Deficit Irrigation Strategies in *Vitis vinifera* L. cv. Cannonau under Mediterranean Climate. Part I - Physiological Responses, Growth, Yield and Berry Composition

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Submitted for publication: October 2012
Accepted for publication: June 2013

Key words: Water stress, photosynthesis, water productivity, source:sink balance, anthocyanin profile

The effect of deficit irrigation strategies on physiological performance, growth, source:sink balance, water productivity and berry composition of field-grown grapevines of *Vitis vinifera* L. cv. Cannonau (syn. Grenache)/1103P were investigated in Sardinia, Italy, in 2009. In two of the treatments, both sides of the root system received 50% and 25% crop evapotranspiration (ETc), referred to as strategies DI50 and DI25, respectively. In the third treatment, which included partial root-zone drying (strategy PRD), ETc was set at 50%. All three treatments were compared to a full irrigation control (strategy FI), thus 100% ETc. No severe water stress was imposed from berry development onwards. Strategies DI25 and PRD induced higher stomatal closure and leaf water-use efficiency. A slightly higher net assimilation rate was recorded in FI before véraison. During ripening, leaf area decreased in DI50 and DI25, but lateral shoots continued to grow in FI and PRD. Yield and pruning weight were higher in FI, but in all the treatments the vines were source:sink balanced and supported ripening. Irrigation water productivity was higher in DI25, and no significant differences in yield or water productivity were observed between PRD and DI50 irrigated with a similar volume of water. Full irrigation produced berries with a significantly higher fresh and dry weight, lower °Brix and higher malic acid at harvest, while PRD berries weighed less and had less titratable acidity, lower phenol content and a higher pH. Total anthocyanin contents were consistently lower in DI25 and PRD, with highest values measured in DI50. The treatments showed different anthocyanin profiles, with a higher concentration of acylated anthocyanin in DI25 and PRD.

INTRODUCTION

Water stress is one of the most important factors limiting crop growth and yield worldwide, especially in Mediterranean areas (Escalona *et al.*, 1999; Flexas *et al.*, 2002; Fereres & Soriano, 2007). Despite this, the positive and negative impact of irrigation on wine grapes is still much debated. This is probably due to a lack in understanding the interactions between grapevine physiology, yield and berry quality (Bravdo *et al.*, 2004; Bindon *et al.*, 2008b; Palliotti *et al.*, 2009; Schultz & Stoll, 2010).

Vine water stress is known to reduce vegetative growth, berry weight and yield, while enhanced accumulation of anthocyanins and tannins in berry skin is often reported (Hardie & Considine, 1976; Matthews & Anderson, 1988; Roby *et al.*, 2004; Castellarin *et al.*, 2007). This has spurred interest in allowing mild water stress to occur in the vineyards by reducing soil moisture availability using deficit irrigation techniques (Dry & Loveys, 1998; Chaves & Oliveira, 2004; Bowen *et al.*, 2011). These techniques involve watering at levels less than that required for maximum consumption by the vine at key stages in the growth season, reducing watering for definite periods of time (Matthews & Anderson, 1988; Dry *et al.*, 2000; Williams *et al.*, 2010), or even withholding watering as prescribed by the regulated deficit irrigation strategy, RDI (Chalmers *et al.*, 1981; Battilani, 2000; Poni *et al.* 2007).

Under Mediterranean climate conditions, a particular type of deficit irrigation technique has been studied, referred to as partial root-zone drying, PRD (Dry & Loveys, 1999; Dry *et al.*, 2000; Stoll *et al.*, 2000). According to this strategy, water is applied alternatively to half of the root system to enable part of the root system to dehydrate and reduce stomatal conductance via chemical signals such as ABA (Zhang & Davies, 1990; Davies *et al.*, 1994). This allows for control of vegetative vigour and of plant transpiration, without the severe water stress periods as in RDI (McCarthy

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Aknowledgements: The authors would like to acknowledge the financial contribution of the Convisar - Vino e Sardegna Consortium (project SQFVS APQ P6), the Sella & Mosca Winery, and the laboratory staff – Filippo Virdis and Giovanni Ligios

et al., 2002; Santos et al., 2003; Chaves et al., 2007). Several studies have shown that ABA root signalling, stemming from PRD, is at the origin of stomatal closure and reduction in vegetative growth (Zhang & Davies, 1990; Dry & Loveys, 1999; Bravdo, 2005). Despite this, application of the PRD strategy remains controversial, as studies have indicated that improvements in yield per unit of applied water may be due to lower rates of water supplied and not from the PRD strategy itself (Bravdo et al., 2004; Sadras, 2009; Myburgh, 2011b).

The desired effect of water deficit is to obtain smaller berries with more skin and seeds and thus higher concentration of phenolics and other extractable compounds that contribute to wine quality (Matthews et al., 1990; Kennedy et al., 2002; Roby et al., 2004). Such effects are potentially promoted by PRD, since the reduction of lateral shoot growth and leaf area improves light penetration into the fruit zone (Du Toit et al., 2003; Santos et al., 2007). However, light is not necessarily a limiting factor in the production of phenols through anthocyanin synthesis if light conditions within the canopy are above a given threshold (Keller & Hrazdina, 1998). An additional benefit of imposing mild water stress might be the enhancement of abscisic acid concentration in the fruit, which appears to increase the production of phenolics, including skin anthocyanins (Owen et al., 2009; Gambetta et al., 2010; Santesteban et al., 2011; Zarrouk et al., 2012). Furthermore, the study by Bindon et al. (2008c) provided evidence that, under water deficit, the light microclimate might influence the accumulation of berry anthocyanin derivatives in a different manner. These authors observed that malvidin derivatives in grape berries were more resistant to possible changes in vine physiology or in bunch microclimate induced by PRD treatment compared to other anthocyanin types. Yet, different berry phenolic profiles can be caused by altered light microclimate conditions in the fruit zone, hence different PAR and UV radiation intensities (Schultz, 2000; Spayd et al., 2002; Tarara et al. 2008; Cohen et al., 2012). The extent to which water deficits alter fruit and wine composition through direct changes in fruit metabolism, versus indirect effects associated with fruit growth or microclimate, is difficult to establish (Roby & Matthews, 2004).

The main goal of this work was to investigate the physiological performance and source:sink balance of cv. Cannonau under different deficit irrigation strategies. Cannonau is the main red grape variety cultivated in Sardinia, and is genetically similar to Grenache (Cipriani et al., 2010; Meneghetti et al., 2011; Nieddu et al., 2011), a variety cultivated in France, Spain and northen Italy out of a total of 200 thousand hectares (Fregoni, 2010). The Grenache group, of Mediterranean origin, is one of the most cultivated red grape varieties in the world and adapts remarkably well to drought. This is described as near-isohydric behaviour (Schultz, 2003), characterised by a strong stomatal control over transpiration to maintain a nearly constant leaf water potential during water stress. This physiology is probably linked to the higher hydraulic conductance of the stem and petioles in near-isohydric cultivars (Schultz, 2003; Soar et al., 2006). However, better stomatal control has not been recorded in other experiments (Chouzouri & Schultz, 2005). Hydraulic conductance may be affected by other features that influence cell structures and osmotic adjustments, like the rootstock (Poni et al., 2007; Chaves et al., 2010; Alsina et al., 2011). Stomatal sensitivity to vapour pressure deficit varies with the duration of water deficit, temperature and atmospheric demand (Maroco et al., 1997), but in isohydric varieties stomata seem to be less responsive to VPD than in anisohydric species (Rogiers et al., 2009). In our experiment, particular attention was also given to soil moisture dynamics, vegetative growth and berry composition to evaluate the effects of different irrigation strategies and to optimise the use of such management techniques under Mediterranean climate conditions.

MATERIALS AND METHODS

Experimental site and irrigation scheduling

The study was carried out during 2009 in a twelve-year-old commercial vineyard located in the Nurra wine region, near Alghero (40° 38' N; 8° 18' E), North Sardinia, Italy. *Vitis vinifera* L. cv. Cannonau grapes, grafted onto 1103P rootstock, were planted in northeast-southwest oriented rows. Vines were spaced 2.5 m between rows and 1.0 m along the row, and trained to an unilateral cordon 1.2 m in height, head trained and mechanically hedge-spur pruned.

The vineyard was located in a landscape of alluvial deposits and aeolian sandstones of the Pleistocene (Aru et al., 1990). The soil, classified as Halpic Nitosol (FAO, 1988), is sandy clay loam and consists of a 30 cm thick A horizon on a 30 cm Bt-C horizon, moderately permeable, alkaline and desaturated. Soil physical and moisture properties are reported in Table 1.

Irrigation was scheduled according to the water balance method, following the methodology proposed by Allen et al. (1998). Full irrigation (FI), deficit irrigation (DI) and partial root-zone drying (PRD) strategies were set, based on weekly estimations of crop evapotranspiration (ETc). Four treatments were imposed: FI, DI50 and DI25 (supplying 100%, 50% and 25% of ETc respectively, simultaneously to

TABLE 1

Physical and moisture properties at 2 soil layers (CRAS, 2005).

Slope (%)	Soil depth (cm)	Sand ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)	pH	OM	C/N	BD	CEC	FC	WP	SAW
0	0-30	340	213	447	8.3	24	20.0	1.70	14.21	24.1	13.9	10.9
	30-60	332	261	407	8.3	22	18.6	1.65	16.70	22.6	12.2	10.4

OM = organic matter ($g\ kg^{-1}$), C/N = carbon:nitrogen ratio, BD = bulk density ($g\ cm^{-3}$), CEC = cation exchange capacity (Meq 100 g^{-1}), FC = field capacity (% pF 2.5), WP = wilting point (% pF 4.2), SAW = soil available water (% vol/vol) both sides of the root system), and PRD (supplying 50% of ETc to one side of the root system, allowing the other side to dry, and alternating the watered side every 15 days).

Meteorological data from the closest weather station (Alghero Fertilia, 40° 37' 52" N; 8° 17' 19" E), gathered by the "Aeronautica Militare" (2009), were used to compute reference evapotranspiration (ETo). The different phenological stages were taken into account for estimating ETc, and a crop coefficient of 0.8 was set up for each treatment (Williams & Ayars, 2005). The water supply was then calculated considering the effective rainfall of each given period and 55% soil available water (SAW). Finally, to determine the watering volume, a 10% loss of efficiency of the irrigation system was used (Brouwer *et al.*, 1989). Irrigation was applied with a subsurface drip system with two lines per row, laterally spaced 40 cm from the plant row and placed at a depth of 40 cm. On each line, drippers were spaced 50 cm apart and the discharge rate for each emitter was 2 $L\ h^{-1}$. The experimental design consisted of three blocks with three contiguous rows per treatment. The experimental plots consisted of 10 plants per replicate, with an interval of 20 plants between replicates. In each replicate, data collection was performed on the central row, while the adjacent rows were considered buffer rows. The trial was installed for the first time in 2005 (Mameli *et al.*, 2012), and we here present the results of the fifth year of the experiment. Irrigation started after fruit set (mid-June) and ended three weeks before harvest (on 20 August). ETo, ETc, number of irrigation applications and total water volumes supplied are reported in Table 2.

Soil water content and plant physiological status

A portable capacitance probe (Profile Probe PR2, Delta-T Devices Ltd., UK) was used to monitor soil moisture content during the growing season and to analyse the patterns of moisture distribution in the soil profile at different depths (10, 20, 30, 40 cm). To provide representative and periodical measurements of soil moisture content close to the root system for each treatment, two access tubes were carefully placed between plants, without disturbing or compacting the nearby profile. The outputs recorded by the sensors are expressed in electric potential (mV) units and were converted to percentage of water content, θ (vol/vol), following the calibration curve provided by the manufacturer.

To evaluate plant water status, stem water potential (Ψs) in each replicate was monitored on two well-exposed adult leaves from the middle part of the main shoot, on days with a clear sky, using a Pump Up pressure chamber (PMS Instruments Co., USA). The intact leaves were covered with aluminium foil-coated plastic bags for at least 30 minutes prior to the measurement to allow for the equilibration of leaf water potential with Ψs. These measurements were performed weekly from fruit set until harvest, at solar noon.

Leaf gas exchange was also monitored at midday on two adult, well-exposed mid-shoot leaves per replicate, on the same days of the Ψs measurements, using a portable photosynthesis system Ciras-2 (PP systems, UK). For each sampling date, direct measurements of net photosynthetic rate (Pn), stomatal conductance (g_s) and transpiration (E) were taken, and intrinsic water use efficiency (WUEi) was then calculated using the ratio between net photosynthesis and stomatal conductance. Data were collected at a reference CO_2 concentration similar to ambient (370 $\mu mol\ mol^{-1}$); during the measurements, leaf chamber temperature (T_{air}) ranged from 28.1°C to 33.0°C, and relative air humidity varied between 62% and 77%.

Vegetative growth, yield and pruning weight

Empirical models for leaf area estimation (Lopes & Pinto, 2005) were developed and validated for Cannonau and then used to evaluate vegetative growth during the season. Four representative shoots were selected for each treatment per block to estimate main and lateral leaf area per shoot. At harvest, yield components were computed by weighing the clusters and recording the number of clusters and yield per vine. During winter, pruning weight, shoot number and average shoot weight were recorded. Finally, irrigation water productivity (IWP) was estimated through the ratio between yield and amount of supplied water. ELA/LA (Smart *et al.* 1990), LA/yield (Kliewer & Dokoozlian, 2005) and the Ravaz indices were calculated to further evaluate the plants' source:sink balance.

Ripening controls and berry composition analysis

From véraison until harvest, representative cluster fractions from different positions within the canopy were sampled every two weeks for the purpose of monitoring ripening. Berry weight, total soluble solids (°Brix), pH, titratable

TABLE 2

Reference evapotranspiration (ETo), crop evapotranspiration (ETc) (ARPAS, 2010) and irrigation supply (number and volume) according to the different irrigation strategies in 2009.

	Phenological stage				
Evapotranspiration estimates (mm)	Flowering to fruit set	Fruit set to véraison	Véraison to ripening		
ETo	124.0	230.0	240.0		
ETc	49.6	135.2	111.2	Irrigation supply	
Treatment		% ETc		No. of irrigations	Total volume (mm)
FI	100	100	100	12	250
DI50	100	50	50	12	144
PRD50	100	50	50	12	132
DI25	100	25	25	12	80

acidity (g tartaric acid L^{-1}), total anthocyanin (mg malvin L^{-1}) and total phenol (mg catechin L^{-1}) contents were analysed (OIV, 1990). The anthocyanin profile was evaluated using the method proposed by Di Stefano & Cravero (1991).

Statistical analysis

Statistical data analysis was performed using SPSS software v. 16 for analysis of variance (ANOVA) and the least significant difference (LSD) test for mean separation. Significant differences are represented in the tables by letters, and in the figures the symbols *, **, ***, and ns represent significant differences for P-values < 0.05, 0.01 and 0.001, and non-significant difference respectively.

RESULTS AND DISCUSSION

Seasonal hydrologic balance

Monthly averages for precipitation (PP), potential evapotranspiration (ETP) and soil water available reservoir (SWAR) during the growing season in 2009, as well as long-term (30-year) averages for PP and ETP are reported in Table 3. From October 2008 until April 2009, precipitation values were the highest of the last four decades, both in terms of quantity and number of rainy days (ARPAS, 2010). The heavy precipitation registered in December and January promoted progressive soil water saturation and a hydrologic surplus higher than during the previous year. In February and March this pattern was inverted due to the absence of rain and the increase of ETo, leading to a deficit in soil water balance until the mid-April precipitation (ARPAS, 2010). The rain inputs decreased during May and June, with no more than 20 mm being registered, and from the beginning of July until the end of August precipitation practically ceased (Table 3). In September, precipitation reached 116 mm in two days (80 mm and 36 mm on 2009-09-14 and 2009-09-15 respectively).

The high ETo values during May gave rise to an elevated deficit of the hydro-meteorological balance, reaching -180 mm in several locations. In July and August, ETo was high, but remained within the mean climatologic values. When computing ETo for the period of May to September, high values were observed (approximately 594 mm, Table 2). Nevertheless, the precipitation in April and September favoured soil water availability.

Soil moisture content and depletion patterns

The mean soil water content (θ, vol/vol) during the irrigation season and the mean soil moisture along the profile are presented in Figs 1A and 1B, respectively. Differences between treatments were high and constant throughout the season. The mean volumetric soil moisture content was maintained close to field capacity in FI, and varied between 21% and 17% until mid-September. In the deficit-irrigated treatments, average θ remained below field capacity until the September rain events. Thereafter, the volumetric soil water reached saturation in all treatments (nearly 28% vol/vol) (Fig. 1A). DI50 and PRD constantly presented twice the average water volume of the DI25 treatment, averaging 14% against the 7% of DI25. Most plant-available water is lost to drainage within a few days of irrigating, and most of that held can readily be taken up by crops (Hillel, 2004). However, depletion of soil moisture to below 10% (vol/vol), as recorded in DI25, substantially increases matric tensions and the potential for extreme stress (Kramer & Boyer, 1995).

DI25 consistently had less water in all soil layers monitored, and the average content remained slightly inferior to the average soil available water (~ 10% vol/vol, Table 1) until mid-September (Fig. 1B). On two of the measuring dates (2009-07-10 and 2009-08-24), soil moisture depleted to 0% in DI25 and to nearly 10% in DI50 at the water application depth (40 cm), while the soil retained a volumetric water content of 15% in PRD. The intense precipitation of mid-September led to soil saturation at the surface. The final measurement on 2009-09-21 showed a high soil water content (nearly 30% vol/vol at 10 cm depth) in almost all the soil profiles (25% vol/vol in DI50 and PRD, and 12% vol/vol in DI25, at 40 cm depth).

The higher water supply in FI, DI50 and PRD effectively increased soil moisture at the root zone, particularly in the soil layers from 20 to 40 cm, where θ was maintained close to field capacity in FI, while it varied between 20% and 10% (vol/vol) in DI50 and PRD. Significantly different moisture distribution patterns were observed along the different depths and between treatments (Fig. 1B) throughout the season. The main variations were observed within the 20 to 40 cm zone, close to the zone where the water was applied (40 cm) and where the root extraction was intense. These soil layers might have contributed the most to plant water extraction, as the percentage of water stored varied greatly.

TABLE 3

Hydrologic balance variables during the growth season in 2009 and long-term monthly averages (30-year) in Alghero, Sardinia, Italy (Aeronautica Militare, 2009; ARPAS, 2010).

	Mean PP (mm)		Mean ETP (mm)		SWAR (mm)
	2009	30-year	2009	30-year	2009
Apr	76.5	49.2	84.9	77.5	158.3
May	7.6	27.2	145.6	111.9	56.3
Jun	11.7	17.0	158.6	141.2	8.5
Jul	0.0	5.3	191.4	164.0	0.0
Aug	0.0	24.7	170.8	150.0	0.0
Sep	138.2	38.1	111.6	99.7	1681.6

PP = precipitation (mm); ETP = potential evapotranspiration (mm); SWAR = soil water available reservoir (mm), estimated from meteorological data.

The higher θ variation from 20 to 40 cm was recorded in DI25 (where θ ranged from 0% to 10%). In a vineyard, the pattern of soil water extraction is normally similar to the root distribution pattern, and the volume of watered soil under drip irrigation depends upon soil characteristics, initial moisture content, volume of water supplied, dripper flow and location (Van Zyl & Weber, 1981; Rodrigues, 2011). Fig. 1B shows clearly different water distribution patterns in PRD compared to DI50, with a sharper decrease of soil water volume from 20 to 40 cm in PRD and a higher

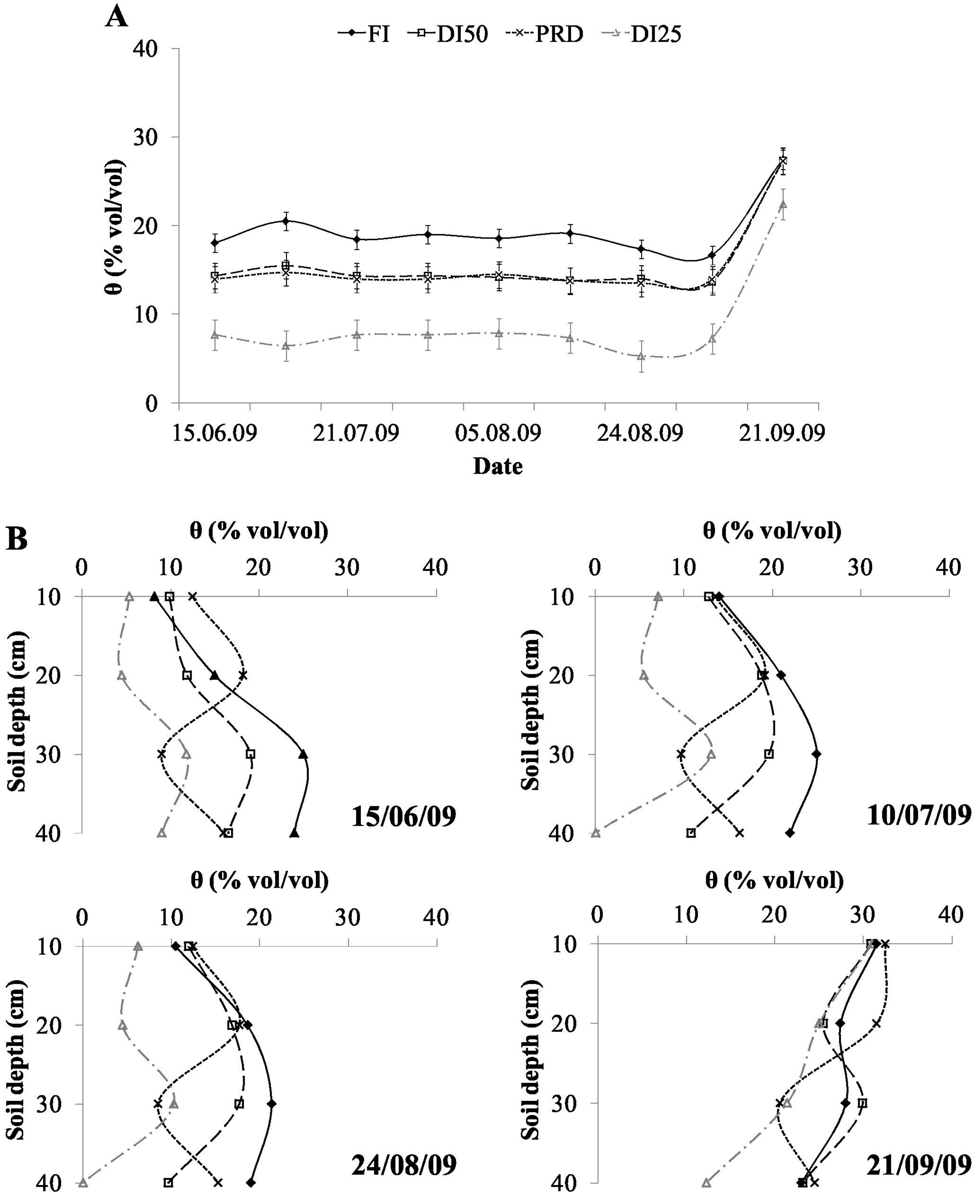

FIGURE 1

Effect of irrigation treatments on average soil moisture content (**A**); and moisture distribution at 10, 20, 30 and 40 cm of soil depth on different dates of the irrigation season (2009-06-15, 2009-07-21, 2009-08-24 and 2009-09-21) (**B**). Mean values ± S.E. (n = 9).

content in DI50. These contrasting patterns reflect both the concentration of the supplied water volume in a smaller zone and the longer watering and drying cycles of the PRD treatment, which amplified the differences between watered and dry zones (Dry & Loveys, 1999). Important capillary movement occurred in all treatments, but to a greater extent in FI, DI50 and PRD. The water ascension may also have been facilitated by the absence of slopes, which would easily promote the driving out of water and percolation from the desired wet spot in the root zone.

Plant water relations and photosynthetic performance

Stem water potential

Fig. 2 shows the patterns of stem water potential of each irrigation treatment during the 2009 season. In FI and DI50, the Ψs values remained within mild water stress thresholds (Myburgh, 2011a), ranging from -1.1 MPa to -0.6 MPa in FI and from -0.8 MPa to -0.7 MPa in DI50. The lowest values were recorded at véraison, on 2009-07-31, probably due to the high temperatures and high evapotranspiration rates recorded on this particular day (Aeronautica Militare, 2009). A decreasing trend of Ψs was observed both in PRD and DI25. In these treatments, Ψs reached moderate water stress values (-1.2 MPa in PRD and -1.1 MPa in DI25) at mid-ripening, and recovered rapidly after the heavy rain in September. Similarly, Mercenaro and Nieddu (2006), studying the effects of different irrigation strategies on the physiological status of Cannonau, observed a nearly constant pattern of Ψs in the irrigated control (above -0.6 MPa) and a decreasing trend in the PRD and DI treatments, which dropped to as far as -1.2 MPa by the end of the season. In the present experiment, mild to moderate water stress conditions (Van Leeuwen *et al.*, 2009; Myburgh, 2011a) were maintained during the entire irrigation season, but at harvest time the Ψs completely recovered in all the treatments, to nearly -0.4 MPa, after the heavy rain events in mid-September. The rapid recovery of stem water potential values at harvest indicates that the imposed water deficit did not affect the vines' water status recovery (Schultz & Matthews, 1988; Choné *et al.*, 2001).

Leaf gas exchange

Net photosynthetic rate reached significantly higher values in the FI plants at véraison (approximately 14 µmol CO$_2$ m^{-2} s^{-1}), averaging 12 µmol CO$_2$ m^{-2} s^{-1} in the DI50, PRD and DI25 plants (Fig. 3A). During ripening, Pn decreased progressively to similar values in every treatment (down to 7 µmol m^{-2} s^{-1} in FI and DI25 and to 6 µmol m^{-2} s^{-1} in DI50 and PRD at harvest time). In DI25, the decreasing trend in Pn was smaller (it reached a 40% lower rate at harvest than at véraison, against the 50% decrease of the other treatments) (Fig. 3A). g_s and E varied little and in low values across the summer (from 50 to 100 mmol m^{-2} s^{-1} and 2.6 to 5.3 mmol m^{-2} s^{-1} respectively). Both PRD and DI25 presented lower values than DI50 and FI and, in the latter treatment, g_s and E were higher than in DI50 only at véraison. At mid-ripening and at harvest, g_s and E values were still significantly lower in the DI25 and PRD plants (Fig. 3B and 3C). High intrinsic water-use efficiency was observed in all treatments, but PRD and DI25 had higher values at véraison and during ripening, averaging 202 and 190 µmol mol^{-1} respectively at mid-ripening (2009-08-24) against the 143 and 140 µmol mol^{-1} of FI and DI50 (Fig. 3D). At harvest, the differences between FI, DI50 and PRD were not statistically significant, but WUEi values were still higher in DI25 than in the other treatments, with only a 15% reduction from the values at véraison against the nearly 30% decay observed in the other treatments. As expected, a strong stomatal control over transpiration was observed in all the treatments, due to the isohydric behaviour of Cannonau (Fig. 3B and C). The PRD strategy induced higher stomatal closure than that observed

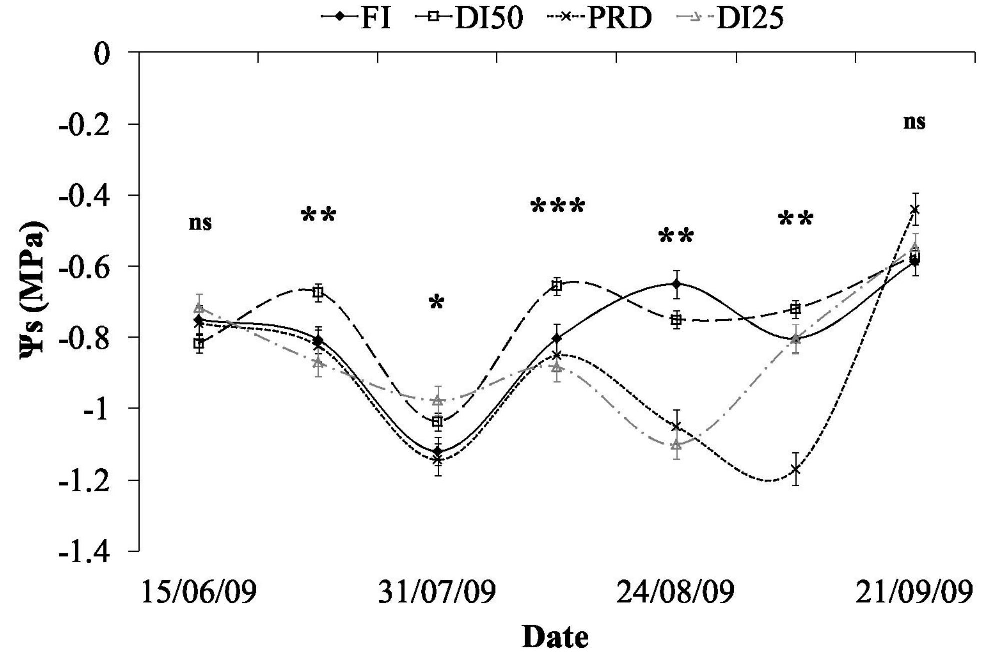

FIGURE 2

Effect of irrigation treatments on stem water potential (Ψs) during the irrigation season. Mean values ± SE and ANOVA (n = 9; *, ** and *** represent significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$; ns = not significant).

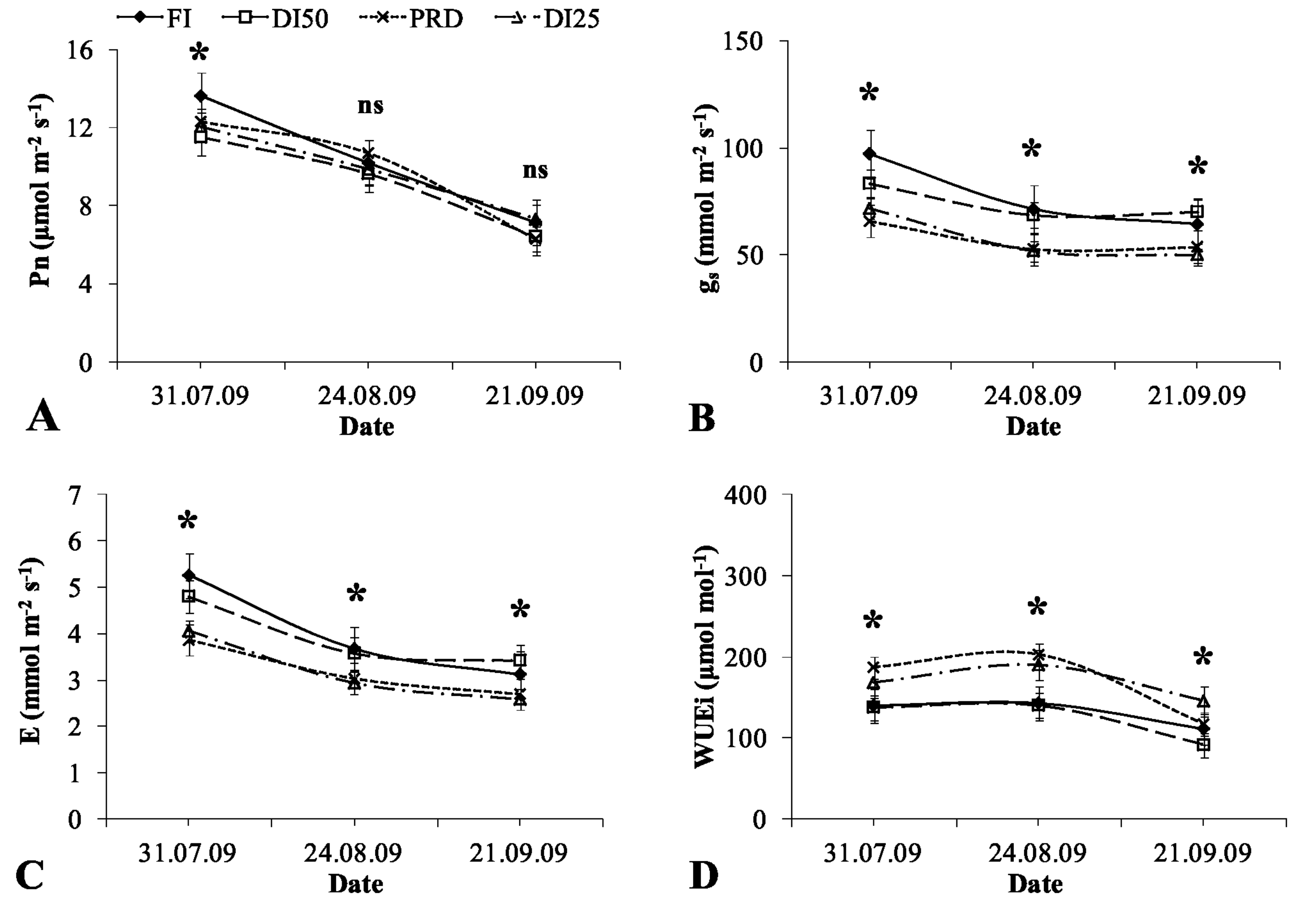

FIGURE 3

Effect of irrigation treatments on leaf gas exchanges measured at solar noon during the 2009 irrigation season. (A) Pn = net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), (B) gs = stomatal conductance (mmol H_2O m^{-2} s^{-1}), (C) E = transpiration rate (mmol H_2O m^{-2} s^{-1}), (D) WUEi = intrinsic water-use efficiency (µmol CO_2 mol^{-1} H_2O). Mean values ± SE and ANOVA (n = 9, * = $P < 0.05$, ns = not significant).

in the DI plants irrigated with the same water volume: this led to higher intrinsic water-use efficiency values in PRD, which varied close to those observed in the DI25 plants. Finally, no significant differences between treatments were observed for leaf temperature (T_{leaf}) during gas exchange measurements. Furthermore, the leaf-to-air vapour pressure deficit ($VPD_{leaf-air}$) at midday was low, averaging 1.13 kPa on the hottest measuring date (2009-07-31), and as much as 1.60 and 1.00 kPa on 2009-08-24 and 2009-09-21 respectively. The maximum T_{leaf} values were recorded on 2009-07-31 (nearly 33°C), but the relative humidity on this day also was very high (about 77%), which might have allowed for slightly higher g_s.

Overall, high atmospheric humidity, elevated temperatures and low VPD, together with the near-isohydric behaviour, could explain the fact that stomatal conductance varied little and within low values across the summer, even in the FI plants, without major differences being observed between treatments regarding net assimilation rates. These results are in accordance with those obtained by Rogiers *et al.* (2009), who observed that, over a VPD range of 1.5 to 3.5 kPa, cv. Grenache showed lower sensitivity to VPD. The stomata were almost closed across all VPDs, and g_s remained unresponsive to VPD by comparison with the anisohydric variety Semillon, with just a slight decrease with increasing VPD.

Vegetative growth, yield and water productivity

Leaf area development

As far as leaf area development is concerned, the total leaf area of the Cannonau plants reached a maximum of 4.10 m^2 in the FI plants, 3.36 m^2 in PRD, and 3.00 m^2 in DI50 and DI25 at cluster closure stage, on 2009-07-13 (Table 4). At that time, the shoots were composed mostly of main leaves (60% to 80% of the total leaf area), and no significant differences were observed between treatments regarding lateral and main leaf area per plant. At harvest, total leaf area had already decreased in the DI50 and DI25 plants (~ 0.35 and 0.63 m^2 per plant respectively), and no statistical differences were observed between these two treatments. Conversely, in the FI plants, main and lateral leaf area values were similar to those recorded before véraison. Santos *et al.* (2007) observed an increase in lateral leaf area per shoot at véraison in cv. Moscatel due to irrigation, with full-irrigated plants presenting significantly higher total leaf area per vine compared to the non-irrigated and PRD (supplying 50% ETc) treatments. Furthermore, in those studies, the total leaf area of the DI 50% ETc plant was not significantly different from the other treatments, but in many instances vegetative growth was reduced in PRD50. Similar results were also observed by Chaves *et al.* (2007) in cv. Castelão when the vines were supplied with the same irrigation treatments reported in Santos *et al.* (2007). Conversely, in our study, the PRD plants presented significantly higher leaf area at harvest (~ 4.0 m^2), mainly due to an increase in lateral leaf area growth during ripening. Since the PRD strategy would be expected to produce higher control of growth due to an imposed partial root-drying cycle (Dry & Loveys, 1999), leaf area growth data on PRD reinforced the hypothesis of deeper water root extraction promoting vegetative growth (Van Zyl & Weber, 1981; Sadras, 2009; Williams *et al.*, 2010). As a consequence, a high leaf area was produced from fruit set until harvest, particularly as far as secondary shoot growth is concerned. Over the years, the concentration of the supplied water volume on a single side of the root system per irrigation cycle, together with the doubled re-watering intervals on each vine side, have probably stimulated root development in deeper soil layers in the PRD plants compared to DI50.

Despite the hot weather conditions registered in the summer of 2009 (ARPAS, 2010), the watering interventions promoted canopy development in PRD before and after véraison, and although main leaf area senescence had already started by the time the last measurement was taken, lateral shoots were still growing in PRD, as much as in the FI plants. Main leaf area abscission (estimated as the difference in leaf area between measuring dates) was significantly more intense in the DI25 plants – about 24% of the leaf area being measured pre-véraison.

Vine growth and source:sink balance

Several authors have reported a reduction in shoot growth resulting from PRD strategies in comparison to DI (Dry & Loveys, 1998; Chaves *et al.*, 2007; Santos *et al.* 2007; Bindon *et al.* 2008b). In our study, canopy exposed leaf area per vine (ELA) was higher in FI, PRD and DI25 plants, but the proportion of total leaf area that represented canopy surface (ELA/LA) did not differ significantly between treatments (Table 5). DI25 plants presented a slightly thinner canopy, but the ELA/LA ratio values were not statistically different from those of the other two treatments.

As far as yield components and pruning weight are concerned, no significant differences were found between the PRD treatments and DI50. FI and PRD plants presented a significantly higher yield than DI25, mainly due to an increased number of clusters per vine. FI plants had a higher pruning mass than PRD and DI25, but similar to that of DI50 plants. The fact that yield and pruning weight in DI25 did not differ from DI50 suggests that the amount of water applied was enough to reach the optimum yield level and similar vigour under mild water stress conditions. A higher Ravaz index was computed for both PRD and DI25, but the values were not statistically different from those obtained for DI50. In addition, using features of optimum crop loads (Smart *et al.*, 1990; Kliewer & Dokoozlian, 2005), we can conclude that both full and deficit irrigated vines were within the limits of an optimal source:sink balance. FI and PRD plants were closer to the higher limit of an optimal leaf area-to-yield per vine ratio (Kliewer & Dokoozlian, 2005: 0.8 to 1.2 m^2 leaf area kg^{-1} fruit), due to the higher total leaf area (Table 5).

Irrigation water productivity

In our experiment, irrigation water productivity (IWP) was significantly higher in DI25 than in the DI50, PRD and FI treatments (DI25 having nearly two times higher IWP than

TABLE 4

Effect of irrigation treatments on leaf area development pre-véraison (2009-07-13) and prior to harvest (2009-09-21). Mean values (n = 9) and ANOVA for P < 0.05; ns = not significant.

	Pre-veraison				*P*	Pre-harvest				*P*
	FI	DI50	PRD	DI25		FI	DI50	PRD	DI25	
Primary LA (m^2 vine^{-1})	2.42	2.36	2.06	2.20	ns	2.14^{a}	2.11^{a}	2.24^{a}	1.67^{b}	< 0.05
Lateral LA (m^2 vine^{-1})	1.68	0.69	0.94	1.17	ns	1.89	0.59	1.77	1.06	ns
Total LA (m^2 vine^{-1})	4.10	3.05	3.00	3.36	ns	4.02^{a}	2.70^{b}	4.01^{a}	2.73^{b}	< 0.05

Primary leaf area (LA), lateral leaf area and total leaf area per vine.

TABLE 5

Effect of irrigation treatments on vine growth, source:sink balance and irrigation water productivity in 2009. Mean values (n = 9) and ANOVA for P < 0.05; ns = not significant.

	FI	DI50	PRD	DI25	*P*
ELA (m^2 vine^{-1})	0.86^{a}	0.62^{b}	0.91^{a}	0.89^{a}	< 0.05
ELA / Total LA	0.22	0.23	0.24	0.38	ns
Yield (kg vine^{-1})	4.00^{a}	3.10^{b}	3.62^{ab}	3.19^{b}	< 0.05
No. clusters vine^{-1}	19.8^{a}	13^{b}	16^{ab}	15^{ab}	< 0.05
Cluster weight (kg)	0.22	0.23	0.23	0.22	ns
Pruning weight (kg vine^{-1})	0.42^{a}	0.30^{ab}	0.25^{b}	0.26^{b}	< 0.05
Total LA/yield	1.00^{a}	0.94^{ab}	1.10^{a}	0.84^{b}	< 0.05
Ravaz Index	9.87^{b}	10.4^{ab}	15.0^{a}	12.3^{a}	< 0.05
IWP (g berry L^{-1})	3.4^{c}	8.6^{b}	11.0^{b}	16.0^{a}	< 0.05

ELA = exposed leaf area; ELA/LA = exposed leaf area (ELA) to total leaf area (LA) ratio; IWP = irrigation water productivity.

DI50 and PRD, and five times higher than FI). There were no significant differences between DI50 and PRD (re-watered with the same amounts), since these two treatments achieved a similar yield, while a significantly low IWP was obtained in the FI plants (Table 5). Previous studies have shown that PRD improves IWP when compared to fully irrigated plants (Souza *et al.*, 2005; Santos *et al.*, 2007), but comparable water productivity could be achieved by applying similar irrigation volumes using a DI strategy (Sadras, 2009). The DI25 strategy further improved irrigation water productivity, and the fact that the yield of DI25 plants did not differ from that of DI50 and PRD suggests that the amount of water applied was enough to reach the optimum yield level under mild water stress conditions.

Berry ripening and composition

Berry composition

The irrigation treatments caused significant differences in both berry fresh weight and composition (Table 6). PRD berries had the lowest fresh weight during ripening, and the other three treatments showed similar berry fresh and dry weight before harvest. In the last two weeks of ripening, the differences between PRD and the two DI became significant; at harvest time, PRD and DI25 had significantly lower berry fresh and dry weight than the FI and DI50 plants. The differences are probably related to the higher crop load registered in the PRD plants (Table 5), while the lower berry weight in DI25 could in fact be ascribed to the moderate water stress conditions. In their studies, Bowen *et al.* (2011) observed no effects on berry mass caused by deficit irrigation. However, these authors reported a decreasing trend in berry mass during ripening, both in Cabernet Sauvignon and Merlot.

Regarding berry composition, FI berries had a significantly lower total soluble solids content at harvest, while the three deficit irrigated treatments had similar °Brix. PRD and DI treatments showed similar berry composition, except for tartrate contents, which were significantly higher in the PRD berries at the beginning of ripening but became the lowest from mid-ripening until harvest, leading to a significantly higher pH in the must (Table 6). The treatments did not present significantly different titratable acidity, but FI berries had significantly higher malic acid than DI25 at harvest. Regarding total phenols, the PRD berries presented lower contents from the first ripening controls, although no statistical differences were measured at harvest. On

TABLE 6

Effect of irrigation treatments on berry fresh and dry weight and main composition during ripening and at harvest. Mean values (n = 9) and ANOVA for $P < 0.05$; ns = not significant.

Date	Treatment	BFW	BDW	°Brix	pH	TA	Ma	Ta	TAnt	TPh
	FI	1.52^{a}	0.19	12.67	2.64^{a}	16.24^{b}	5.88	4.01^{c}	50.7	1071.^{1a}
28.07.09	DI50	1.34^{ab}	0.18	12.43	2.49^{b}	21.02^{a}	6.17	4.37^{b}	63.2	898.3^{a}
	PRD	1.12^{b}	0.15	12.60	2.51^{b}	18.16^{ab}	6.02	4.96^{a}	64.8	640.9^{c}
	DI25	1.29^{ab}	0.18	11.53	2.52^{b}	18.38^{ab}	5.67	4.28^{bc}	42.7	686.3^{bc}
	Sig.	*	ns	ns	*	*	ns	*	ns	*
	FI	1.88	0.37	17.93	3.04^{a}	8.00	3.82	4.30^{ab}	170.3	1074.2^{a}
10.08.09	DI50	1.77	0.35	17.07	2.86^{b}	8.01	3.53	4.39^{ab}	162.1	1087.9^{a}
	PRD	1.68	0.34	17.27	2.82^{b}	7.54	3.53	4.65^{a}	163.7	668.3^{b}
	DI25	1.88	0.36	17.87	2.84^{b}	7.78	3.52	4.02^{b}	160.6	1088.5^{a}
	Sig.	ns	ns	ns	*	ns	ns	*	ns	*
	FI	1.85	0.44	20.87	2.96	4.97^{a}	0.87	4.23	202.8	1179.9
24.08.09	DI50	1.97	0.45	20.73	3.00	4.79^{a}	0.90	4.00	278.1	1273.8
	PRD	1.95	0.41	21.60	3.11	4.45^{b}	0.82	3.75	198.0	1183.0
	DI25	2.03	0.44	22.33	3.09	4.98^{a}	0.91	4.13	247.5	1145.7
	Sig.	ns	ns	ns	ns	*	ns	ns	ns	ns
	FI	2.10^{ab}	0.50	23.2	4.34^{a}	3.83	0.59^{a}	4.63^{a}	165.0^{b}	1027.0^{ab}
07.09.09	DI50	2.25^{a}	0.57	24.80	4.19^{ab}	3.98	0.71^{a}	4.09^{b}	289.9^{a}	1286.9^{a}
	PRD	2.06^{b}	0.52	24.27	4.08^{b}	3.76	0.26^{b}	4.76^{a}	225.7^{ab}	754.7^{b}
	DI25	2.25^{a}	0.54	24.07	4.18^{b}	3.94	0.73^{a}	4.17^{b}	208.6^{b}	995.9^{ab}
	Sig.	*	ns	ns	*	ns	*	*	*	*
	FI	2.57^{a}	0.57^{a}	22.93^{b}	4.33^{ab}	3.32	0.87^{a}	4.47^{a}	112.3^{b}	811.3
22.09.09	DI50	2.47^{a}	0.59^{a}	24.47^{a}	4.35^{ab}	3.28	0.66^{ab}	4.22^{a}	197.7^{a}	1171.8
	PRD	2.10^{b}	0.52^{b}	24.53^{a}	4.38^{a}	3.08	0.66^{ab}	3.87^{b}	139.9^{ab}	931.9
	DI25	2.27^{b}	0.53^{b}	24.47^{a}	4.28^{b}	3.39	0.44^{b}	4.13^{a}	136.8^{b}	1180.5
	Sig.	*	*	*	*	ns	*	*	*	ns

BFW = berry fresh weight (g), BDW = berry dry weight, TSS = total soluble solids (°Brix), TA = titratable acidity (g tart. ac. L^{-1}), Ma = malic acid (g L^{-1}), Ta = Tartaric acid (g L^{-1}), TAnt = Total anthocyanins (mg L^{-1}), TPh = total phenols (mg L^{-1}).

the last two sampling dates, fully irrigated together with the less irrigated plants presented significantly lower total anthocyanin contents. The highest values at harvest were measured in DI50 berries, and no significant differences were detected between PRD and DI25. Both Chaves *et al.* (2007) and Romero *et al.* (2010) reported increased extractable anthocyanin and polyphenols contents in deficit-irrigated vines compared to the more irrigated controls, but the differences between treatments were not significant in all the years of the trial, and some authors have reported only slight increases in total anthocyanin content due to PRD (Bindon *et al.*, 2008a).

In conclusion, DI25 and PRD did not significantly improve total berry anthocyanin content. The effects of irrigation strategies depend upon the variety, the climatic conditions during the growing season (Chaves *et al.* 2007) and environmental factors (such as PPFD, temperature or VPD) that influence shoot physiological processes and interact with factors that affect the rhizosphere, determining the final nature and intensity of the varietal response. When higher concentrations of anthocyanins and polyphenols are observed in less irrigated treatments, this can be explained by a better light microclimate in the fruit zone and higher cluster exposure in more open canopies (Santos *et al.*, 2007; Intrigliolo & Castel, 2008). Conversely, excessive sunlight and heat exposure during crucial berry ripening stages might block anthocyanin synthesis and lead to the degradation of that accumulated previously. Hence, the lower anthocyanin contents observed in DI25 in our trial were likely a consequence of the high berry exposure to critical thermal and light microclimate conditions, rather than an effect of the imposed water stress (Fernandes de Oliveira & Nieddu, 2013).

Must anthocyanin profile

The 15 anthocyanin derivatives commonly found in *V. vinifera* were analysed in order to evaluate the effect of irrigation treatments on the accumulation patterns of each anthocyanin derivative and on the overall content of mono-, acetyl- and coumaroyl-glucosides forms (Table 7 and Fig. 4). At the beginning of ripening, no significant differences were detected in monoglucoside and acetyl-glucoside forms. Total levels of malvidin-glucosides are generally reduced under shaded microclimates (Haselgrove *et al.*, 2000; Spayd *et al.*, 2002; Downey *et al.*, 2004). This could explain the lower content of malvidin derivatives in the FI, DI50 and PRD berries in our study (Fernandes de Oliveira & Nieddu, 2013). Furthermore, according to Bindon *et al.* (2008c), changes in malvidin derivatives in grape berries during ripening are more resistant to possible changes in vine physiology or bunch microclimate induced by the PRD treatment than other anthocyanin types. In the present study, total acetyl-glucoside forms were higher in DI25 on the first sampling dates, but the differences tended to be smoother as ripening advanced, with no significant differences being detected between treatments at harvest. Conversely, coumaroyl derivatives were significantly higher in DI25, on almost all sampling dates (Fig. 4). PRD and DI25 presented a significantly higher content of some of the derivatives, although at variable levels throughout ripening (specifically cyanidin mono- and acetyl-glucosides, delphinidin and petunidin-acetyl-glucoside, and malvidin-coumaroyl-glucosides, and less malvidin-glucoside). These results are in accordance with those obtained by Bindon *et al.* (2008c), who found that changes in the anthocyanin profile in PRD-treated berries relative to the DI treatment irrigated with the same amount of water were characterised by an increase in acylated anthocyanins (acetyl and coumaroyl-glucoside forms) compared to malvidin-glucosides. The acylated anthocyanins are known to be more stable than the non-acylated forms (Rodriguez-Sanoa *et al.*, 1999; Tarara *et al.*, 2008). An increased proportion of such forms may help improving berry quality in a red wine grape variety such as Cannonau, of which many accessions often present feeble red colour. Further analysis was carried

TABLE 7

Effect of irrigation treatments on berry skin anthocyanin profile. Anthocyanin forms present in berry homogenates during ripening and at harvest in 2009. Mean values (n = 9) and ANOVA for $P < 0.05$; ns = not significant.

Date	Anthocyanin forms (mg/100 g)	FI	DI50	PRD	DI25	P
28.07.09	3-Monoglucosides	38.3	27.8	38.2	34.6	ns
	3-Acetyl-glucosides	1.94	1.34	1.92	1.92	ns
	3-p-Coumaroyl-glucosides	2.12^{b}	1.59^{b}	4.71^{a}	1.57^{b}	< 0.05
10.08.09	3-Monoglucosides	123.4^{ab}	108.2^{ab}	104.6^{b}	145.5^{a}	< 0.05
	3-Acetyl-glucosides	6.32^{ab}	5.26^{b}	6.21^{ab}	8.23^{a}	< 0.05
	3-p-Coumaroyl-glucosides	16.9^{a}	10.9^{ab}	5.9^{b}	17.4^{a}	< 0.05
24.08.09	3-Monoglucosides	61.6	75.3	69.7	92.4	ns
	3-Acetyl-glucosides	5.82	4.77	5.19	5.85	ns
	3-p-Coumaroyl-glucosides	4.4^{b}	10.8^{a}	10.7^{a}	12.5^{a}	< 0.05
07.09.09	3-Monoglucosides	63.6	84.1	80.0	78.2	ns
	3-Acetyl-glucosides	5.04	5.12	5.9	6.03	ns
	3-p-Coumaroyl-glucosides	11.0^{b}	13.7^{ab}	16.3^{a}	15.3^{a}	< 0.05
22.09.09	3-Monoglucosides	81.3	71.7	68.0	77.7	ns
	3-Acetyl-glucosides	5.74	5.28	5.00	5.87	ns
	3-p-Coumaroyl-glucosides	13.6^{b}	13.8^{b}	11.3^{b}	19.2^{a}	< 0.05

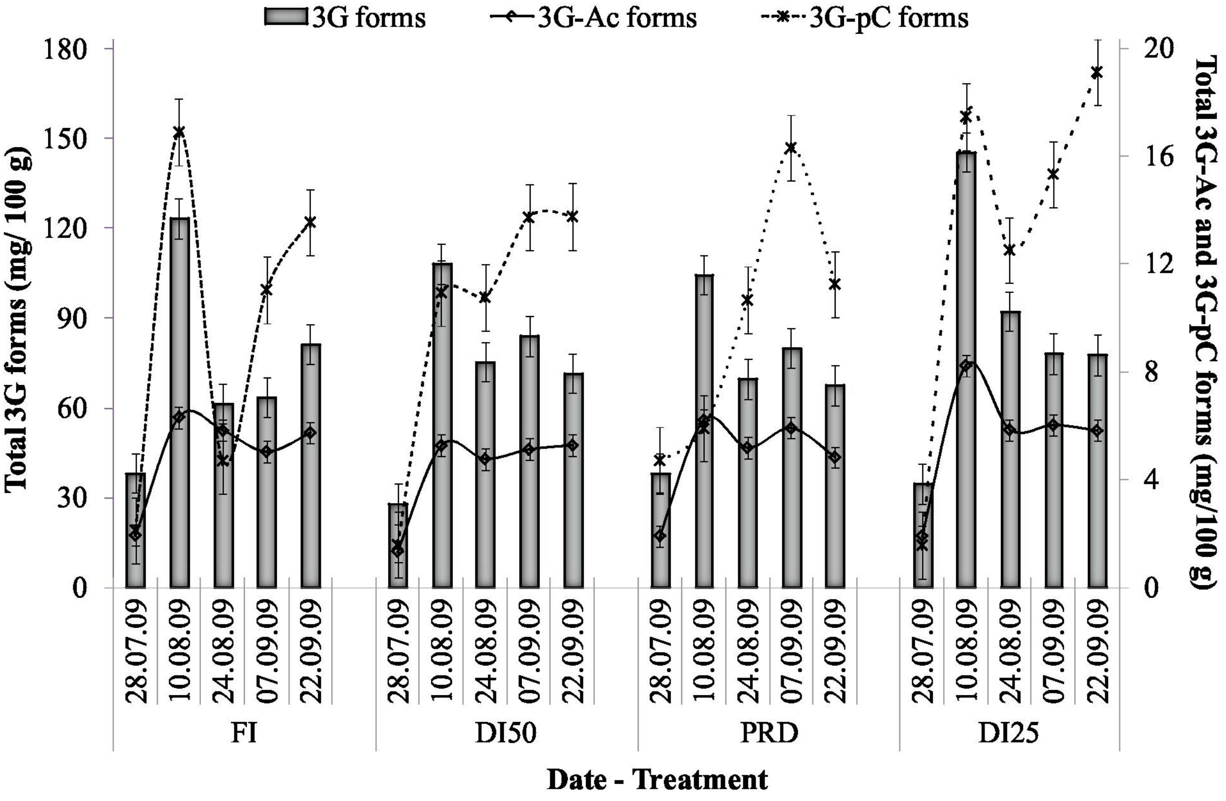

FIGURE 4

Effect of irrigation treatments on berry skin anthocyanin profile. Anthocyanin forms: 3G forms = monoglucosides; 3G-Ac forms = acetyl-glucosides; 3G-pC forms = coumaroyl-glucosides – all in berry homogenates during ripening and at harvest in 2009. Mean values ± S.E. (n = 9).

out (Fernandes de Oliveira & Nieddu, 2013) regarding light and thermal microclimate conditions and anthocyanin accumulation in order to better address and understand this issue.

CONCLUSION

Despite the hot and rainless weather conditions in the 2009 season, the cv. Cannonau subjected to drip subsurface deficit irrigation did not undergo severe water stress. During ripening, stem water potential reached moderate deficit values in PRD and DI25, while FI and DI50 experienced only mild water stress. Stomatal conductance and transpiration varied within low values in every treatment, and PRD induced higher stomatal closure and leaf water-use efficiency compared to the DI treatment irrigated with the same amount of water. Both high temperatures and atmospheric humidity, together with the isohydric behaviour of Cannonau, could explain the slight differences in net assimilation rate and the low stomatal conductance, also recorded in FI plants. In our trial, the increased leaf area of PRD plants during ripening could possibly be related to a higher density of absorbing roots in deeper soil layers, stimulated by fairly profound water infiltration into the subsoil in a PRD strategy applied with an underground drip irrigation system. The absence of differences in yield between DI50 and PRD indicates that high water productivity can be reached with a less complex deficit irrigation system.

The DI25 and PRD treatments proved to be better strategies for the studied terroir, giving good responses from both agronomic and crop quality points of view. The reduced total anthocyanin contents observed in the DI25 and PRD berries could be balanced by a higher proportion of more stable, acylated derivatives. A favourable berry skin colour composition can be achieved in Cannonau with deficit irrigation management, applying only 25% of ETc, without compromising yield or the plant's physiological status during periods of water stress.

However, it is yet to be established whether the different anthocyanin profiles obtained in PRD and DI25 are a direct result of the deficit irrigation strategies, hormonally mediated, or whether they are indirectly caused by a shadier microclimate in the fruit zone. The evaluation of the clusters' light and thermal conditions and the implications of these for berry skin anthocyanin accumulation were investigated as part of the ongoing study. Further consideration will be given to this issue on the completion of this study.

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