0.4 and -0.6 MPa in 0% ETP plants. From 1995–1999, drought was less severe, leading to Ψ_{PD} between -0.2 and -0.3 MPa in 0% ETP plants. However, it is not our purpose here to analyse in depth year-to-year variations in grapevine physiology. Rather, we benefit from such an amount of data to strengthen the links among different physiological processes that are regulated by drought, from leaf photosynthesis to grape yield and quality.

Three parameters (Ψ_{PD} , Ψ_{MD} and SWA) were calculated as indicative of water availability and/or water stress. A Pearson's correlation analysis was performed to relate each of these three parameters (integrated over the season) to all the other variables analysed, in order to decide which of the three best reflected the drought-induced variations in grapevine physiology. Table 1 shows the correlation factors for some of these variables, representative of water losses (g_s , E_D), photosynthesis (A_D), and grape yield, and quality (SS and TPC). It is clear that Ψ_{PD} and SWA correlated better with any parameter than Ψ_{MD} . This was expected, since ANOVA revealed that the treatment effect on Ψ_{MD} was only marginally significant (not shown). This is also consistent with the report by Flexas *et al.* (2002a), who showed that leaf relative water content was not correlated with any photosynthetic parameter. The isohydric behaviour of grapevines implies a high capacity for this species to maintain its water status above a certain value even under very high water deficit conditions. Consequently, Ψ_{MD} variations during summer are narrow and only marginal differences are observed between irrigated and non-irrigated plants (Choné *et al.* 2001). Only when drought is extremely severe is such regulation capacity broken, causing Ψ_{MD} to drop (see Fig. 2 for an example of this extreme situation).

In contrast, both SWA and Ψ_{PD} were significantly correlated with most of the parameters analysed. The latter is likely to reflect the equilibrium between soil and leaf water potentials before sunrise. This suggests that, for isohydric genotypes such as those studied here, most of the plant responses to drought are triggered by soil water availability, in addition to tissue water status. This may be mediated by chemical signals synthesised in the roots in response to soil drying. Correia *et al.* (1995) indeed demonstrated in field-grown grapevines that the maximum daily g_s (although not its diurnal fluctuations) was determined by the xylem ABA concentration. In addition, it has been shown that partial root drying in grapevines, a process that involves a 5-fold increase in leaf ABA content, induces stomatal closure and reduction in shoot growth without modifying leaf water potential (Stoll *et al.* 2000). It has also been shown that most of the drought-induced down-regulation of photosynthesis depends directly on g_s , and not on the leaf water potential (Flexas *et al.* 2002a).

Based on these results, we considered that SWA or Ψ_{PD} are better indicators of drought than Ψ_{MD} in grapevines. However, we will focus on Ψ_{PD} mainly for two reasons. First, Ψ_{PD} is directly measured while SWA is only a rough estimate of the real water availability in the vineyard during

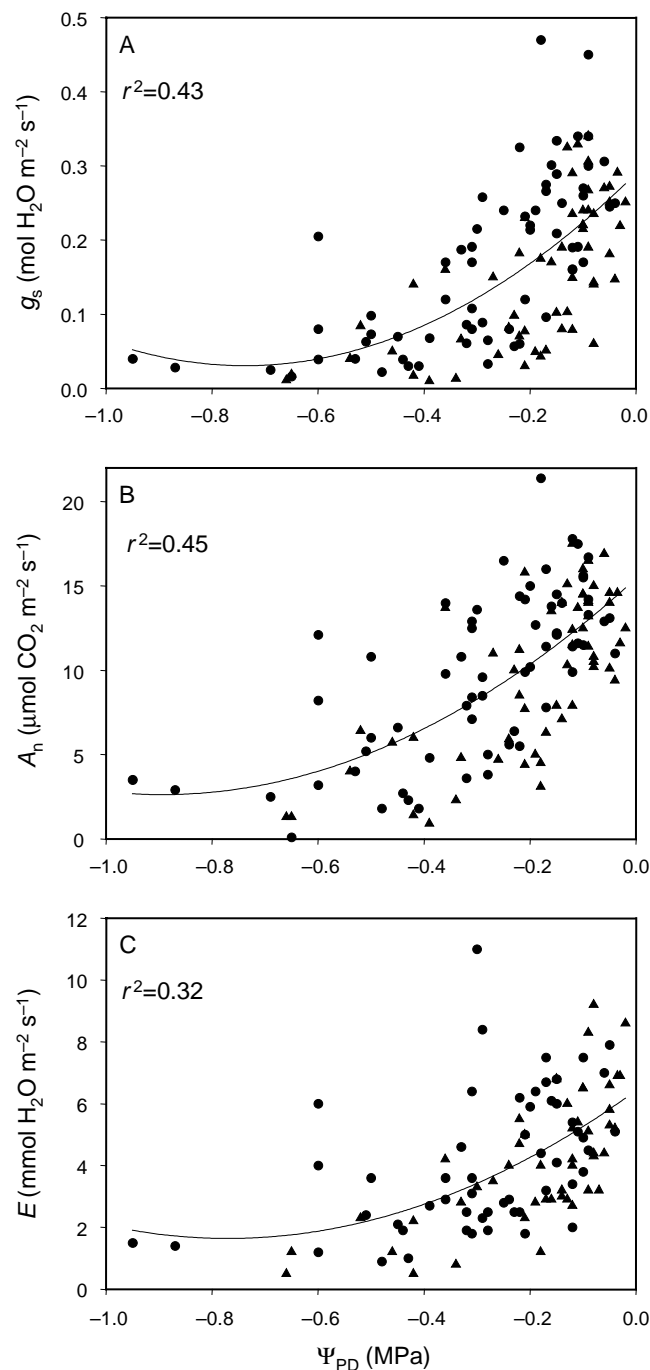


Fig. 1. Variations of (A) stomatal conductance (g_s) (B) net CO_2 assimilation (A_N) and (C) leaf transpiration (E) in relation to pre-dawn leaf water potential (Ψ_{PD}). Data are monthly averages of six replicates for Tempranillo (●) and Manto Negro (▲). All the treatments and years were included. r^2 of the best-fit regressions are shown as inserts.

the growing period; second, we obtained three sets of Ψ_{PD} data per year but only one set of SWA data, so using Ψ_{PD} data will strengthen some of the following correlations.

Drought induces parallel down-regulation of water losses and CO₂ assimilation, but the latter is due to both stomatal and non-stomatal factors

A clear correlation was found between Ψ_{PD} and g_s , A_N , and E (Fig. 1). These relationships improved when adjusted to a second-order correlation instead of a linear one, as previously shown in grapevines (Poni *et al.* 1994) and other species (Schulze and Hall 1982; Tardieu and Simonneau 1998). This behaviour reflects the high degree of adaptation of grapevines to drought; a small variation in Ψ_{PD} induces a tight stomatal closure, so plant water status can be maintained within a narrow range. Similar correlations were observed between Ψ_{PD} and A_D or E_D (not shown), as also shown by Schultz (1998) in Grenache and Syrah.

In spite of parallel down-regulation of transpiration and photosynthesis by drought, the latter was affected by both stomatal and non-stomatal factors. If decreased photosynthesis is dominated by stomatal closure, increased water-use efficiency is expected but if it is mainly determined by non-stomatal factors, decreased water-use efficiency should be observed (Flexas *et al.* 2002a). Taking all the data from ten years, we observed an absence of irrigation treatment effect on A_N/E , and only a minor effect on A_N/g_s , in spite of significant effects in A_N , E and g_s (Table 2), which suggests a co-regulation of stomatal aperture and photosynthetic metabolism. Differences among cultivars in drought-induced regulation of photosynthesis and transpiration were not important, also confirming previous reports (Flexas *et al.* 2002a). The treatment effect on ETR confirms the presence of some metabolic limitations to photosynthesis. It has to be stressed that ETR correlated better with g_s (fitting a curvilinear relationship) than with any parameter reflecting water status (not shown), thus, confirming early findings

that in this species most of the photosynthesis metabolic regulation is in turn related to stomatal closure (Flexas *et al.* 2002a). Pre-dawn F_v/F_m was unaffected by irrigation treatment (Table 2), thus, confirming that photoinhibition is not a major factor in drought-induced photosynthesis depression (Flexas *et al.* 1998).

The pattern of progressive down-regulation of photosynthesis with increasing drought intensity was clear when comparing diurnal time courses of several photosynthetic parameters among different treatments. Fig. 2 shows an example that includes data from the treatment 100% ETP in addition to 30% ETP and 0% ETP plants (data correspond to August 2000, a sampling time at which a very severe drought had developed). For both cultivars, very important differences between treatments were observed in Ψ_{PD} , as well as in Ψ_{MD} (Figs 2A, G). As discussed, the latter is unusual in these two cultivars (see data described by Flexas *et al.* 1998), but this example serves to cover the entire range of drought severity observed during those years. The lack of recovery of Ψ in the afternoon in the most stressed plants confirms that a very severe stress was experienced, possibly involving xylem cavitation (Schultz and Matthews 1988; Escalona *et al.* 2002). Daily time courses of g_s and A_N closely reflected water stress. For instance, maximum g_s and A_N rates were similar for Tempranillo at 100% ETP and Manto Negro at 30% ETP (Figs 2B, C, H, I), owing to similar Ψ_{PD} . Also, as drought intensity increased, peak values of both g_s and A_N were displaced towards the early hours of the day, as typically observed in many species (Damesin and Rambal 1995; Vadell *et al.* 1995).

In contrast, the ETR response to drought was not so marked, either in absolute maximum values or in time-displacement, except in the most stressed plants (Figs 2D, J). As a result, the ratio ETR: A_N progressively increased under drought (not shown), which has previously been shown in grapevines and interpreted as an increased photorespiration to photosynthesis ratio (Flexas *et al.* 1999a, b, 2002a).

Table 2. Mean values of stomatal conductance (g_s), midday net CO₂ assimilation (A_N), intrinsic water use efficiency (A_N/g_s), midday leaf transpiration (E), instantaneous water use efficiency (A_N/E), electron transport rate (ETR) and efficiency of excitation capture by open PSII at pre-dawn (F_v/F_m)

Irrigation treatments (0% ETP and 30% ETP) are evaluated separately for each cultivar (Manto Negro and Tempranillo). Different superscript letters within the same row indicate statistically significant differences between treatments ($P < 0.05$)

	Manto Negro 0% ETP	Manto Negro 30% ETP	Tempranillo 0% ETP	Tempranillo 30% ETP
g_s (mol H ₂ O m ⁻² s ⁻¹)	0.08 ^a	0.18 ^b	0.09 ^a	0.22 ^b
A_N (μmol CO ₂ m ⁻² s ⁻¹)	6.03 ^a	11.94 ^c	7.00 ^{ab}	12.18 ^c
A_N/g_s (μmol CO ₂ mol ⁻¹ H ₂ O)	89.3 ^d	76.4 ^{bcd}	79.9 ^{cd}	60.7 ^{ab}
E (mmol H ₂ O m ⁻² s ⁻¹)	2.56 ^a	4.67 ^b	2.85 ^a	4.71 ^b
A_N/E (μmol CO ₂ mmol ⁻¹ H ₂ O)	2.76 ^a	2.70 ^a	2.89 ^a	2.69 ^a
ETR (μmol e ⁻ m ⁻² s ⁻¹)	86.6 ^a	125.8 ^b	96.7 ^a	119.8 ^b
F_v/F_m	0.79 ^a	0.81 ^a	0.79 ^a	0.80 ^a

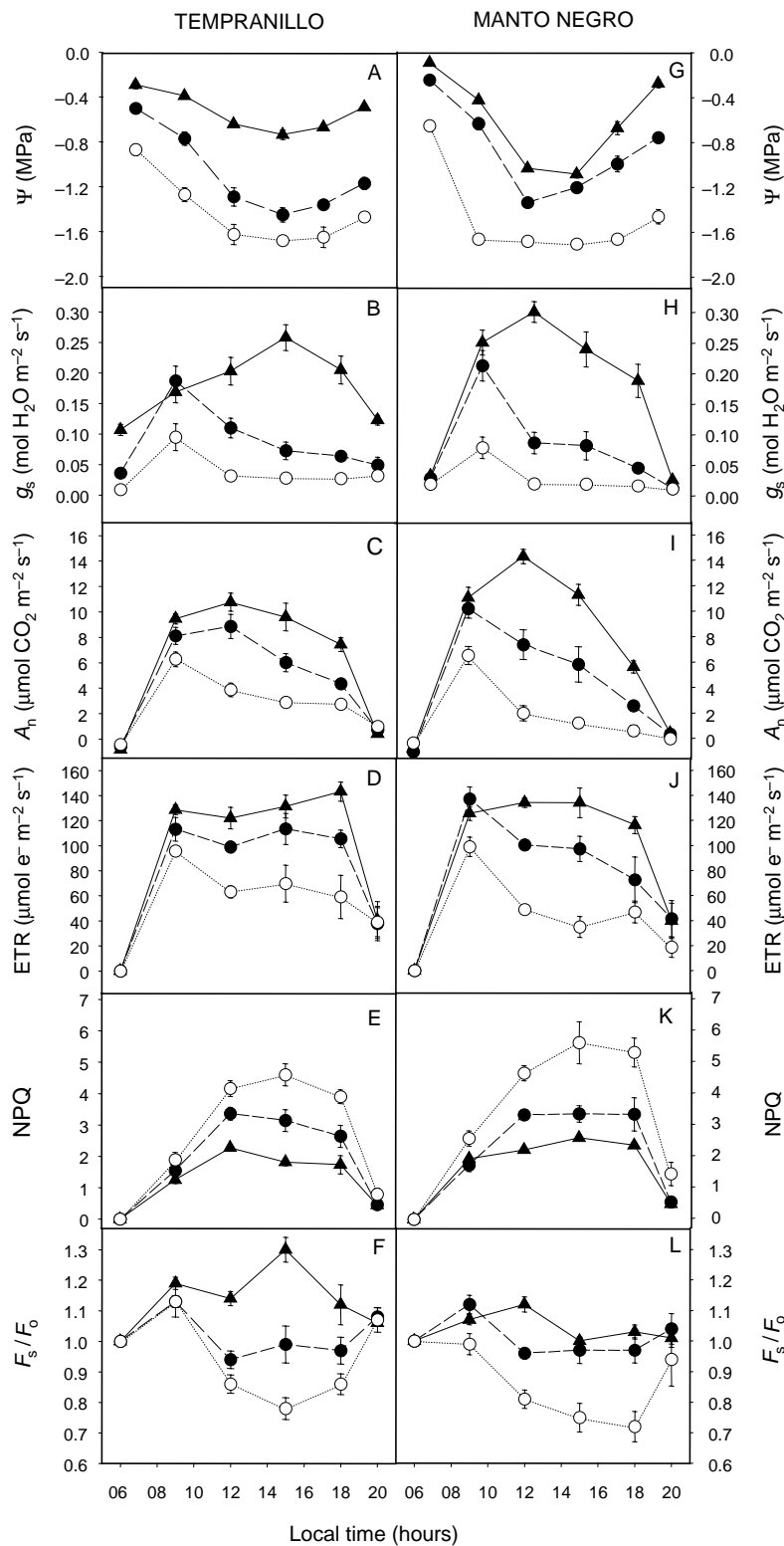


Fig. 2. Diurnal time courses of (A, G) leaf water potential (Ψ), (B, H) stomatal conductance (g_s), (C, I) net CO_2 assimilation (A_n), (D, J) electron transport rate (ETR), (E, K) non-photochemical quenching of chlorophyll fluorescence (NPQ) and (F, L) steady-state chlorophyll fluorescence normalised to basal fluorescence at pre-dawn (F_s/F_0) in three different treatments: 100% ETP (\blacktriangle), 30% ETP (\bullet) and 0% ETP (\circ). Two cultivars are shown: Tempranillo (left panels: A, B, C, D, E, F) and Manto Negro (right panels: G, H, I, J, K, L). Data are means \pm standard error (s.e.) of six to eight replicates per cultivar and treatment (data from August 2000).

The data corresponding to Tempranillo at 30% ETP showed a midday and afternoon depression in A_N that is not accompanied by any depression in ETR. This fact supported the conclusion that absolute photorespiration rates may increase under mild drought (Wingler *et al.* 1999), especially in the early afternoon (Valentini *et al.* 1995; Flexas *et al.* 1999a). NPQ increased as drought intensity increased (Fig. 2E, K), as already shown for potted grapevines (Flexas *et al.* 1999a). This is indicative that, as drought progresses, available light exceeds that which can be used for photosynthesis by an increasing amount (Cornic 1994). A slightly higher capacity for thermal dissipation in Manto Negro than in Tempranillo, together with the maintenance of slightly higher Ψ_{PD} during the season, are in accordance with the reputation of Manto Negro as a cultivar better adapted to drought than Tempranillo. Thermal dissipation in Manto Negro plants is strongly related to de-epoxidation of the xanthophyll cycle, but in Tempranillo the relationship was less clear (Medrano *et al.* 2002a). The diurnal patterns of steady-state chlorophyll fluorescence (F_s , Figs 2F, L) also accurately reflected drought intensity, as was observed in potted plants (Flexas *et al.* 1999a, 2002b). This confirms the possibility that this parameter could be used for remote sensing of plant water status, thus, facilitating rational irrigation scheduling (Flexas *et al.* 2002b).

Non-stomatal regulation of photosynthesis is due to reduced capacity for RuBP regeneration, but not to decreased Rubisco activity

Overall, the present results are consistent with our previous reports about photosynthetic down-regulation under drought: the photosynthetic control changes from being predominantly stomatal at early steps of drought to being non-stomatal at more severe drought intensity (Escalona *et al.* 1999a; Flexas *et al.* 2002a). The most important non-stomatal regulations are decreased light- and CO_2 -saturated photosynthesis (A_{SAT}) (Quick *et al.* 1992; Escalona *et al.* 1999a), reduced photochemistry (Flexas *et al.* 1999a, 2002a), and, possibly, decreased mesophyll conductance (Flexas *et al.* 2002a). From our previous results, it remained to be determined (i) whether decreased A_{SAT} was due to decreased capacity for RuBP regeneration or simply related to decreased electron transport, and (ii) whether depressed activity of photosynthetic enzymes could account for some of the observed metabolic down-regulation, as recently suggested by Maroco *et al.* (2002) for field-grown grapevines.

To evaluate these aspects, RuBP content and Rubisco activity were analysed for Tempranillo plants at several sampling times in 2000 and 2001. Although data were scarce in comparison with other parameters, the results were relevant (Fig. 3). The RuBP content was reduced in 0% ETP plants, and a very strong correlation was observed between RuBP content and Ψ_{PD} (Fig. 3A). Moreover, the RuBP response to decreasing Ψ_{PD} was quite similar to that of A_{SAT} (Fig. 3A, data

from Escalona *et al.* 1999a). The similarity between the responses of RuBP content and A_{SAT} to Ψ_{PD} strongly suggests that the latter was down-regulated by decreased capacity for RuBP regeneration, and not by decreased electron transport. This would be consistent with the fact that A_{SAT} decreases more steeply in response to drought than ETR (Medrano *et al.* 2002b), as well as with reports in other species that RuBP regeneration is strongly affected by drought (Giménez *et al.* 1992; Gunasekera and Berkowitz 1993). According to recent results reported by Tezara *et al.* (1999), decreased capacity for RuBP regeneration may be related to drought-induced impairment of chloroplastic ATPase. This was regarded as the main point of photosynthetic down-regulation under drought (Tezara *et al.* 1999). Further studies will be necessary to confirm this hypothesis.

In contrast, both total and initial Rubisco activities (expressed on a soluble protein basis) were unaffected by

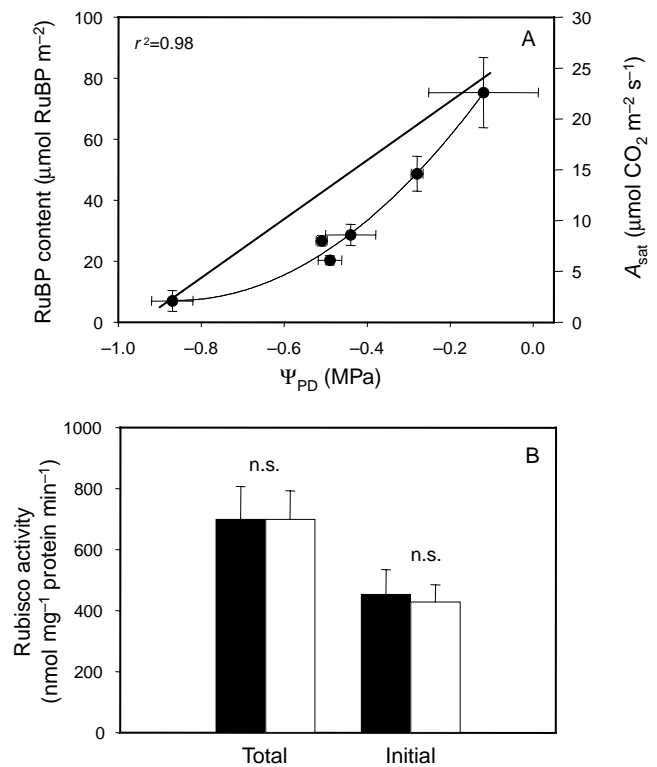


Fig. 3. (A) The relationship between the ribulose-1,5-bisphosphate content (RuBP) and the pre-dawn leaf water potential (Ψ_{PD}) in Tempranillo plants in August 2000 and July and August 2001 (r^2 is shown as an insert). Data are average \pm s.e. of four to eight replicates. The line represents the best-fit regression between A_{SAT} and Ψ_{PD} described by Escalona *et al.* (1999a) for field-grown Tempranillo plants. (B) Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) initial and total activities in Tempranillo plants. Data for each of two treatments (irrigation at 30% ETP, filled bars and 0% ETP, empty bars) are averaged from three different sampling times (August 2000 and July and August 2001), when the Ψ_{PD} values ranged from -0.12 to -0.51 MPa in 30% ETP plants, and from -0.44 to -0.87 MPa in 0% ETP plants. Data on 100% ETP were not available. Data are means \pm s.e. of 15 replicates.

drought (Fig. 3B). This is consistent with the high stability of the photosynthetic machinery in response to drought, reported for many species (Flexas and Medrano 2002). The present results do not contradict those of Maroco *et al.* (2002), since their water stressed plants presented much lower Ψ_{pD} than those analysed in the present study, possibly owing to the fact that their vines were growing on a sandy soil. Moreover, in their data, although Rubisco activity was depressed by about 35%, the difference was not statistically significant. Thus, it is clear that although Rubisco activity may probably be somewhat depressed under very severe drought, this does not seem to be the key point of drought-induced metabolic regulation.

Scaling up from midday measurements to whole-day estimations of carbon gain and water losses

Photosynthesis has been shown to correlate with growth and yield in different crops, but only when properly scaled,

i.e. considering both diurnal and seasonal variations, as well as differing contributions from leaves occupying different positions within the canopy (Intrieri *et al.* 1997). For half of the years studied, diurnal time courses of A_N and E were followed, and used to determine A_D and E_D . A strong correlation was observed between A_N and A_D (Fig. 4A), as well as between E and E_D (Fig. 4C), regardless of the cultivar. Such a correlation is of predictive value, and allows an accurate estimation of daily-integrated leaf carbon assimilation and transpiration from gas-exchange data determined only at midday. When compared on a percentage basis (i.e. each value expressed as a percentage of the maximum value obtained during the 5-year period) it is clear that, for high values of photosynthesis (i.e. non-stressed plants), decreases in A_N and A_D were similar (Fig. 4B). However, as drought progressed, A_N became more depressed than A_D . This is expected from the daily patterns of A_N (Fig. 3), since under drought, A_N is depressed mainly from midday. Transpiration

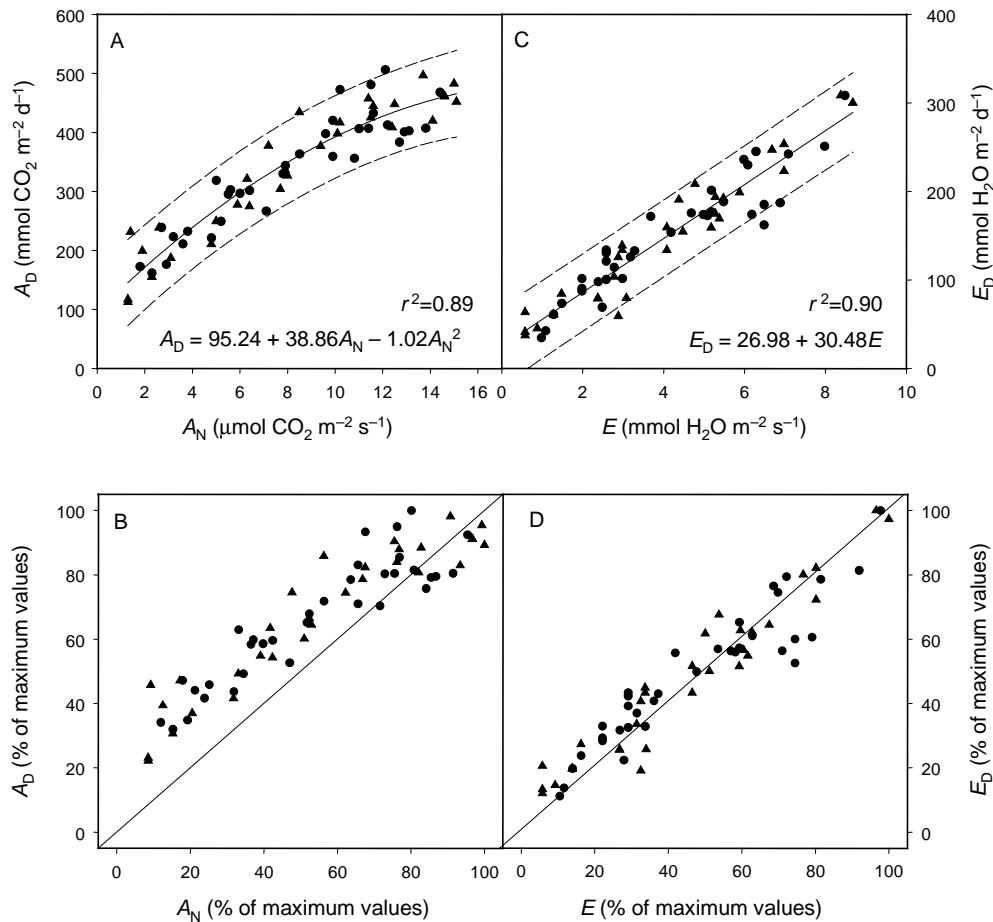


Fig. 4. The relationships between (A, B) net CO₂ assimilation (A_N) and its integration over the day (A_D) and (C, D) between leaf transpiration (E) and its integration over the day (E_D), for Tempranillo (●) and Manto Negro (▲). Monthly-averaged data are shown both in absolute values (A, C) or expressed as a percentage of maximum values (B, D). All the treatments and years were included. Regression lines and prediction intervals ($P < 0.05$) are drawn in upper panels (A, C). The best-fit regressions and their r^2 are shown as inserts. The line in lower panels (B, D) represents the 1 : 1 relationship.

Table 3. Mean values of unitary grape yield and berry weight
 Irrigation treatments (0% ETP and 30% ETP) are evaluated separately for each cultivar (Manto Negro and Tempranillo). Different superscript letters within the same row indicate statistically significant differences between treatments ($P < 0.05$)

	Manto Negro 0% ETP	Manto Negro 30% ETP	Tempranillo 0% ETP	Tempranillo 30% ETP
Unitary grape yield (kg plant ⁻¹)	2.47 ^a	3.63 ^b	2.49 ^a	5.41 ^c
Berry weight (g per 100 berries)	218.0 ^a	254.5 ^b	210.6 ^a	229.3 ^a

data, by contrast, lie close to the 1:1 relationship (Fig. 4D). Therefore, the high degree of correlation between A_N and A_D , and between E and E_D , makes the midday data alone a good basis on which to analyse the relationships between photosynthesis, transpiration, and yield.

Scaling up from the single-leaf to the whole-canopy level results in more complexity. One approach involves determining diurnal time courses of gas exchange not only in fully-exposed leaves, but in different canopy layers and positions, and then scaling up to whole-canopy

photosynthesis by measuring the total leaf area of each layer. This estimate was made during three years (1997, 1999 and 2000; Escalona *et al.* 1999b, 2003). Those data showed that daily-integrated drought-induced depressions of photosynthesis and transpiration per unit leaf area, analysed at the whole-canopy level, were similar to those observed for fully exposed leaves on a percentage basis. Miller *et al.* (1997) found similar correlations between single-leaf and whole-canopy photosynthesis measurements in Chambourcin grapevines.

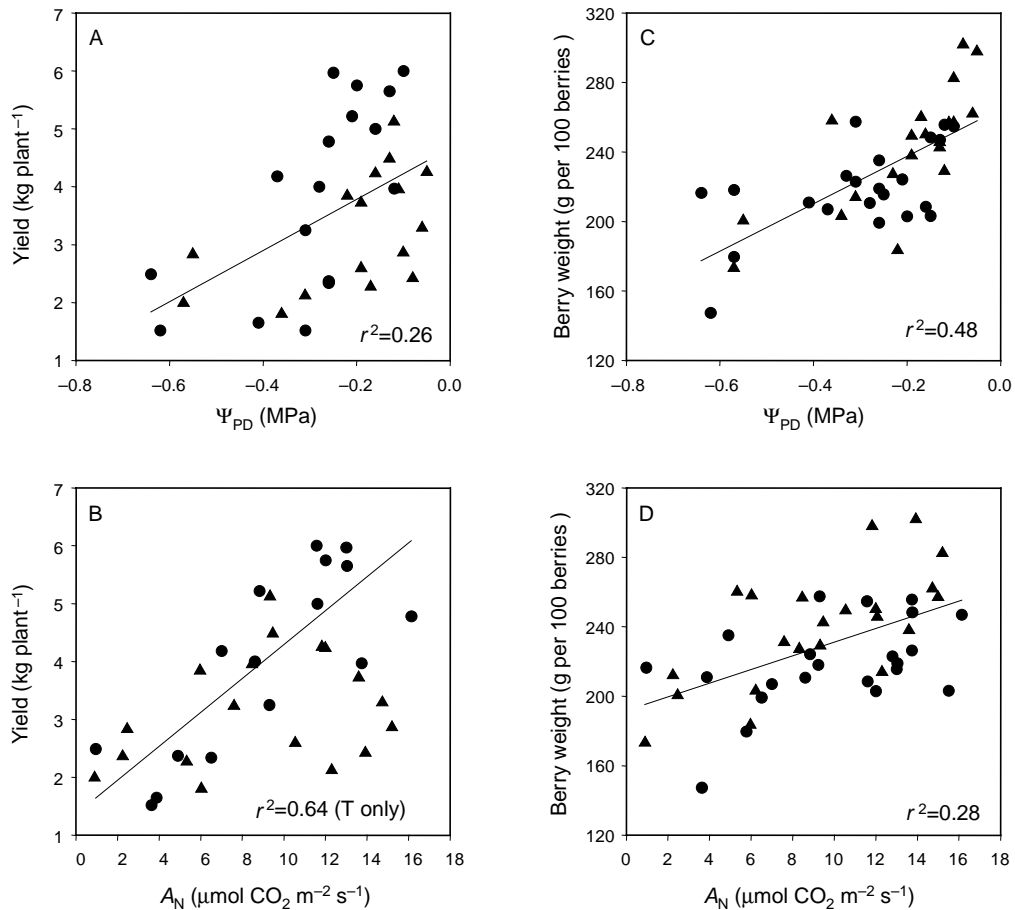


Fig. 5. Variations of unitary grape yield (A, B) and berry weight (C, D) with respect to Ψ_{PD} (A, C) and A_N (B, D). Data are averages of six replicates for Tempranillo (●) and Manto Negro (▲). All the treatments and years were included. r^2 of the best-fit regressions are shown as inserts. In B only Tempranillo was included in the regression.

Another approach involves measuring sap-flow rates of the entire plant, using sap-flow meters (Ginestar *et al.* 1998a, b), and then scaling them to gas-exchange units by measuring the total leaf area of the plant. A highly significant linear correlation was found between midday E values and sap flow in potted grapevines (Escalona *et al.* 2002), which favours the use of E and E_D as a first approach to estimating whole canopy water losses.

The regulation of grape yield under drought is cultivar-dependent

Grape yield (kg vine^{-1}) was strongly affected by the irrigation treatment (Table 3). On average, irrigation doubled yield in Tempranillo but increased it only by 45% in Manto Negro. The results obtained in Tempranillo were similar to previous reports by Esteban *et al.* (1999, 2001) for this cultivar with the same training system. In contrast, the treatment effects on berry weight were only significant in Manto Negro. However, the differences between treatments were small, so most of the variation in yield was attributed to increased fertility under irrigation (increased number of clusters and/or berries per cluster), rather than to differences in berry weight. Schultz (1996) reached a similar conclusion for both Grenache and Syrah vines.

Both grape yield and berry weight correlated significantly with Ψ_{PD} (Figs 5A, C). Although the relationship between yield and Ψ_{PD} appeared steeper in Tempranillo than in Manto Negro, covariance analysis failed to detect any interaction of the relationships with the cultivar. A high degree of correlation between Ψ_{PD} and berry weight was also observed by Ginestar *et al.* (1998b) in Shiraz vines. In contrast, Williams (2000) reported a linear dependence of grape yield on applied water, but yield response became saturated when the water supplied accounted for *ca* 60% of water losses (estimated with a lysimeter). Similarly, in the two years in which two irrigation treatments were compared (30% ETP and 100% ETP), grape yield differed little and non-significantly between the treatments (not shown).

Once a close correlation between A_D and A_N , and a reasonable relationship between whole canopy photosynthesis

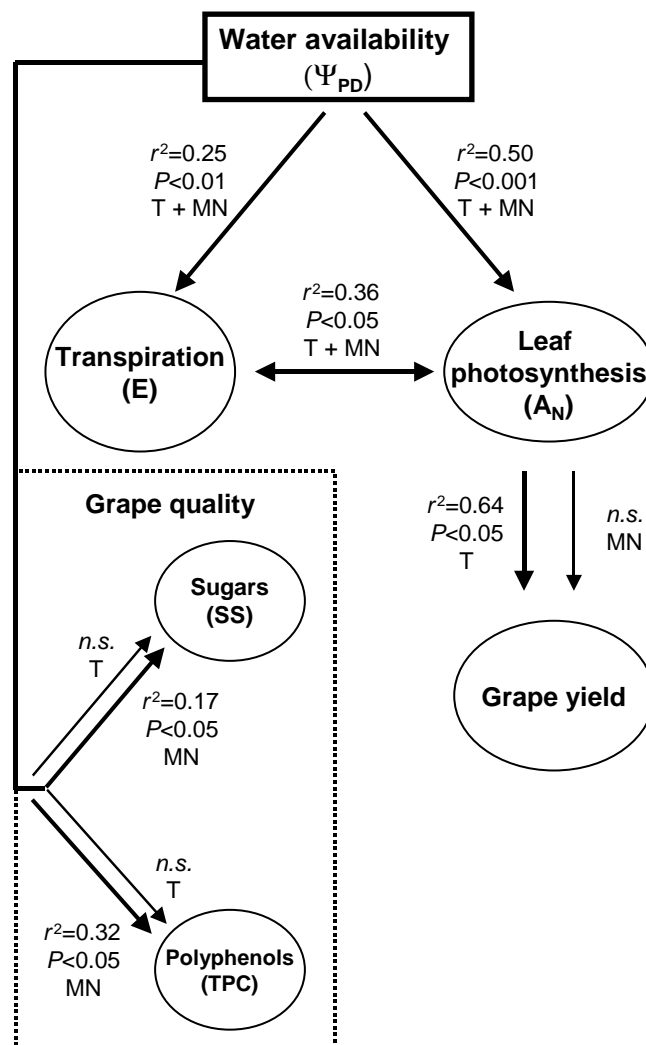


Fig. 6. Summary of interrelationships between water availability, gas-exchange parameters (leaf transpiration, E and net CO_2 assimilation, A_N), unitary grape yield and grape quality parameters (soluble solids, SS and total polyphenol content, TPC). r^2 and P values from linear regression adjustments are shown where appropriate for each separate cultivar, Tempranillo (T) or Manto Negro (MN), or for both pooled (T + MN).

Table 4. Mean values of soluble solids (SS), titratable acidity (TA), pH, total polyphenol content (TPC) and total anthocyanin content (TAC)

Irrigation treatments (0% ETP and 30% ETP) are evaluated separately for each cultivar (Manto Negro and Tempranillo). Different letters within the same row indicate statistically significant differences between treatments ($P<0.05$)

	Manto Negro 0% ETP	Manto Negro 30% ETP	Tempranillo 0% ETP	Tempranillo 30% ETP
SS ($^{\circ}$ Brix)	21.79 ^a	22.90 ^b	19.76 ^a	19.81 ^a
TA (g tartaric acid L^{-1})	4.24 ^a	4.83 ^a	4.69 ^a	5.11 ^a
pH	3.63 ^{ab}	3.76 ^b	3.58 ^a	3.55 ^a
TPC (mg g^{-1} DM)	6.32 ^a	5.35 ^a	11.02 ^b	9.33 ^b
TAC (mg g^{-1} DM)	1.66 ^a	0.94 ^a	2.96 ^b	2.87 ^b

and A_N was demonstrated, the latter could be used to study the dependency of grape yield on photosynthesis (A_N was preferred to A_D , since it was determined in 10 instead of only 5 years). A significant correlation between grape yield and A_N was observed in Tempranillo, but no correlation was found in Manto Negro (Fig. 5B). This could be due to differences in total leaf area per plant, which is clearly higher in Manto Negro than in Tempranillo, owing to the different training systems used. When total area per vine was considered and whole-canopy photosynthesis was estimated, it was significantly correlated with grape yield in Manto Negro, but not in Tempranillo (Escalona *et al.* 1999b, 2003). It was concluded that factors other than carbon assimilation, such as fertility and fruit set, strongly determined grape yield in Manto Negro. Manto Negro maintains an active vegetative growth during ripening, but not Tempranillo, so Manto Negro reaches total leaf areas of 5–8 m² vine⁻¹ while leaf area of Tempranillo reaches only 4–6 m² vine⁻¹ at harvest (Escalona *et al.* 1999b, 2003). This vegetative growth, therefore, competes for carbon with fruit ripening. Also, Manto Negro maintains a higher Ψ_{PD} than Tempranillo during the season, suggesting that this cultivar may allocate more photosynthates to roots than Tempranillo. Overall, it seems that Manto Negro expends much larger amounts of carbon in vegetative growth (leaves, shoots and roots) than Tempranillo. Therefore, less carbon is available for fruits at any given level of carbon accumulated. The physiological behaviour of Manto Negro in respect to Tempranillo was comparable to that of Grenache in respect to Syrah (Schultz 1996). According to this author, Grenache, which optimised survival at the expense of yield, was considered drought-avoiding, while Syrah, which invested large amounts of resources on fruits but was subjected to occasional plant death under severe drought, was considered drought-tolerant. Occasional plant death did indeed occur during the most severe years in 0% ETP Tempranillo vines, but not in Manto Negro.

The correlation between berry weight and A_N (Fig. 5D) was also significant, as were those between both grape yield and berry weight, and E (not shown).

Drought-induced variations of grape quality are cultivar-dependent, and do not depend on photosynthesis or yield

The effect of treatment on fruit quality at harvest time depended on the parameter analysed (Table 4). SS (°Brix) was significantly increased in 0% ETP Manto Negro plants compared with irrigated ones, but no effect was observed in Tempranillo. It seems a general feature that drought effects on soluble sugar content are cultivar-dependent. For instance, Schultz (1996) observed that drought decreased soluble sugar content in Grenache, as did Williams (2000) in Thomson seedless, but not either Schultz (1996) or Ginestar *et al.* (1998b) in Syrah.

The pH and TA were little affected by drought, as already reported by Freeman and Kliewer (1983) and Ginestar *et al.* (1998b). TPC and TAC presented significantly higher values in Tempranillo than in Manto Negro, although the effect of irrigation treatment was not significant in either cultivar. This lack of treatment effect was likely due to high inter-annual variability in these parameters within each treatment. A recent report comparing TPC and TAC in irrigated and 0% ETP Tempranillo plants also highlighted the fact that year-to-year variability was larger than variability due to the irrigation treatments (Esteban *et al.* 2001). Such a large inter-annual variability has often been described in grapevines, and is due to a complex interaction of different factors, mainly related to cluster microclimate (Jackson and Lombard 1993). For most of the years, irrigated Manto Negro plants showed a significant reduction of TPC and TAC, while Tempranillo plants were unaffected. In fact, TPC was significantly correlated with Ψ_{PD} ($r^2 = -0.32$, $P < 0.05$) in Manto Negro, but no correlation was observed in Tempranillo. Drought-induced decreases in colour parameters were described by Freeman and Kliewer (1983) in Carignane vines and by Ginestar *et al.* (1998b) in Syrah, but Schultz (1996) found no stress effect on colour in either Grenache or Syrah.

None of the fruit quality parameters analysed showed significant correlations with photosynthesis, transpiration or yield parameters (not shown).

Concluding remarks

This is, to the best of our knowledge, the first attempt to statistically correlate water availability, photosynthesis, water losses, grape yield and quality in grapevines under water stress. A summary of these interrelationships is shown in Fig. 6.

In summary, the present results suggest that there is a close link from water availability to grape yield, through water stress effects on photosynthesis. By contrast, grape quality seems to be regulated somewhat independently of photosynthesis and yield, although somewhat linked to water availability. To confirm this would be of practical interest, since it would mean that irrigation scheduling could be used to regulate grape yield and grape quality in an independent manner. The different responses observed in Tempranillo and Manto Negro suggest that regulation of grape quality is also dependent on the cultivar and/or training system.

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