Physiological Thresholds for Efficient Regulated Deficit-Irrigation Management in Winegrapes Grown under Semiarid Conditions

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Abstract: The effects of two regulated deficit-irrigation (RDI) strategies pre- and postveraison on soil-plant water relations and their influence on leaf area development, cluster microclimate, yield, and berry quality were evaluated during two years in field-grown Monastrell grapevines under semiarid conditions in southeastern Spain. Three treatments were applied. The control was irrigated at 60% ET_c (crop evapotranspiration), or 319 mm water over the full season. Regulated deficit-irrigation treatment 1 (RDI-1) received the same irrigation as the control before fruit set, 30% ET_c from fruit set to harvest, and 45% ET_c postharvest. Regulated deficit-irrigation treatment 2 (RDI-2) was the same as RDI-1, but with 15% ET_c from fruit set to harvest. RDI-1 maintained soil water content and vine water status adequate for sustaining leaf gas exchange, without affecting sugar accumulation or increasing polyphenols at harvest. Moreover, RDI-1 reduced yield and berry size and improved cluster microclimate by reducing leaf area and increased water use efficiency. However, RDI-2 suffered more stress, mainly postveraison. This severe water stress substantially reduced root-vine hydraulic conductance and leaf gas exchange, decreasing gas exchange efficiency, leaf nitrogen, and chlorophyll content. Excessive postveraison water stress advanced leaf abscission, reducing leaf area development and yield. Lower leaf photosynthesis and higher leaf abscission significantly decreased yield and sugar in RDI-2 berries compared with RDI-1. Polyphenol and anthocyanin content also decreased significantly in RDI-2 compared with RDI-1. To avoid severe root and leaf function damage and increase polyphenols in this cultivar, we identified optimum physiological thresholds for several vine water indicators pre- and postveraison.

Key words: gas exchange efficiency, phenolic composition, physiological thresholds, soil-plant-water relationships, sugar accumulation

Irrigation management is the largest, most controllable determinant of grape and wine quality in arid areas (Fereres and Evans 2006). One of the most promising irrigation-management techniques for vineyards in semiarid areas is regulated deficit irrigation (RDI) (McCarthy et al. 2002, Kriedemann and Goodwin 2003, Keller 2005, Chaves et al. 2007). The effect of RDI depends on vine phenological stage and the severity of the stress imposed (McCarthy et al. 2002). RDI in winegrapes is commonly applied during two periods to increase berry quality. Water deficits early in the season, from fruit set to veraison (preveraison), control berry size and reduce vine vigor (McCarthy et al. 2002, Keller 2005). Water deficits after veraison, during fruit ripening, increase the biosynthesis of anthocyanins and other phenolic compounds (Kennedy et al. 2002). Both practices can reduce yield and vegetative development compared to full irrigation (Kriedemann and Goodwin 2003) and can benefit berry and wine quality in different ways (McCarthy et al. 2002, Cortell et al. 2005, 2007).

A disadvantage of RDI is that it requires water status to be maintained within a narrow tolerance range. Overirrigation undermines the advantages of RDI and increases water use, while underirrigation can lead to severe yield or quality losses (Jones 2004). Thus, efficient scheduling of RDI requires defining several threshold values for plant stress indicators, beyond which irrigation is necessary to avoid deleterious effects on vines and to achieve specific objectives in crop management. However, the variable responses of different cultivars to different climatic conditions and water stress levels make it difficult to use only one indicator for vine water status or irrigation scheduling.

A common and practical indicator used in grapevine to manage irrigation is water potential, measured as predawn leaf water potential, stem water potential at midday or midmorning (Keller et al. 2008), or leaf water potential (Shellie

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2006). However, there is some disagreement concerning whether stem or leaf water potential is better correlated to vine physiology and concerning which time of day best reflects vine performance (Baeza et al. 2007). In isohydric species such as some grapevine cultivars, leaf water status is stable over a wide range of soil water potentials (Schulze 2003). In these instances, water potential or other tissue water status indicators cannot be used as a sensitive measure of water stress (Jones 2004, 2007). Recent results indicate that leaf water potential is a good indicator of both vine water status and agronomic response, but not of must composition (Baeza et al. 2007).

At present, there is little definitive information about the relationship between different vine water status parameters and berry composition, particularly phenolic composition (Sousa et al. 2006, Keller et al. 2008). An optimum threshold for stomatal conductance (g_s) between 0.05 and 0.15 mol m^{-2} s^{-1} was suggested to increase water use efficiency in grapevines (Cifre et al. 2005), but investigators did not study the relationship between leaf photosynthesis, g_s, or other physiological parameters and berry composition.

Here we describe the effects of RDI on some important physiological processes and their influence on berry composition, particularly of phenolics. Over two years, two different RDI strategies were applied pre- and postveraison to achieve three objectives traditionally related to increased berry and wine quality: (1) to control excessive vegetative development, (2) to reduce berry size, and (3) to stimulate the direct accumulation of anthocyanins and other phenolic compounds by postveraison water deficit.

The study focused on finding significant relationships between physiological indicators and berry composition under RDI and identifying the threshold limits or vine-specific optimums of these indicators during different phenological stages to maximize berry phenolic composition at harvest. We studied the soil water status, leaf function, leaf area, and cluster microclimate and their relationships to berry composition under different water stress severity pre- and postveraison. The aim was to identify physiological thresholds for efficient long-term RDI strategies for premium red wines and to improve water use efficiency under semiarid conditions.

Materials and Methods

Field conditions, plants, and irrigation treatments. This research was carried out from 2006 to 2007 at the CIFEA experimental station in Jumilla, Murcia (southeastern Spain) (lat. 38°23'40''N; long. 1°25'30''W; 350 m asl). The soil was a 60 cm deep fine clay (48% clay, 30% silt, 22% sand), with 1.36% organic matter, 18.8% active $CaCO_3$, EC_{sat} (electrical conductivity) 5.04 dS m^{-1}, and pH 7.6. The irrigation well water had EC_{sat} 1.6 dS m^{-1}.

The climate was semiarid Mediterranean, with hot dry summers and daily maximum summer temperatures ~38 to 39°C. There are 12 to 14 days per summer of extreme heat (>35°C), mainly in July and August. Annual rainfall at the experimental site was 285 mm in 2006 and 287 mm in 2007, mainly in the spring and fall, and the total annual reference evapotranspiration (ET_o) was 1248 mm in 2006 and 1250 mm in 2007 (Table 1).

The study was performed on 12-year-old *Vitis vinifera* L. Monastrell (syn. Mourvedre) red wine grapevines grafted onto 1103 Paulsen rootstock and planted in 1997. A bilateral cordon training system trellised to a three-wire vertical system was used. Vine rows ran N-NW to S-SE, and planting density was 2.5 m between rows and 1.25 m between vines (3200 vines/ha). Six 2-bud spurs (12 nodes) per vine were retained at pruning. In May, green nonproductive shoots were removed from each vine according to local grower practice.

Three different irrigation treatments were applied during two consecutive years (2006–2007). The control treatment was irrigated at 60% crop evapotranspiration (ET_c) throughout the season (319 mm/year). Regulated deficit-irrigation treatment 1 (RDI-1) received 30% ET_c while RDI treatment 2 (RDI-2) received 15% ET_c. In RDI treatments, irrigation reduction was applied from fruit set (pea-size berries; treatments initiated on 7 June in 2006 and 6 June in 2007) until harvest (15 Sept in 2006 and 26 Sept in 2007), coinciding with phases I, II, and III of berry growth and development. A recovery of irrigation at 45% ET_c was applied in the RDI treatments from harvest to leaf fall (end of October). The mean annual irrigation water applied was 207 mm in RDI-1 and 156 mm in RDI-2. ET_c was estimated using varying crop coefficients (k_c) ($ET_c = ET_o \times k_c$) based on those proposed by the FAO and adjusted for the Mediterranean area and ET_o values. The ET_o was calculated weekly from the mean values of the preceding 6 to 7 years using the Penman-Monteith–FAO method (Allen et al. 1998) and the daily climate data collected in the meteorological station located at the experimental vineyard. The applied k_c values were 0.35 in April, 0.45 in May, 0.5 in June, 0.75 in July to

Table 1 Climate measures during different phenological stages, 2006 to 2007.

	2006[a]				2007[a]			
Stage	Rainfall (mm	ET_0 (mm)	VPD (KPa)	T^amax (°C)	Rainfall (mm)	ET_0 (mm)	VPD (KPa)	T^amax (°C)
Budburst–fruit set	102	284	1.04	23.8	69.7	283	1.07	22.5
Fruit set–veraison	4.3	309	2.02	32.2	1.5	306	1.98	31.6
Veraison–harvest	35.9	264	1.81	31.4	37.6	255	1.68	30.5
Postharvest	25.6	139	1.24	26.1	84.6	112	0.83	21.9
Whole cycle (Apr–Oct)	167.8	997	1.53	28.4	193.4	957	1.39	26.6

[a]Rainfall and reference evapotranspiration (ET_0): cumulative values during different stages. Vapor pressure deficit (VPD) and maximum daily air temperature (T^amax): mean values during different stages.

mid-August, 0.60 for the end of August to mid-September, and 0.45 for mid-September to the end of October.

Each treatment was replicated four times in a completely randomized, four-plot design. Each replicate consisted of 164 vines. Irrigation was applied three to five times per week, depending on the phenological period, and was controlled automatically. All treatments received the same annual application of fertilizer: 40 kg N, 20 kg P, 60 kg K, and 16 kg Mg/ha, and 1.6 g Fe chelate per vine, supplied through the irrigation system. The amount of water applied in each treatment was measured with flow meters. Water was applied by one pressure-compensated emitter per plant (4 L h^{-1}) in one drip-irrigation line per row. Drip-irrigation lines were placed ~40 cm aboveground.

Soil water content. Volumetric soil water content (θ_v) was measured three to four times per week in 2006 and 2007 over the course of the experiment with a Diviner 2000 portable soil moisture probe (Sentek, Stepney, Australia). One-meter single PVC access tubes were installed to a 70 cm depth in one side of the root zone. Readings were taken 10 to 15 cm from the drip head and oriented perpendicularly to the vine row at 10 to 70 cm depths. Scaled frequencies were converted to θ_v using a capacitance probe calibration equation for similarly textured clay soil, as proposed elsewhere (Rose et al. 2001): $V = 47.38\ SF^{3.12}$, $r = 0.93$.

Vine water status and leaf gas exchange. Each year, midday stem water potential (Ψ_s) was determined weekly from the beginning of vegetative growth until leaf fall. Eight fully exposed and expanded mature leaves were taken per treatment (two leaves per plot). The leaves were enclosed within aluminium foil-covered plastic at least 2 hr before the midday measurement. The Ψ_s was measured at midday (12:00–14:00 hr) using a pressure chamber (model 3000; Soil Moisture Equipment, Santa Barbara, CA). Relative water content (RWC) was obtained under the same conditions as Ψ_s. Rehydration was carried out by submerging leaf petioles in distilled water for 24 hr in the dark at 4°C. RWC was calculated using the equation RWC (%) = [(fw - dw)/(tw - dw)] × 100, in which fw, dw, and tw refer to fresh, dry, and turgid weight, respectively. Dry weight was calculated after drying the leaves to constant weight in an oven at 65°C for 48 hr.

Gas exchange was measured between 09:00 and 11:00 hr every 7 to 14 days from April to October in 2006 and 2007 on selected clear days. Measurements were made on healthy, fully expanded mature leaves exposed to the sun (one leaf on each of 12 vines per treatment), from main shoots located on the exterior canopy. The leaf photosynthesis rate (A), g_s, and transpiration rate (E) were measured with a portable LI-6400 photosynthesis measurement system (LI-COR, Lincoln, NE) equipped with a broadleaf chamber (6.0 cm^2). During measurements, leaf chamber temperature was maintained between 25 and 32°C, leaf to air vapor pressure deficit (VPD_l) at 2.0 ± 0.5 kPa, and relative humidity at 40 to 50%. Molar air flow rate inside the leaf chamber was 350 µmol mol^{-1}. All measurements were taken at a reference CO_2 concentration similar to ambient (380 µmol mol^{-1}) and at a saturating photosynthetic photon flux of 1500 µmol m^{-2} s^{-1}, by using a red/blue light source (6400-02B LED) attached to the leaf chamber.

Leaf nitrogen and chlorophyll content. For chlorophyll analysis, 12 fully expanded leaves per treatment were sampled (on three vines per treatment per plot) during pre- and postveraison periods in 2006. Chlorophyll concentration was determined spectrophotometrically at 647 and 664.5 nm after extraction in 80% acetone. Chlorophyll concentration was calculated as previously described (Inskeep and Bloom 1985). Total nitrogen was measured using a LECO FP-528 elemental analyzer (LECO Corp., St Joseph, MI) both preveraison (2–23 July) and postveraison (1 August) in 2006 and 2007. Twelve leaves per treatment (three leaves per plot) were analyzed.

Root-vine hydraulic conductance. An estimate of plant hydraulic conductance (K_{plant}) in different phenological periods in 2006 was obtained using the evaporative flux method (Nardini and Salleo 2000), which is based on an Ohm's law hydraulic analog:

$$K_{plant} = E_{md}/(\Psi_{soil} - \Psi_{lmin})$$

where E_{md} is the maximum transpiration rate and Ψ_{soil} - Ψ_{lmin} are soil and minimum diurnal leaf water potential, respectively. Predawn leaf water potential (Ψ_{pd}), measured before sunrise in unbagged leaves, was assumed to be in equilibrium with the soil water potential (Richter 1997). In this condition, Ψ_{soil} is equivalent to the predawn leaf water potential. Thus (Ψ_{pd} - Ψ_{lmin}) represents the driving force for the water flow from the soil to the leaf, and K_{plant} is the ratio of the flow through the plant to the driving force for the flow (Lo Gullo et al. 2003). Maximum values for E and minimum values for Ψ_{lmin} (measured between 12:00–17:00) were used to calculate K_{plant}, because under these conditions plants are likely to have transpired all stored water so that steady-state flows are likely to be established. K_{plant} was then scaled to the total leaf area of the vine.

Conductance from root to stem ($K_{root\text{-}stem}$) was estimated using the following equation (Tsuda and Tyree 2000):

$$K_{root\text{-}stem} = E_{md}/(\Psi_{soil} - \Psi_s)$$

where Ψ_s is the midday stem water potential and Ψ_{soil} is predawn leaf water potential.

Leaf area development. Leaf area per vine was estimated preveraison, postveraison, and at leaf fall in 16 vines per treatment (four per plot) using a nondestructive method. The leaves from main and lateral shoots were separated and measured using a LI-3000 leaf area meter (LI-COR). Initially, the single leaf area of randomly selected leaves (12 shoots per treatment, ~200 leaves) was estimated by developing a polynomial equation relating main vein length (L) to leaf area (LA) ($LA = 22.10L - 89.44$, $r^2 = 0.89$, $p < 0.001$, for main shoots and $LA = 18.39L - 51.04$, $r^2 = 0.74$, $p < 0.001$ for lateral shoots). One representative shoot per vine (16 shoots per treatment) was chosen for leaf area measurements. Leaf area per vine was estimated by multiplying the average shoot leaf area by the number of shoots on the vine.

Bunch exposure and cluster microclimate. Berry temperature was determined on clear sunny days in sunlight-exposed (east facing) and nonexposed bunches (inside the canopy at the cluster zone) at two times during the day: early morning (8:00–10:00 hr) and afternoon (13:00–15:00 hr) at veraison and after veraison. Berry temperature was measured in 20 vines per treatment (two representative bunches per vine) using a Testo 845 infrared thermometer (Testo, Lenzkirch, Germany).

Light in the cluster zone (as incident photosynthetically active radiation [PAR], 400 to 700 nm) was measured inside the canopy, close to fruiting positions and on both sides of the vine during midday on sunny days, pre- and postveraison. In each vine, four determinations were made. The same vines chosen for berry temperature measurements were also used for light measurements in the fruit zone. Readings were taken on the face of clusters facing east-west, using a LI-190 SA external quantum sensor connected to a Li-250A light meter (LI-COR).

Yield response and berry quality. Yield components (crop load and number of bunches) were measured at harvest on 52 vines per treatment (13 vines per plot). To determine berry composition at harvest, five to six berries from randomly selected clusters were sampled from the same vines used for yield component analysis. A randomly selected subsample was collected for must analysis. The weight of 100 berries was determined in each subsample to estimate mean fresh berry weight. Total soluble solids (TSS) (Brix) was determined using an Atago RX-5000 digital refractrometer (Atago, Tokyo, Japan). Juice pH and titratable acidity (TA) were determined by titration with 0.1 N NaOH using a Metrohm 686 automatic titrator (Metrohm, Herisau, Switzerland). Malic and tartaric acids were analyzed using enzymatic kits from Boehringer Mannheim GmbH (Mannheim, Germany). Anthocyanins were determined as described (Saint-Cricq et al. 1998) by macerating the grapes for 4 hr at pH 3.6 or 1.0. The total and extractable anthocyanin content of the two solutions was then chemically assayed by measuring absorbance at 520 nm at pH 1.0 and pH 3.6, respectively, while the total phenol content was calculated by measuring the optical density of the solution at pH 3.6 at 280 nm.

Statistical analysis. The data were analyzed using analysis of variance (ANOVA) and means were separated by Duncan's multiple range test using StatGraphics 2.0 Plus software. Linear and nonlinear regressions were fitted using SigmaPlot 2000 (Systat, Richmond, CA). Schwarz's Bayesian criterion index (SBC) was used to find the best fit for nonlinear regression between parameters. Maximum and threshold values were calculated using these fitted equations.

Results

Soil water status and root-plant hydraulic conductivity. The reduction of irrigation after fruit set produced a decrease in the soil water content (θ_v) in the root zone. In both years from fruit set to harvest, RDI-2 had significantly lower θ_v than the control and RDI-1 (Table 2). However, RDI-1 maintained θ_v values that were not significantly different from the control during postveraison in 2006 and 2007 and during preveraison in 2006. During the months immediately after harvest, no significant differences in θ_v were observed between RDI treatments and the control.

Whole plant hydraulic conductance (K_{plant}) and root to stem hydraulic conductance ($K_{root\text{-}stem}$) were similar among treatments prior to water stress (May) and following harvest (September), but decreased significantly in RDI treatments during water stress (Table 3). RDI-2 had significantly lower K_{plant} and $K_{root\text{-}stem}$ than RDI-1 postveraison. There was a close, significant linear relationship among K_{plant}, $K_{root\text{-}stem}$, and Ψ_s (Figure 1).

Table 2 Mean volumetric soil water content (θ_v) (%) in the highest fine root density zone (0–30 cm) for each treatment at four representative periods, 2006 and 2007.

	No water stress (budburst–fruit set)		Water stress (fruit set–veraison)		Water stress (veraison–harvest)		Recovery (harvest–leaf fall)	
Treatment	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**
Control	28.8	28.7	26.7a	27.3a	27.5a	27.3a	25.7	28.0
RDI-1	27.7	29.8	26.0a	25.6b	27.8a	26.4a	27.4	29.5
RDI-2	26.9	27.5	22.3b	20.5c	21.7b	21.4b	25.2	28.1
ANOVA[a]	ns	ns	***	***	***	***	ns	ns

[a]*** and ns indicate significance at $p < 0.001$ and not significant, respectively. Separation by Duncan's multiple range test at 95% confidence level.

Table 3 Mean values of whole plant hydraulic conductance (K_{plant}: g MPa^{-1} s^{-1}) and hydraulic conductance from root to stem ($K_{root\text{-}stem}$: g MPa^{-1} m^{-2} s^{-1}) for each treatment in different phenological stages, 2006.

	Flowering–fruit set (22 May)		Veraison (1 Aug)		Postveraison (22 Aug)		Postharvest (20 Sept)	
Treatment	**K_{plant}**	**$K_{root\text{-}stem}$**	**K_{plant}**	**$K_{root\text{-}stem}$**	**K_{plant}**	**$K_{root\text{-}stem}$**	**K_{plant}**	**$K_{root\text{-}stem}$**
Control	0.99	0.71	0.50a	0.13a	0.19a	0.052a	0.13	0.031
RDI-1	0.90	0.63	0.20b	0.06b	0.17b	0.052a	0.11	0.031
RDI-2	0.96	0.67	0.24b	0.08b	0.12c	0.038b	0.10	0.024
ANOVA[a]	ns	ns	**	**	***	**	ns	ns

[a]**, ***, and ns indicate significance at $p < 0.01$, 0.001, and not significant, respectively. Separation by Duncan's multiple range test at 95% confidence level.

Vine water status and leaf function. In both years, from budburst to fruit set (full irrigated conditions), Ψ_s and RWC were greater than -1 MPa and 94%, respectively, in all treatments (Figure 2A, B). In 2006, when soil water deficits were applied after fruit set (phase I of fast fruit growth period), Ψ_s decreased significantly in RDI-2 vines compared to the control. However, in 2007, after only three days of irrigation reduction, Ψ_s decreased significantly in all RDI vines. Mean Ψ_s decreased progressively during all phase I, reaching minimum values at the end of the phase (end of July). In 2007, leaf RWC was significantly lower at the end of phase 1 in RDI vines than in control vines.

Leaf gas exchange was also significantly reduced in RDI vines in phase I in both years (Figure 2C–H). Only six or seven days after irrigation was reduced in phase I, significant reductions in g_s and E were observed among the treatments. Thus, in 2007, average values of A during phase I were reduced 11% in RDI-1 and 19% in RDI-2 compared with the control. However, stomatal closure (g_s) was further reduced by water stress by 22% and 35% in RDI -1 and RDI-2, respectively (Figure 2F).

The differences between RDI treatments were accentuated during water stress after veraison during mid-August and early September (Figure 2). During the postharvest recovery period (at 45% ET_c), vine water status recovered faster than gas exchange in RDI, with no significant differences observed in Ψ_s, between 15 and 21 days after recovery depending on the year (Figure 2A, B). In 2006, g_s in RDI-2 vines was significantly lower than in control and RDI-1 vines 17 days after recovery. Similarly, in 2007, nearly full recovery was observed in RDI-1, only 6% lower A than the control. However, A did not recover completely in RDI-2 vines.

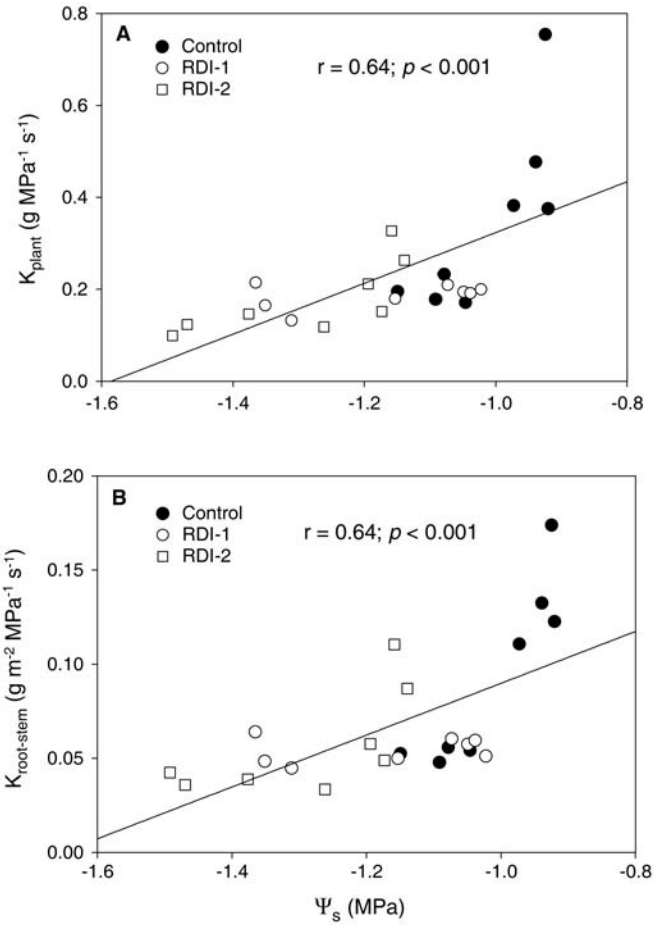

Figure 1 Relationship between midday stem water potential (Ψ_s) and (**A**) whole plant hydraulic conductance (K_{plant}) during pre- and postveraison (K_{plant} = 0.88 + 0.55 Ψ_s) and (**B**) root to stem hydraulic conductance ($K_{root\text{-}stem}$) ($K_{root\text{-}stem}$ = 0.23 + 0.14 Ψ_s) in 2006.

Intrinsic (A/g_s) and instantaneous water use efficiency (A/E) increased with water stress to reach a maximum A/g_s (91 µmol mol^{-1}) at -1.4 MPa of Ψ_s and 0.11 mol m^{-2} s^{-1} of g_s and a maximum A/E at -1.3 MPa and 0.14 mol m^{-2} s^{-1} (Figure 3A, C, D, E). Below these threshold values, leaf gas exchange efficiency did not increase or dropped slightly. When g_s was <0.05 mol m^{-2} s^{-1} as a consequence of severe water stress, A/g_s decreased sharply due to a strong decline in A for small changes in g_s (Figure 3B). Moreover, the relationships between g_s and Ψ_s and A and Ψ_s pre- and postveraison also suggested early and progressive stomatal closure as Ψ_s decreased, with a threshold of approximately -1.1 MPa, above which photosynthesis was not clearly affected (Figure 3F, G). Below -1.1 MPa, stomatal closure intensified, with a subsequent decrease in photosynthesis. In both years, A and g_s showed a typical exponential relationship of $A = 19.4 * (1 - e^{-6.3gs})$ ($p < 0.001$; r = 0.95) (Figure 4).

Leaf chlorophyll decreased significantly more during postveraison in RDI-2 vines than in control and RDI-1 vines (Table 4). Leaf nitrogen decreased significantly in both RDI treatments pre- and postveraison. Thus, photosynthetic nitrogen use efficiency (NUE_{ph}) decreased in the two RDI treatments compared to the control, and RDI-2 also had significantly lower NUE_{ph} than RDI-1.

Leaf area development and cluster microclimate. Leaf area reached a maximum of >6 m^2 vine^{-1} at the end of June, and no significant differences were observed in leaf area among treatments (Figure 5). Following veraison, total leaf area per vine was significantly higher in the control than in RDI-1 and RDI-2. These differences were mainly due to differences in the main shoot leaf area, since lateral leaf area between treatments was similar. From June to early August, the leaf area of the main shoots was reduced by 14% and 19% in RDI-1 and RDI-2, respectively, compared with only 2% in control vines. In this period, lateral leaf area was reduced between 3% and 5% in the RDI treatments.

After veraison, the reduction in leaf area during ripening (early August up to the end of September) was due mainly to intense leaf abscission and leaf senescence in RDI vines. During postveraison, the main leaf area was reduced by 38% and 51% in RDI-1 and RDI-2, respectively, compared to only 19% in control vines (Figure 5B, C). Lateral leaf area was reduced by 26% in RDI-1 and 38% in RDI-2 compared with only 13% in the control. Consequently, cluster zone microclimate was clearly altered by irrigation via alterations in leaf area (Figure 6A, B). The lowest incident PAR values at the cluster zones were in control vines

(Figure 6C). Moreover, the morning berry temperatures of internal clusters were significantly greater in berries from RDI vines than from control vines (Figure 6D).

Yield response and berry quality–vine physiology relationships. Yield (kg vine^{-1}) was significantly reduced by RDI treatments compared to the control in the two years. Mean yield reductions in RDI-1 and RDI-2 were 31% and 44%, respectively (Table 5). Berry weight was an important yield component affected in both years, with a mean reduction of ~24% for RDI-1 and a greater reduction of ~37% for RDI-2 compared to the control. This was also reflected in lower cluster weights in RDI-2, but not in significant differences in berry number per cluster. Also a slight, but significant, decrease was observed in cluster number per vine between control and RDI treatments, but not between RDI treatments.

Total soluble solids (TSS) (as Brix) were significantly reduced in RDI berries (Table 6). There was a close

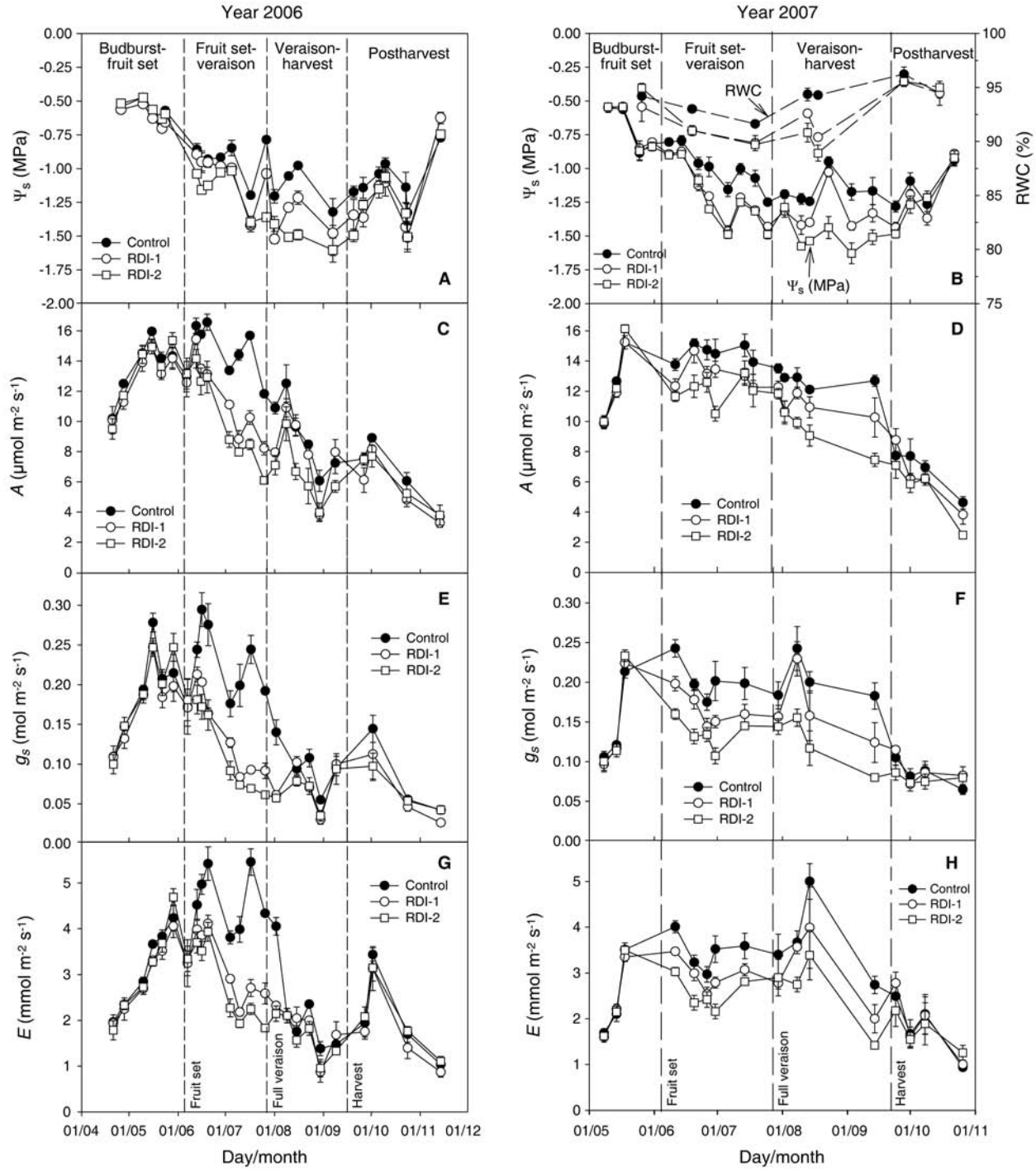

Figure 2 Seasonal patterns during 2006 and 2007 of (**A, B**) midday stem water potential (Ψ_s) and leaf relative water content (RWC); (**C, D**) leaf photosynthesis rate (*A*); (**E, F**) stomatal conductance to water vapor (g_s); and (**G, H**) transpiration rate (*E*). Vertical bars indicate the standard error of the mean. Each point is the average of eight measurements for Ψ_s and RWC and 12 measurements for gas exchange parameters.

relationship between A and Ψ_s postveraison and Brix at harvest (Figure 7A, B). In both years, malic acid decreased significantly in the RDI treatments (Table 6). Other compositional measures, such as juice pH, were not clearly affected by irrigation treatments. In 2006, no significant differences among treatments in total and extractable anthocyanin concentration were observed. However, in 2007 there was a significant increase in total and extractable anthocyanins for RDI-1 and RDI-2 at harvest. In both years, polyphenol concentrations were significantly higher in the RDI treatments, and in 2007 concentrations were significantly higher in RDI-1 than in RDI-2. Extractable anthocyanin and polyphenol concentration increased with greater water stress postveraison until reaching a maximum at the Ψ_s threshold, -1.35 to -1.4 MPa (Figure 7C, D). Below these values, anthocyanin and polyphenol concentration

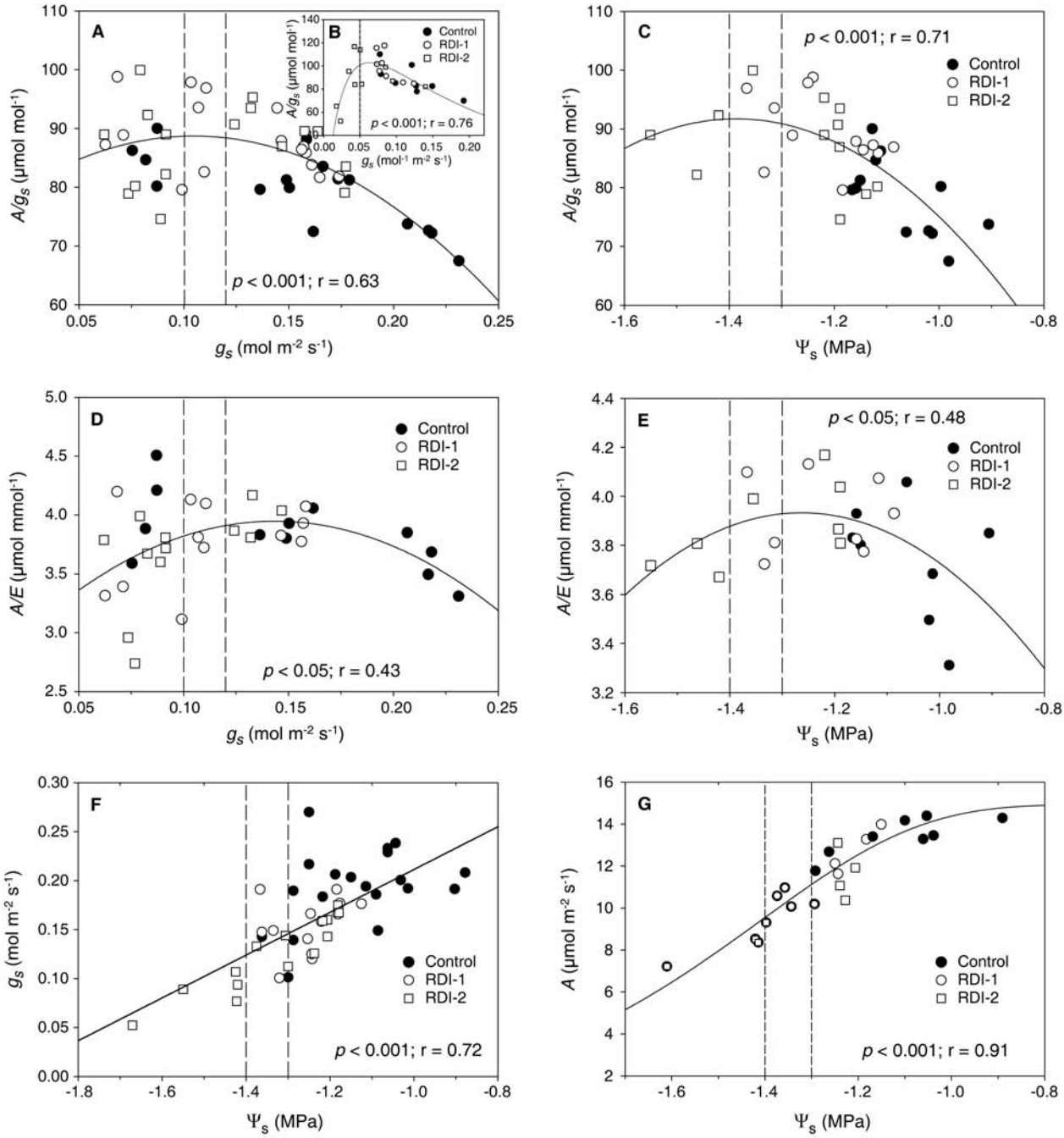

Figure 3 (**A**) Stomatal conductance (g_s) and intrinsic water use efficiency (A/g_s) measured midmorning (9:00–10:30): A/g_s = 74.3 + 277.9g_s - 1327.7g_s^2. (**B**, inset) g_s and A/g_s measured midday (12:30–14:00) during postveraison (most water-stressed period): $A/g_s = 102.9e^{(-0.5\ln(gs/0.0634)/1.1535)^2}$. (**C**) Midday stem water potential (Ψ_s) and midmorning A/g_s: -122.3 - 308.6 Ψ_s - 111.3 Ψ_s^2. (**D**) g_s and instantaneous water use efficiency (A/E) measured midmorning: A/E = 2.6 + 19.2g_s - 66.9g_s^2. (**E**) Midday Ψ_s and A/E measured midmorning: A/E = -0.79 - 7.5 Ψ_s - 2.96 Ψ_s^2. (**F**) Relationship between midday Ψ_s and midmorning g_s (g_s = 0.43 + 0.22 Ψ_s) and (**G**) midmorning leaf photosynthesis rate (A) during postveraison period ($A = 16.3/1 + e^{(-(\Psi s - (-1.5))/0.25)}$). Horizontal and vertical dashed lines indicate different threshold values. Maximum values ranges of different indicators were calculated using the equations. Measurements were taken pre- and postveraison, June–Sept 2007.

did not increase substantially or even decreased. Moreover, the maximum polyphenol concentration reached just after veraison (data not shown) linearly correlated with the Ψ_s maintained during preveraison (Figure 7E). However, the decreased polyphenol observed postveraison, mainly during August (data not shown), also closely correlated with the degree of water stress postveraison (Figure 7F).

Discussion

Root-leaf function and physiological threshold levels under RDI. The linear relationships between $K_{root\text{-}stem}$ and K_{plant}, and Ψ_s suggest that plant hydraulic conductance and root water uptake were progressively reduced during water stress (Figure 1, Table 3). Previous experiments reported that water stress decreases whole-plant hydraulic conductivity in grapevines (Lovisolo and Schubert 1998), in proportion to increased soil water deficit (Schultz 2003). The greater reduction in $K_{root\text{-}stem}$ and K_{plant} observed in RDI-2 postveraison indicates less water supply to shoots, explaining in part the lower vine water status compared to RDI-1 (Figure 2A and 2B). It is well known that water deficits reduce the capacity of roots to take up water (Steudle 2000).

The significant decrease in leaf nitrogen observed in RDI-1 and RDI-2 during pre- and postveraison, but not during the well-irrigated period (April-May) (data not shown),

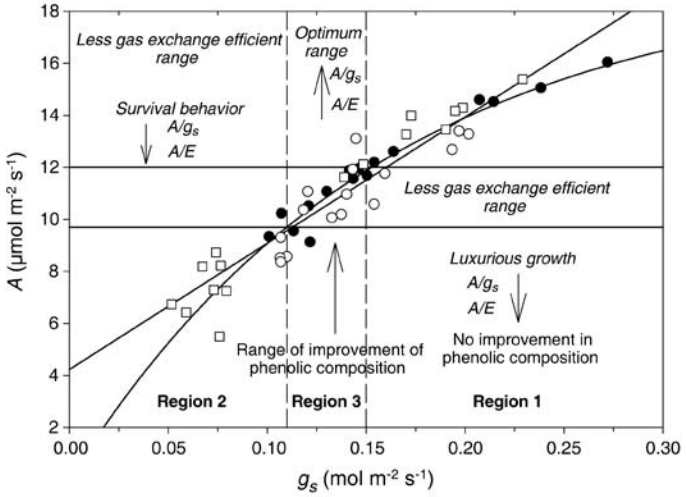

Figure 4 Relationship between photosynthesis (*A*) and stomatal conductance (g_s) (linear, $A = 4.23 + 48.5^{gs}$, $r = 0.94$, $p < 0.001$; exponential, $A = 19.4(1 - e^{(-6.29gs)})$, $r = 0.95$, $p < 0.001$). Each value is the mean per plot calculated before and after veraison (early June–end Sept) for each treatment in the two years.

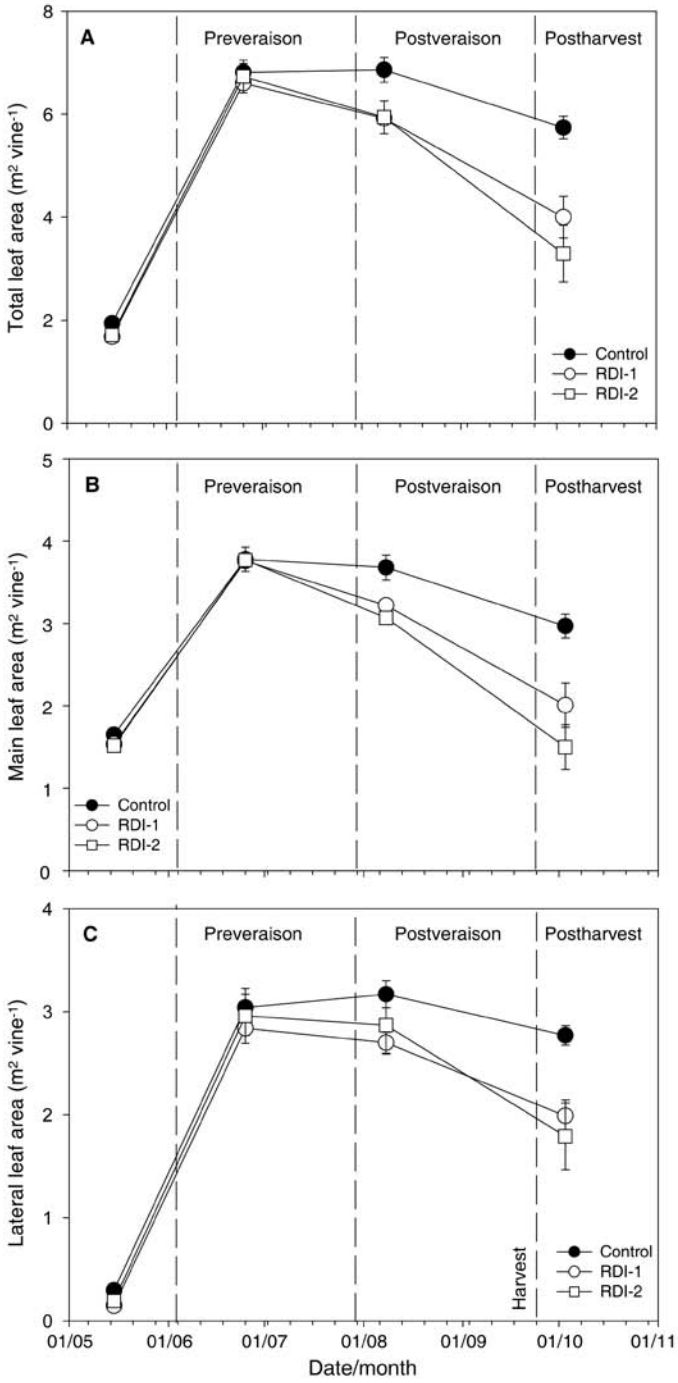

Figure 5 Development of total leaf area (**A**), main leaf area (**B**), and lateral leaf area (**C**) per vine at four representative times in 2007. Vertical bars represent the standard error of the mean. Each point is the average of 16 measurements.

Table 4 Mean leaf chlorophyll and photosynthetic nitrogen use efficiency (NUE_{ph}) for different treatments, pre- and postveraison, 2006 to 2007.

	Preveraison (July)			Postveraison (Aug–mid-Sept)		
Treatment	Chlorophyll (mg dm^{-2})	Leaf N (%)	NUE_{ph} (µmol CO_2 m^{-2} s^{-1}/mmol N kg$^{-1}_{DM}$)	Chlorophyll (mg dm^{-2})	Leaf N (%)	NUE_{ph} (µmol CO_2 m^{-2} s^{-1}/mmol N kg$^{-1}_{DM}$)
Control	2.18	2.59a	7.74a	2.23a	2.06a	8.63a
RDI-1	2.16	2.41b	7.42b	2.23a	1.90b	7.40b
RDI-2	2.12	2.37b	6.86c	2.08b	1.87b	6.24c
ANOVA[a]	ns	**	**	**	**	***

[a]**, ***, and ns indicate significance at $p < 0.01$, 0.001, and not significant, respectively. Separation by Duncan's multiple range test at 95% confidence level.

could indicate reduced N uptake in deficit-irrigated treatments as a consequence of soil water deficit. Nutrient uptake becomes increasingly difficult for drought-stressed grapevines (Conradie 2005), especially if the water deficit is sufficient to slow root growth (Keller 2005). The significantly lower leaf N, leaf chlorophyll, and NUE_{ph}, mainly during postveraison in RDI-2 (Table 4), indicated quantitative losses in the photosynthetic apparatus and/or damage to the biochemical photosynthetic machinery, decreasing photosynthetic capacity. Moreover, long-term photosynthetic capacity was also reduced in RDI-2 compared to RDI-1. This was confirmed by the lower leaf N (data not

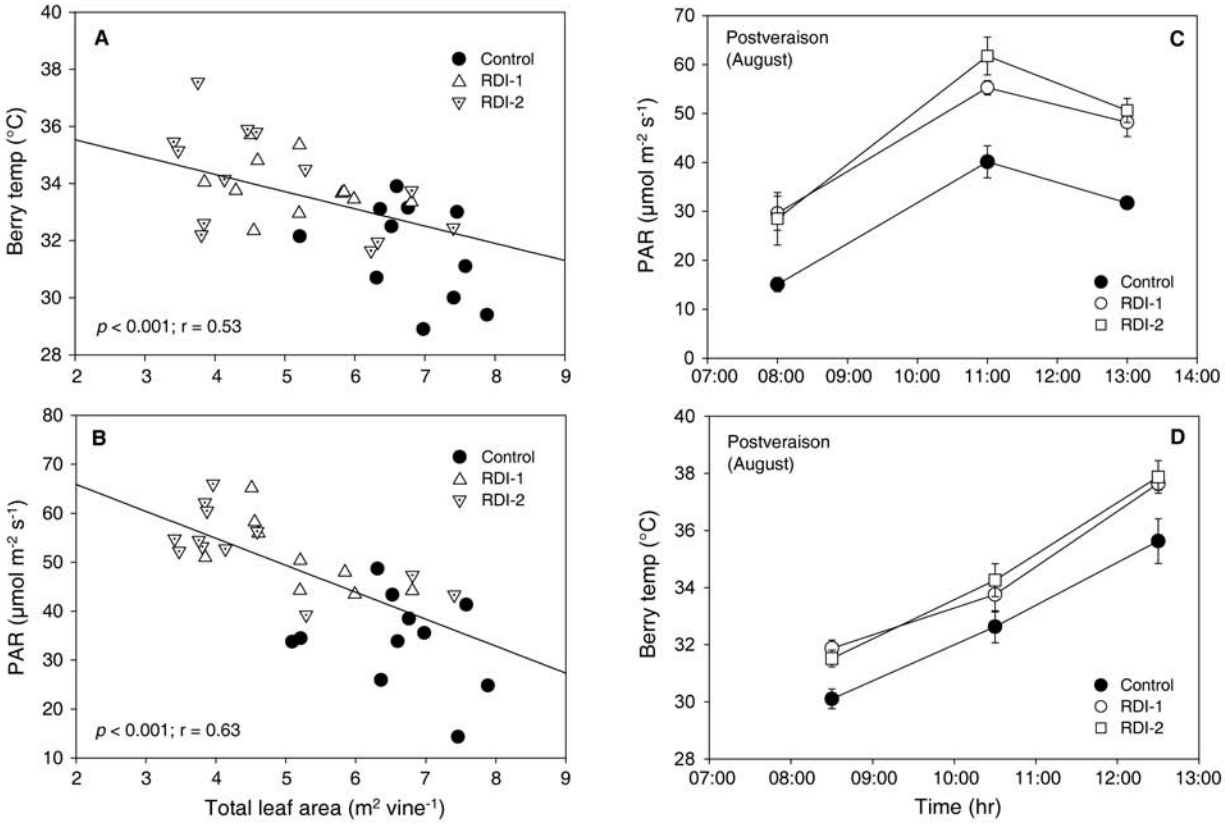

Figure 6 (**A**) Mean total leaf area and mean berry temperature in non-sun-exposed bunches, July–Sept 2007. Each point is a single measurement per vine. (**B**) Mean total leaf area and photosynthetically active radiation (PAR) in fruiting positions, July–Sept 2007. Each point is a single measurement per vine. (**C**) Evolution (morning) of PAR in fruiting positions for each treatment, postveraison Aug 2006–2007. Each point is the average of 12 measurements/treatment. (**D**) Evolution (morning) of berry temperature in non-sun-exposed bunches (inside canopy) for each treatment, preveraison July 2006–2007. Each point is the average of 20 measurements/treatment. In C and D, vertical bars represent standard error of the mean.

Table 5 Yield response for each treatment, 2006 and 2007.

	Yield (kg vine^{-1})			Cluster number/ vine		Cluster wt (g)		Berry fresh wt (g)			Berry number/ cluster	
Treatment	**2006**	**2007**	**Mean**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**Mean**	**2006**	**2007**
Control	5.93a	5.77a	5.85a	22a	21a	271a	275a	1.93a	1.70a	1.81a	140	162
RDI-1	4.49b	3.53b	4.01b	21ab	19b	219b	186b	1.52b	1.22b	1.37b	145	154
RDI-2	3.67c	2.83c	3.25c	20b	19b	187c	148c	1.24c	1.04c	1.14c	152	143
ANOVA[a]	***	***	***	*	***	***	***	**	***	***	ns	ns

[a]*, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. Separation by Duncan's multiple range test at 95% confidence level.

Table 6 Berry composition parameters at harvest, 2006 and 2007.

	TSS (Brix)		pH		Tartaric acid (g L^{-1})		Malic acid (g L^{-1})		Color intensity		Extractable anthocyanins (mg L^{-1})		Total anthocyanins (mg L^{-1})		Extractable polyphenols	
Treatment	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**
Control	21.9a	23.3a	4.05a	4.05	5.64	5.34a	1.32a	1.38a	5.33a	4.98a	378.4	452.7a	623.0	771.4a	40.6a	40.5a
RDI-1	20.7b	22.4b	4.09b	4.07	5.56	5.74b	1.34a	1.10b	7.04b	5.91b	408.7	520.7b	596.8	823.4b	44.0b	52.2b
RDI-2	19.7c	21.3c	4.04a	4.08	5.48	5.99c	1.19b	1.05c	7.34b	5.75b	416.5	492.7ab	603.4	859.5b	46.4c	46.7c
ANOVA[a]	***	***	*	ns	ns	***	**	**	***	***	ns	*	ns	**	***	***

[a]*, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. Separation by Duncan's multiple range test at 95% confidence level.

shown) and significantly lower midmorning *A* maintained postharvest (mid-September–October) in 2007 in RDI-2 vines (Figure 2C and 2D), possibly indicating earlier leaf senescence (Schreiner et al. 2006).

Analysis of seasonal leaf gas exchange and the relationships among g_s, *A*, and Ψ_s indicated that stomatal closure in response to water stress occurred before detectable changes in Ψ_s or RWC (Figure 2, Figure 3F, G). This suggests that g_s is a more precise and sensitive indicator of water stress than Ψ_s and RWC, or even θ_v, when mild or moderate soil water deficit was applied under RDI (Cifre et al. 2005). Moreover, g_s was more sensitive to mild to moderate water stress (Ψ_s > -1.2 MPa) than *A*. As a consequence, in pre- and postveraison periods there was a significant increase in gas exchange efficiency, A/g_s, and A/E in RDI vines when Ψ_s was between -1.3 and 1.4 MPa and when g_s was between

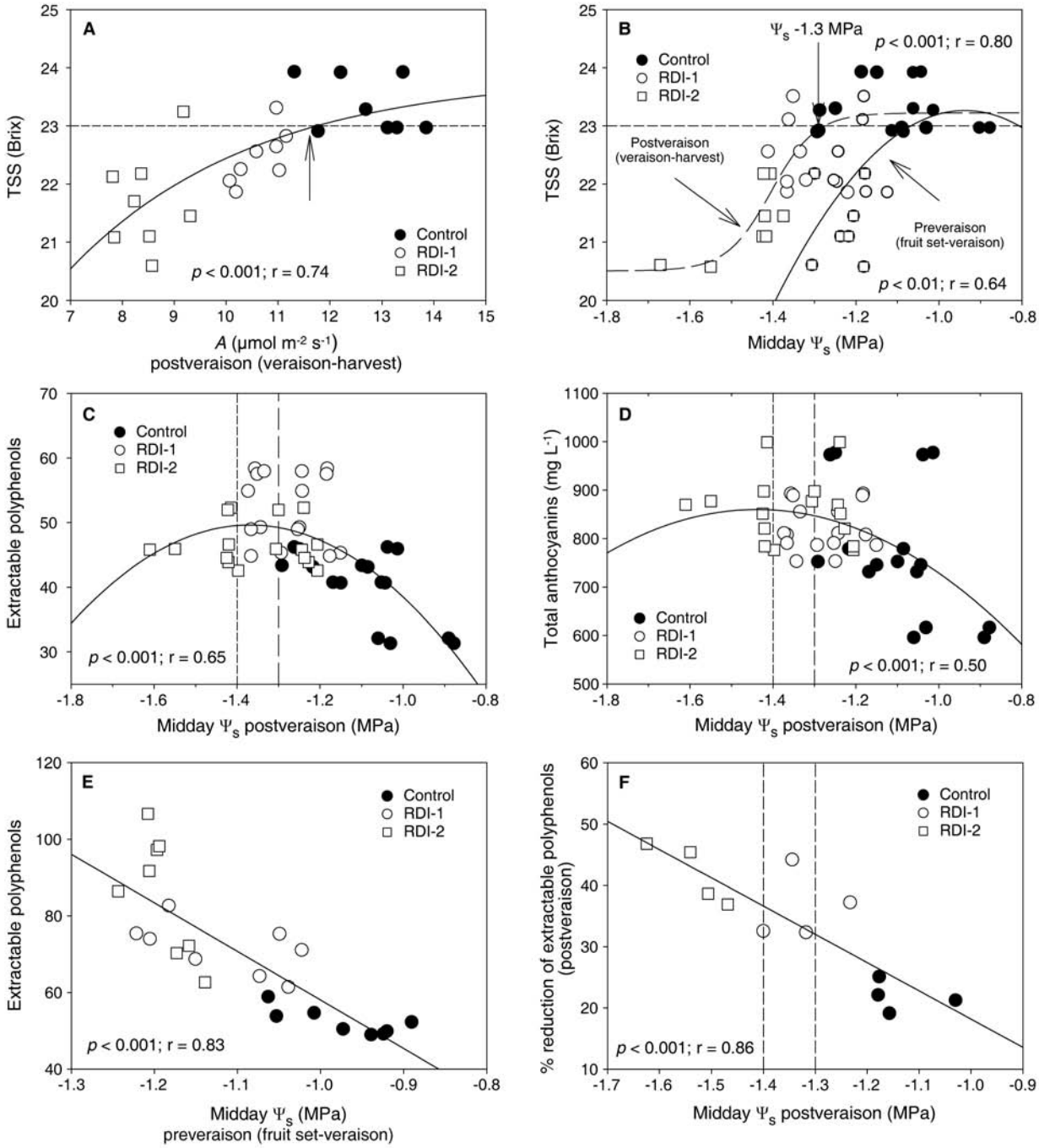

Figure 7 (**A**) Midmorning leaf photosynthesis rate (*A*) at postveraison and total soluble solids (TSS) at harvest: TSS = 23.9(1 - $e^{(-0.28A)}$). (**B**) Midday stem water potential (Ψ_s) and TSS at pre- and postveraison: postveraison TSS = 20.5 + 2.7/(1 + $e^{(-(\Psi s-(-1.4))/0.0535)}$). (**C**) Midday Ψ_s and extractable polyphenols: $poly_{ext}$ = -103.9 - 224.2 Ψ_s - 81.9 Ψ_s^2. (**D**) Ψ_s and total anthocyanins: $anth_{tot}$ = -555.4 - 1968.6 Ψ_s - 684.5 Ψ_s^2. (**E**) Preveraison midday Ψ_s and extractable polyphenols just after veraison: $poly_{ext}$ = -68.9 - 126.9 Ψ_s. (**F**) Postveraison (Aug–early Sept) midday Ψ_s and % reduction of extractable polyphenols: $\%reducpoly_{ext}$ = -27.9 - 46.1 Ψ_s. In A–E, each point is the average of one plot for 2006 and 2007. Thresholds and maximum values calculated using the equations. Vertical and horizontal dashed lines represent different threshold values proposed.

0.11 and 0.14 mol m^{-2} s^{-1}, as has been shown previously (Costa et al. 2007).

The analysis of the changes in the slope of the A/g_s relationship (plotting all data of the two years) showed three different water use efficiency regions (Figure 4). In region 1, g_s > 0.15 mol m^{-2} s^{-1} did not exhibit a substantial improvement in photosynthesis compared to g_s while E (water consumption), and gas exchange efficiency started to decrease ($\Delta A/\Delta g_s exp < \Delta A/\Delta g_s linear$ or $\Delta A/\Delta g_s exp - \Delta A/\Delta g_s linear < 0$). This stomatal behavior corresponds with phase 1 of the photosynthesis response shared by different grapevine cultivars (Flexas et al. 2002). At this stage, stomatal closure is probably the only limitation on photosynthesis, with a progressive increase in A/g_s (Cifre et al. 2005). In our study, mean g_s > 0.15 mol m^{-2} s^{-1} at midmorning was maintained only in the control treatment during preveraison in 2006 and the entire pre- and postveraison in 2007 (Figure 2E, F), indicating less plant water use efficiency than the RDI treatments.

In region 2, to maintain g_s less than or equal to 0.11 mol m^{-2} s^{-1}, vines did not exhibit improved gas exchange efficiency (Figure 4). Under these conditions, A decreased proportionally more than E and g_s ($\Delta A/\Delta g_s exp > \Delta A/\Delta g_s linear$ or $\Delta A/\Delta g_s exp - \Delta A/\Delta g_s linear > 0.10$). Moreover, the relationships between g_s and A/g_s at midday during mid-August–early September, the period of greatest water stress, also showed a drastic decrease in A/g_s at g_s < 0.05 mol m^{-2} s^{-1} and Ψ_s < -1.7 MPa) (Figure 3B, F), which coincided also with an increase in intercellular CO_2 concentration (Ci) (data not shown). This stomatal response coincided with phase 3 of severe water stress: g_s < 0.05 mol m^{-2} s^{-1} as reported (Flexas et al. 2002, 2004).

In region 3, according to our data, g_s between 0.12 and 0.15 mol m^{-2} s^{-1} ($\Delta A/\Delta g_s exp > \Delta A/\Delta g_s linear$ or $0 < \Delta A/\Delta g_s exp - \Delta A/\Delta g_s linear < 0.10$) would increase leaf water use efficiency (up to 86–89 µmol mol^{-1}) without having a detrimental effect on A, maintaining rates between 10 and 12 µmol m^{-2} s^{-1} during pre- and postveraison (Figure 4). This stomatal behavior (with a slight shift) corresponds to phase 2 of moderate water stress, as proposed (Flexas et al. 2002). These results suggest that intermediate g_s values could be used as physiological indicators to improve vine water use efficiency in Monastrell grapevines under semiarid conditions (Table 7).

Vine physiology–berry quality relationships. Microclimate in the cluster zone was clearly altered by RDI, as it reduced canopy leaf area (Figure 6A, B). Intense leaf abscission during postveraison severe water stress increased fruit sunlight exposure in RDI treatments, as reported in Shiraz grapevines (Ginestar et al. 1998a). The degree of defoliation was closely correlated with the severity of water stress postveraison (data not shown), as previously observed (Ginestar et al. 1998a, Wample and Smithyman 2002). In warm, semiarid growing regions such as Jumilla, increased cluster exposure to direct solar radiation as a consequence of reduced leaf area increases cluster temperature and explains the increased malic acid degradation under RDI (Ginestar et al. 1998b). Greater cluster exposure under RDI increased color intensity and anthocyanin and polyphenol content above control vines, confirming previous studies (Santos et al. 2005, 2007). These results are in accordance with the increased anthocyanins and other phenolic compounds found in berries of vines subjected to water deficit and different edaphoclimatic conditions (Ginestar et al. 1998b, Kennedy et al. 2002, Castellarin et al. 2007a, 2007b). The differences in phenolic composition between RDI-1 and RDI-2 (mainly in 2007) cannot be explained by changes in the cluster environment, as significant differences in canopy exposure between RDI-1 and RDI-2 were not found.

The positive correlation between A during postveraison and TSS at harvest (Figure 7A) indicates that the low total soluble solids under RDI can be explained by low carbohydrate accumulation in the berries postveraison. This likely resulted from lower A compared to the control and reduced leaf area as a consequence of intense leaf abscission. Reduced seasonal and daily CO_2 assimilation and more intense leaf abscission during ripening in RDI-2 as a consequence of more severe water stress would explain the lower sugar accumulation in theses berries compared to RDI-1 (Table 6). Sugar concentration seems to be related to functional leaf area available for fruit growth (Mabrouk and Sinoquet 1998) and decreased A and sugar export from the leaves under severe water stress reducd berry sugar accumulation (Keller 2005, Conde et al. 2007).

The relationships between TSS and A and between TSS and midday Ψ_s (Figure 7A, B) suggest that by maintaining Ψ_s at -1.3 MPa and A at ~10.5 to 12 µmol m^{-2} s^{-1} during postveraison (Table 4), Brix could be 22.5 to 23 at harvest,

Table 7 Threshold ranges for optimum and dangerous water stress proposed for pre- and postveraison in RDI Monastrell grapevines to avoid severe damage in root and leaf function and to improve berry quality under these edaphoclimatic conditions. Mean values of different physiological indicators maintained pre- (early June–end July) and postveraison (Aug–mid-Sept) in 2007.

Indicator[a]	Optimum threshold Pre- and postveraison	RDI-1 Before veraison	RDI-1 After veraison	RDI-2 Before veraison	RDI-2 After veraison	Dangerous threshold Pre- and postveraison
g_s	0.12–0.15	0.15–0.16	0.15	0.14–0.13	0.11	$g_s \leq 0.11$
Ψ_s	-1.25–1.4	-1.21	-1.32	-1.23	-1.45	$\Psi_s \leq -1.4$
A	10–12	12.7–12.0	10.6	11.6–11.2	8.7	$A < 10$
A/g_s	85–90	83.3–90	81	90–93	85	-
A/E	3.87–4.0	3.97	3.5	4.17	3.5	-

[a]Units: midmorning g_s: mol m^{-2} s^{-1}; midday Ψ_s: MPa; midmorning A: µmol m^{-2} s^{-1}; A/g_s: µmol mol^{-1}; A/E: µmol $mmol^{-1}$.

which is appropriate for red wine production in the Jumilla region. Moreover, the close linear relationship between midday Ψ_s and leaf area reduction postveraison (data not shown) suggests that these threshold values should be maintained (though not exceeded) to reduce the intense leaf abscission during ripening.

Reduced berry size in RDI-2 (15–18% lower than in RDI-1) and consequent lower yields (Table 5) did not improve phenolic composition compared to RDI-1 in either year (Table 6). The lower leaf area development and crop load in 2006 (1.02 and 1.12 m^2/kg in RDI-1 and RDI-2, respectively) compared to 2007 (1.55 and 1.51 m^2/kg in RDI-1 and RDI-2, respectively) and the lower gas exchange rates postveraison (exceeding the proposed threshold limits) in both RDI treatments in 2006 (Figure 2) could explain the similar anthocyanin concentrations in 2006 (Table 6). In 2007, the RDI-1 treatment had more extractable polyphenols and anthocyanins than RDI-2. The combination of intense leaf abscission and substantially decreased photosynthetic capacity in RDI-2 vines likely resulted in decreased accumulation of all metabolites, primary (sugars and organic acids) and secondary (flavonoids), as suggested elsewhere (Downey et al. 2006, Joscelyne et al. 2007). The relationships between several physiological measures and polyphenolic concentrations allowed for calculation of physiological thresholds for improved phenolic composition of Monastrell berries at harvest. Moderate preveraison water stress as reached in 2007 in RDI-1 (mean Ψ_s = -1.21 MPa, g_s = 0.15 to 0.16 mol m^{-2} s^{-1}) and even in RDI-2 (Ψ_s = -1.23 MPa, g_s = 0.13 to 0.14 mol m^{-2} s^{-1}) were within the range proposed to increase A/g_s without substantially affecting soil water status, root function, and leaf function (gas exchange and chlorophyll content) (Table 7). Total polyphenol increased just after veraison as a consequence of moderate preveraison water stress, compared to well-irrigated vines (Figure 7E), without negatively impacting other berry quality measures. Similarly, a preveraison leaf water potential of -1.3 MPa is associated with beneficial yield and berry quality attributes (Shellie 2006). Midday Ψ_s of approximately -1.3 to -1.4 MPa postveraison (but never $\Psi_s \leq$ -1.4 MPa) and midmorning g_s between 0.14 and 0.12 mol m^{-2} s^{-1} maintained high leaf photosynthetic capacity and increased anthocyanin and polyphenol concentrations at harvest (Figure 3F, Figure 7C, D). These g_s values were also associated with mean levels of midmorning A >10 µmol m^{-2} s^{-1} (11.4 to 10 µmol m^{-2} s^{-1}) (Figure 4) and with maximum A/E approximately 4 µmol $mmol^{-1}$ and A/g_s approximately 90 µmol mol^{-1} (stage 2 of moderate water stress proposed by Flexas et al. 2002). Maintaining A, g_s, and Ψ_s within the optimum range and above the threshold values presented (Table 7) would also avoid drastic decreases in extractable polyphenols and sugar accumulation postveraison.

Conclusions

The moderate water stress applied in RDI-1 maintained adequate soil water to sustain vine water status and gas exchange within the range of optimum threshold values. This increased fruit sugar content and phenolic composition at harvest compared with well-watered or control vines. However, RDI-2 suffered higher stress, mainly postveraison, when the proposed threshold values were clearly exceeded. The severe water stress substantially reduced soil water, root function (reducing root-plant hydraulic conductivity), and leaf gas exchange (decreasing A, leaf N and chlorophyll, and NUE_{ph}). Moreover, excessive water stress postveraison produced excessive leaf abscission, reducing leaf area development and yield. Lower A and greater leaf abscission significantly decreased sugar, malic acid, polyphenols, and anthocyanins in RDI-2 berries compared to RDI-1.

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