Scientia Horticulturae 179 (2014) 239–247

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Optimizing deficit irrigation strategies to manage vine performance and fruit composition of field-grown ‘Sangiovese’ (*Vitis vinifera* L.) grapevines

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ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
15 September 2014
Accepted 16 September 2014
Available online 7 October 2014

Keywords:
Photosynthesis
Leaf water potential
Stem water potential
Soil water content
Grape composition

ABSTRACT

Vineyard irrigation practices in viticultural regions with substantial fluctuations in summer precipitation require the development of efficient water management strategies focused on time of application and irrigation volumes, especially for the widely planted ‘Sangiovese’ grape variety in Central Italy (Marche region). In this research, key to each irrigation strategy was the monitoring of soil water content (SWC) underneath the vine canopy by capacitance probe. The probe was utilized as a tool for scheduling irrigation and for evaluating vine physiological performance under different environmental conditions. Control non-irrigated vines (I_0) were compared to deficit irrigation (DI) treatments based on replacement of 33% (I_1) or 70% (I_2) of crop evapotranspiration of the previous week, applied between pea-size (4–5 mm fruit diameter) and veraison phenological stages. Vines were irrigated when midday leaf water potential (Ψ_l) was near −0.9 MPa, net photosynthesis (Pn) was still high (about 11 μmol CO_2 m^{-2} s^{-1}), and soil water content (SWC) in the first 0.6 m depth beneath the vine canopy had reached the wilting point (WP) threshold. The driest season (2007) resulted in low yield (8 t/ha) and excessive total soluble solids (TSS; 25.7 °Brix) in the grapes at harvest for the I_0 treatment. The limited amount of water supplied to I_1 treatment benefited yield (12 t/ha) and lowered TSS (about 22 °Brix) without reducing anthocyanins and phenols concentration, while saving 25 mm of water as compared to I_2 treatment. Consequently, a strategy providing irrigation with a DI approach was a useful tool to limit excessive alcohol levels in wines produced from berry grown in 2007. However, DI treatments did not influence vine yield and grape composition in the 2008 and 2009 seasons. Those seasons were characterized by moderate and slight water scarcity, respectively. In addition, the I_2 treatment did not improve any of the parameters measured in this experiment. When compared to I_1, midday Ψ_l and Ψ_{stem} were strongly correlated to SWC in the first meter of soil depth below the vine canopy, measured by a capacitance probe. SWC and midday Ψ_l were also positively correlated to leaf photosynthesis. Further, SWC can be easily monitored in the vineyard, and therefore could be potentially used as an effective indicator of grapevine water status and photosynthetic performance.

1. Introduction

Increased variability in rainy events and reduced summer precipitation are the major environmental concerns related to modern viticulture (IPCC, 2007). Worldwide vineyards are commonly located in areas featuring progressive soil water deficits resulting from reduced rainfall and high evaporative demand; this was exacerbated by both increasing in canopy leaf area and warmer air temperature during the growing season (Chaves et al., 2010). Further, the increase of mean annual temperature in Western Europe beginning around 1980, likely amplified the potential

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Abbreviations: DI, deficit irrigation; ET_o, reference evapotranspiration; ET_c, crop evapotranspiration; Kc, coefficient cultural; FWC, field water capacity; GDD, growing degree days; I_0, non-irrigated vines; I_1 and I_2, replacement of 33% and 70% of estimated ET_c of the previous week from berry-set to full veraison, respectively; Ψ_l, leaf water potential; Pn, net photosynthesis; SWC, soil water content; Ψ_{stem}, stem water potential; WP, wilting point; WUE, water use efficiency; TSS, total soluble solids; Ψ_{lpd}, predawn leaf water potential.

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http://dx.doi.org/10.1016/j.scienta.2014.09.032

for summer drought stress (Werner et al., 2000). Consequently, deleterious limitations in vine growth and yield have occurred with increased frequency in several viticultural areas traditionally dry farmed. Often, yield reduction was also coupled with unbalanced must composition, due to the increase in pH and sugar accumulation, resulting in lower total acidity and excessive wine alcohol. While severe water stress induces low yield and reduced berry color at harvest, mild water deficit is useful to enhance anthocyanins and phenols concentration in the fruit (Chaves et al., 2007); but the possible increase in structural complexity of phenolic compounds may reduce their extractability during fermentation (Sivilotti et al., 2005). Alternatively, fully irrigated vines exhibited lower concentrations of berry skin anthocyanins and total phenols when compared to those deficit irrigated (Chaves et al., 2007; Bucchetti et al., 2011). According to these results would be interesting to optimize strategies based on deficit irrigation in order to maintain the yield and improve the grapes quality also under variable climatic condition (wet and dry periods).

In Central Italy, an increase in air temperature coupled with a decrease in rainfall during the growing season was observed in several viticultural areas in the last three decades (Di Lena et al., 2012). In particular, the seasonal pattern of the last decade in Central Italy was quite variable and characterized by hot and dry summers (i.e. 2003, 2007, 2011 and 2012 seasons) alternating with rainy ones (i.e. 2005, 2006, 2010 and 2013). Prior to the last decade, vineyard irrigation in Central Italy was unusual; however, the striking summer drought experienced in 2003 forced grape-growers to consider irrigation as a cultural tool in viticultural areas historically dry-farmed. Thus, there was expanding interest in the development of effective water management strategies, designed to take into account the sequences of wet and dry periods, with the ultimate goal of maintaining yield and fruit quality.

In comparison with not irrigated vines, DI, currently used in dry areas for improving yield and grape quality while optimizing water use, is supported by several vine physiology studies (McCarthy et al., 2002; Medrano et al., 2002; Cifre et al., 2005; Chaves et al., 2007). Practical field applications of DI take into account soil moisture status and/or plant water status as crucial indicators for establishing irrigation strategies. Different physiological indicators for DI scheduling in grapevines include: stomatal conductance, leaf temperature and pressure chamber measurements (Bernard et al., 2004; Cifre et al., 2005). These are widely used as practical tools to estimate vine water status (Williams and Araujo, 2002; Girona et al., 2006; Van Leeuwen et al., 2008) and they also allow uniform methodology in data collection. Predawn leaf water potential (Ψ_{lpd}) is considered a good indicator of vine water status at different development stages because of its correlation with leaf photosynthesis (Deloire et al., 2004; Santos et al., 2005). Williams and Araujo (2002) reported close correlation among Ψ_{lpd}, midday leaf water potential (Ψ_l) and stem water potential (Ψ_{stem}) measured at noon, thereby indicating a common relationship among the three parameters. However, Choné et al. (2001) and Patakas et al. (2005) reported that midday Ψ_l and Ψ_{stem} seem to define vine water status better than Ψ_{lpd}, especially in warm growing regions. This discrepancy may be tied to soil moisture profile, environmental conditions, genotype (scion and rootstock cultivar) and phenological stages in which water stress occurred (intensity and duration). In fact, in response to water deprivation, some grapevine genotypes have shown improved control of stomata (anisohydric), whereas others have demonstrated lower stomata sensitivity (isohydric) (Chaves et al., 2010). In open field conditions, under mature vineyard conditions, it was quite common to find a wide range of stomatal conductance for a limited range of Ψ_l (Jones, 1990). Bota et al. (2001) ranked 22 *Vitis vinifera* L. cultivars into two different groups: "alarmist" cultivars, showing strong reduction of stomatal conductance in response to a relatively small decrease in Ψ_l; and "luxurious" cultivars showing lower reduction in stomatal conductance under water stress. However, application of these physiological indicators (Ψ_l, Ψ_{stem} and Ψ_{lpd}) requires well trained operators, making adoption difficult where irrigation was not commonly practiced.

The aim of this study was (1) to evaluate the impacts of DI treatments upon vine yield, grape quality, leaf gas-exchange and carbon isotope discrimination of mature 'Sangiovese' grapevines, major black-berried cultivar grown in Italy and, (2) to investigate the application of plant and soil water status indicators for irrigation strategies under variable climatic conditions.

2. Materials and methods

2.1. Plant material, experimental conditions and experimental design

This research was carried out over 3 years (2007–2009) in a hillside commercial vineyard located at Monteprandone (Marche region, Central Italy, lat. 42°55′ N, long. 13°50′ E, elevation 273 m a.s.l.). The soil (8% slope) was without gravel in the first 1 m of depth, and with a 2 mm-sieved fraction composed of 7.5% sand, 55.0% silt and 37.5% clay. The vineyard was planted in 2004 with Sangiovese (clone VCR23) cultivar grafted onto 1103 Paulsen rootstock. The vines were spaced 1.0 m within rows and 3.0 m between rows, oriented NNE to SSW. Vines were hand-pruned leaving one cane per vine with 6–13 nodes and trained to a VSP (vertically shoot-positioned) trellis. The cane wire was set at 0.9 m aboveground with two pair of surmounting catch wires allowing a canopy growth to a height of approximately 1.2 m. During the three years of trial, shoots were mechanically trimmed at 0.3 m above the top pair of wires in mid-July. Recommended crop protection practices were followed and the pest management program was based on scouting, experience, and weather conditions.

The experimental design consisted of two main blocks, each with nine rows, 60 m in length. Each block was divided into three plots of three rows each (two buffer rows and a central row for data collection). Two of them were equipped with irrigation systems consisting of pressure compensating emitters of low capacity (1.6 L/h) spaced 0.6 m apart and positioned in a drip line suspended 0.6 m above the ground on a wire. Next to a control non-irrigated plot (I_0), two DI treatments, based on the replacement of 33% (I_1) and 70% (I_2) of the crop evapotranspiration (estimated $\mathrm{ET_c}$) recorded the previous 7 days. DI treatments were initiated in the 2007 season. The irrigation system was manually operated by opening and closing the valves corresponding to each experimental plot at the desired time. $\mathrm{ET_c}$ was calculated as follows:

$$\mathrm{ET_c} = \mathrm{ET_o} \times K_c \quad (\text{where} K \text{ is the crop coefficient}) \tag{1}$$

$\mathrm{ET_o}$ was estimated by the Penman-Montheith method (Allen et al., 1998) using data from an automated weather station located near the vineyard. K_c were estimated on the basis of actual canopy growth by applying the following equation proposed by Williams and Ayars (2005):

$$K_c = -0.008 + (0.017 \times \mathrm{SA}), \tag{2}$$

where SAS is the soil surface area shaded by grapevine canopy. Water volumes were computed by applying the $\mathrm{ET_c}$ equation (1) and subtracting rainfalls. In the 2007 and 2008 seasons, two irrigation applications were implemented between berry-set and veraison and an added one was necessary post-veraison. In 2009, however, a single irrigation was required just before veraison (Table 1).

Growing degree days (GDD) or Amerine and Winkler (1944) index were also calculated using data from an automated weather

Table 1
Growing degree days (GDD), rainfall, reference (ET_0) and crop (ET_c) evapotranspiration, water supplied to 'Sangiovese' plots according to irrigation treatments and total water amount in each season.

Parameters	2007	2008	2009
GDD^{x} from bud-burst to harvest	1823	1780	1763
Rainfall from bud-burst to anthesis (mm)	96	102	95
Rainfall from anthesis to veraison (mm)	61	94	276
Rainfall from veraison to harvest (mm)	15	1	97
Rainfall from bud-burst to harvest (mm)	172	197	468
Estimated ET_0^{y} from bud-burst to anthesis (mm)	255	235	160
Estimated ET_0 from anthesis to veraison (mm)	342	326	392
Estimated ET_0 from veraison to harvest (mm)	266	240	220
Estimated ET_0 from bud-burst to harvest (mm)	863	801	772
Estimated ET_c^{z} from bud-burst to anthesis (mm)	75	73	50
Estimated ET_c from anthesis to veraison (mm)	178	227	267
Estimated ET_c from veraison to harvest (mm)	156	155	133
Estimated ET_c from bud-burst to harvest (mm)	409	455	405
Irrigation supplied to I_0 (mm)	30	0	0
Irrigation supplied to I_1 (mm)	55	28	13
Irrigation supplied to I_2 (mm)	80	62	28
Total water amount I_0 (rain + irrigation) (mm)	209	197	468
Total water amount I_1 (rain + irrigation) (mm)	234	225	481
Total water amount I_2 (rain + irrigation) (mm)	259	259	496

[x] Cumulative growing degree days (base 10 °C).
[y] Penman-Montheith methods (Allen et al., 1998).
[z] Crop evapotranspiration.

station located near the vineyard. GDD expresses the sum of all daily temperatures for the active growth and available in an area during the vine growing season between April and October. The mathematical expression is: Ta = Σ(Tmed − 10 °C), where Ta is the active temperature, Tmed is the average air temperature and 10 °C is the 'zero vegetation' for grapevines. Since the vines below 10 °C stops its development, precisely because it is considered the 'zero vegetation', this value was subtracted from the sum, placing equal to zero the contributions of days in which the average temperature drops below this value.

2.2. Soil and vine water status

Diviner 2000® (Sentek Environmental Technologies, Stepney, South Australia) capacitance probe was utilized to monitor soil water content (SWC). Six probes were used in the study each year, two per treatment and one per row. Each probe was placed in an access tube installed to a depth of 1 m between two emitters beneath the drip line, 0.1 m apart. Two access tubes per irrigation treatment were installed in the rows between two vines, with emitters positioned 0.4–0.5 m from the trunk where the highest grapevine root density occurs (Silvestroni et al., 2007). The probe was calibrated with the vineyard's soil type and the water was expressed as a percentage of volumetric water content. Measurements were carried out every 2 weeks with the exception of the drought during the last week of July when measurements were made every day. Soil samples were taken at each tube installation to determine field water capacity (FWC) and wilting point (WP), set at −0.033 MPa and −1.5 MPa, respectively. In 2008 and 2009, midday leaf (Ψ_l) and stem (Ψ_{stem}) water potential measurements (Scholander pressure chamber, PMS Instrument Co., Albany, USA), were conducted periodically during the season to monitor vine water status and to establish timing of irrigation. Measurements of Ψ_l were taken from 12:00 to 13:30 h on six mature and well-exposed leaves per treatment sampled from the central part of the primary shoots. For Ψ_l determinations, the sample leaf blades were covered with a plastic bag, quickly sealed, and their petioles then cut within 1–2 s. The time between leaf excision and chamber pressurization was generally <10 to 15 s. About 2 h before midday measurements, six homologous leaves for determination of Ψ_{stem} were enclosed in black plastic bags covered with aluminum foil.

2.3. Gas exchange and carbon isotope discrimination measurements

In the third year of the trial (2009), beginning at pre-anthesis stage on DOY 136 (day of the year) and every 2 weeks, single leaf gas exchange measurements were taken in the morning (from 09:30 to 11:30 h) until harvest using a portable, open system, LCA3 infrared gas analyzer (ADC BioScientific Ltd., Hoddesdon, Herts, UK). The system was equipped with a broad leaf chamber (6.25 cm^2) and measurements were taken at ambient relative humidity with airflow adjusted to 350 mL min^{-1}. For each treatment, six well-exposed, fully expanded leaves at node positions 6–10 from the base were used for gas exchange measurements under saturating light conditions (PAR > 1400 μmol photons m^{-2} s^{-1}). Just before harvest in 2009 (DOY 245) leaves on different positions on the primary shoot (5, 10, 15 and 20 node from the base) were collected for carbon isotope discrimination measurements after being covered by aluminum foil for 24 h in order to depress leaf photosynthesis, i.e. non-structural carbohydrates formation, thus leaving only constitutional carbohydrates. Three biological replicates consisting of five leaves were taken in I_0 and I_2 treatments. Leaves were then weighed, oven dried at 55 °C until reaching constant weight and grounded. Subsamples of the dried leaves (1 mg) were analyzed for $\delta^{13}C$ using a Europa Scientific ANCA-SL Stable Isotope Analysis System (Europa Scientific Ltd., Crewe, UK). Carbon isotope composition was expressed as the ratio $^{13}C/^{12}C$ of the sample to the $^{13}C/^{12}C$ of the Pee Dee Belemnite standard as described by Farquhar et al. (1989).

2.4. Vine yield and grape composition

Harvest was performed when berry sugar accumulation appeared to level off on the basis of total soluble solids (TSS). Harvest dates were 3, 9, and 8 September in 2007, 2008, and 2009, respectively. Yield per vine was measured at harvest and the number of clusters per vine counted in nine vines per plot (18 vines per treatment). Each year 500 berries per plot were randomly collected from the harvested clusters, weighed to determine berry fresh weight and crushed to obtain a juice sample for measuring TSS (as °Brix), pH, and titratable acidity (TA). A Maselli LR-01 temperature-compensating refractometer (Maselli Misure, Parma, Italy) was used to measure TSS, while TA was measured using a Crison Titrator (Crison Instruments, Barcelona, Spain) with NaOH 0.25 N to a pH 7.00 end point, expressed as g/L of tartaric acid equivalent. pH was measured with a Crison pH meter (Crison Instruments, Barcelona, Spain), and tartaric acid concentration was determined via a reaction of tartaric acid with vanadium acid producing an orange color measured by spectrophotometer at 500 nm. Enzymatic kits (Enzyplus-Raisio) were utilized for assessing malic acid concentration. Concentrations of yeast assimilable nitrogen (YAN), including ammonium and α-aminoacids, were estimated by formol titration to pH 8.10, following the Ogorodnik and Merkureua procedure reported by Gump et al. (2002). Total anthocyanin and polyphenol concentrations were determined after Di Stefano et al. (2002) on 50 fresh berries per sample per treatment randomly collected from the 500 berry sample per plot at harvest.

2.5. Statistical analysis

Basic statistics, analysis of variance, and correlation analysis were performed using Statistica (Statistica 4.3; StatSoft, Inc. 1993) and Sigma Plot (version 10; SPSS, Chicago, IL, USA). Results were tested for homogeneity of variance and subjected to analysis of variance (ANOVA). Linear regression analysis was performed on Pn and on soil and vine water status parameters. DI treatments effects were tested by ANOVA and means separation was calculated by applying the Student–Newman–Keuls test at $P = 0.05$ level. In figures, data are also reported as mean values ± standard errors (SE).

3. Results

3.1. Environmental conditions

The number of growing degree days (GDD, base 10 °C) from bud-burst to harvest were similar in the three seasons, ranging from a minimum of 1763 in 2009 to a maximum of 1823 in 2007 (Table 1). Contrarily, precipitation showed ample fluctuations from bud-burst to harvest ranging from a minimum of 172 mm in 2007 to a maximum of 468 mm in 2009. In spite of similar heat summation among the three growing seasons, the maximum air temperature was higher in 2007 compared to 2008 and 2009 and resulted in a maximum weekly ET_0 summation that peaked over 50 mm near veraison, i.e. from DOY 189 to DOY 215 (Fig. 1 and Table 1).

The driest growing season (2007) featured the highest evaporative demand associated with the lowest precipitation, even in the first part of the year. From bud-burst to harvest, rainfall was only 172 mm, corresponding to 20.6% of the reference evapotranspiration (ET_0) and to 43% of the crop evapotranspiration (estimated ET_c). In such environmental conditions, with the vineyard in its first year of full production, 30 mm of water were supplied also in the I_0 treatment to support grapevine growth and yield. Considering the entire growing season, I_0 received 51% and 43% of the estimated ET_c respectively in 2007 and 2008, while I_2 had 63% and 57% of the estimated ET_c respectively in 2007 and 2008 (Table 1).

The wettest growing season occurred in 2009 when 468 mm of rain fell from bud-burst to harvest, 2.7 times the 2007 rainfall. During 2009, the total amount of rainfall between anthesis and veraison, i.e. from DOY 140 to DOY 210, reached 276 mm. I_0 received 104% of the 2009 growing season estimated ET_c, thus suggesting a lack of water stress. However, no measurable rainfall occurred for 20 consecutive days in 2009, from DOY 195 to DOY 215, when the maximum air temperatures averaged near 30 °C and the weekly ET_0 summation approached 40 mm. For that reason, a single irrigation event was considered necessary in I_1 and I_2 treatments (Table 1 and Fig. 1), both for grapevine and for soil water status indicators, and initiated when shoot growth had slowed.

3.2. Effects of DI on vine and soil water status and gas exchange relationships

In the third year of the trial vine and soil water status and leaf gas exchange were extensively monitored. In the 2009 season midday Ψ_l was similar in the three treatments until DOY 204 (pre-veraison stage) when it lowered to −0.9 MPa (Fig. 2A), which, according to Van Leeuwen et al. (2008), can be considered the beginning of slight water stress. At that time, Ψ_{stem} values averaged −0.6 MPa (Fig. 2B). Leaf Pn of 'Sangiovese' vines increased until DOY 156 (post-anthesis stage), peaking at 14.4 μmol CO_2 m^{-2} s^{-1}, then slowly declining simultaneously with the Ψ_l until DOY 204 reaching values of about 10.6 μmol CO_2 m^{-2} s^{-1} (Fig. 2C). Micro-irrigation was scheduled on DOY 205 and it led to an increase of Ψ_l from −0.81 to −0.72 MPa in I_1 and I_2 treatments, respectively. One week after irrigation, on DOY 212, the vines subjected to both DI treatments showed Ψ_l values higher than those of non-irrigated plots. Later, irrespective of treatments, Ψ_l progressively decreased to values around −1.1 and −1.2 MPa indicating a moderate water stress that persisted through berry ripening to harvest (Fig. 2A and B).

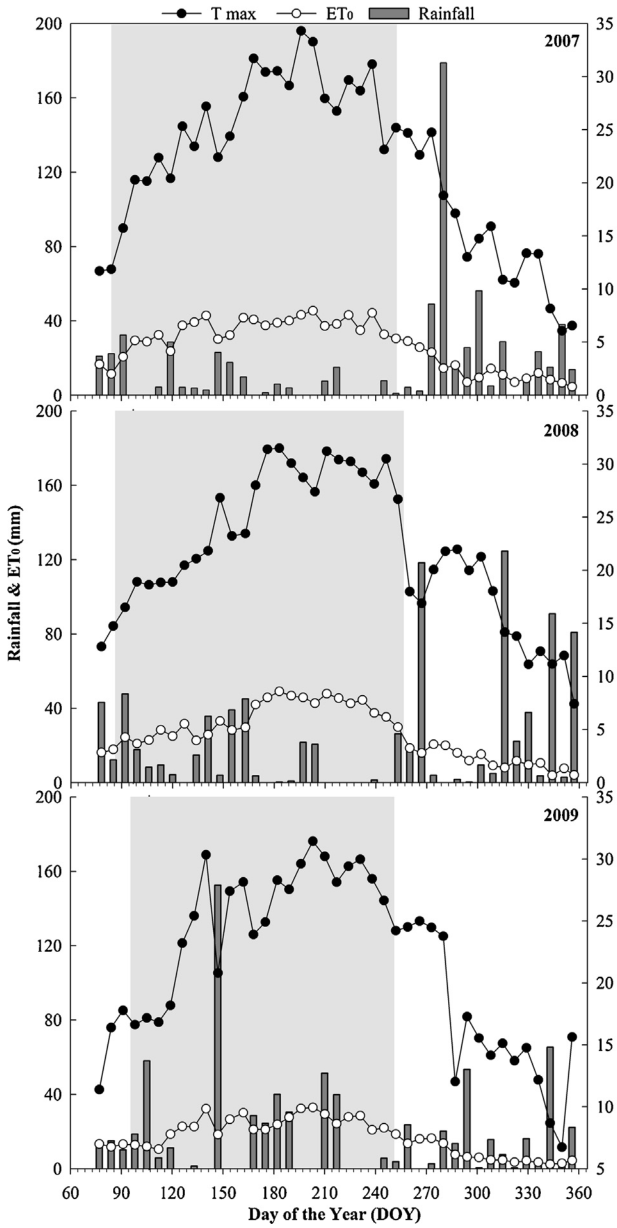

Fig. 1. Seasonal evolution of cumulative weekly rainfalls, reference evapotranspiration (ET_0) and maximum air temperature (Tmax) in 2007, 2008 and 2009 seasons. The period between budburst and harvest is marked with a light gray rectangle.

An irrigation delivering 28.4 mm of water raised 'Sangiovese' leaf photosynthesis to 12 μmol CO_2 m^{-2} s^{-1} in I_2 treatment (+10% compared to values recorded before irrigation). Meanwhile, (DOY 212), Pn remained almost constant in the I_1 treatment (11 μmol CO_2 m^{-2} s^{-1}), while it decreased to 8.3 μmol CO_2 m^{-2} s^{-1} in the I_0 treatment. Therefore, in comparison to I_0, the Pn of vines subjected to both DI treatments remained higher up to DOY 248 (Fig. 2C) during the entire berry maturation period. A positive effect of drip-irrigation on the photosynthetic performance of medial leaves was evident even in a season with relatively abundant precipitation. Stomatal conductance measurements throughout the season ranged from a minimum of 88 mmol m^{-2} s^{-1} to a maximum of 150 mmol m^{-2} s^{-1} and no DI effects were observed (data not shown). However, differences were found between I_0 and I_2 treatments in carbon isotope discrimination in position 15 of the primary shoot (−1.18‰) where leaves were formed during the mild water stress period (pea-size phenological stage) (Fig. 3). Thus,

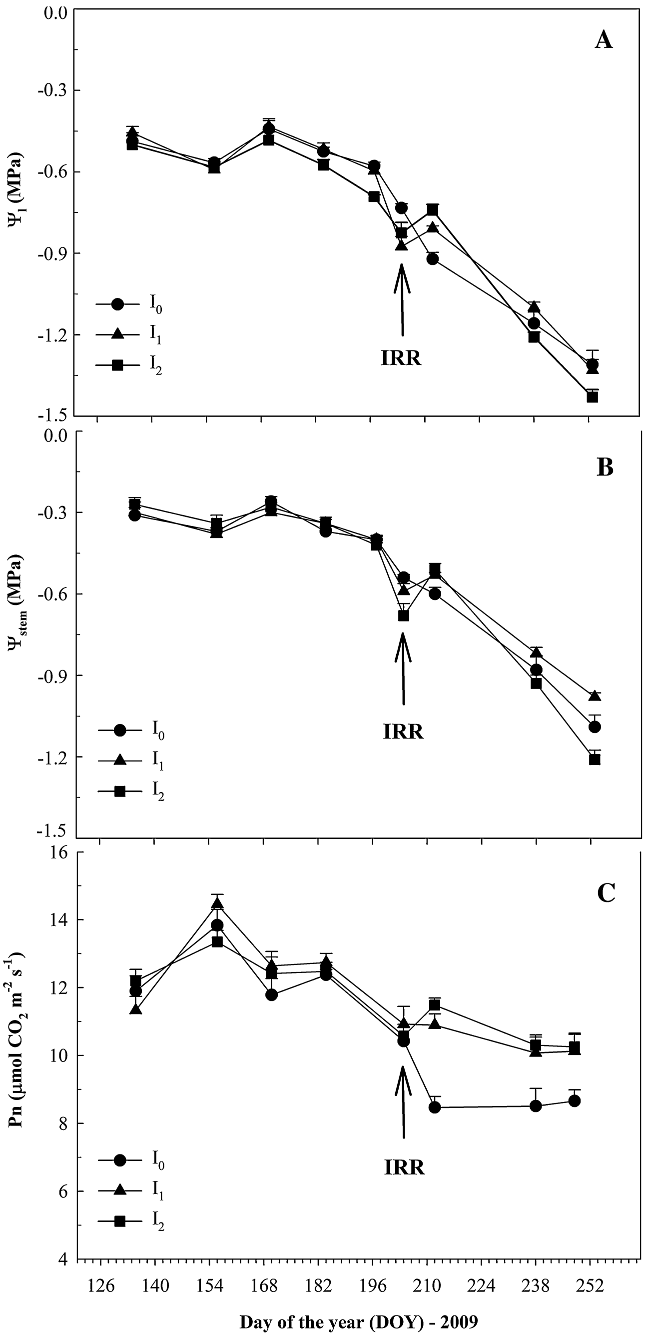

Fig. 2. Evolution of midday leaf (Ψ_l) and stem (Ψ_{stem}) water potentials and net photosynthesis (Pn) in 'Sangiovese' vines grown under different irrigation regimes in 2009 season. I_0, non-irrigated, rain-fed I_1 and I_2 deficit irrigation respectively based on the replacement of 33% and 70% of the previous week estimated ET_c from pea-size berries to veraison. Each point is the mean of six measurements ± standard error (SE). Bud burst, anthesis, veraison and harvest occurred respectively on day of year (DOY) 95, 140, 210 and 252.

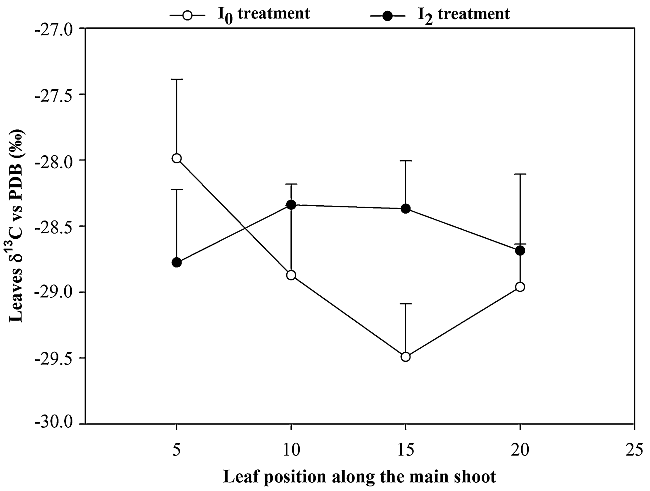

Fig. 3. Carbon isotope discrimination determined just before harvest on 2009 season in leaf samples collected from primary shoots at differentpositions. Leaves were collected from nodes 5, 10, 15 and 20 from treatments I_0 and I_2. I_0, non-irrigated, rain-fed; I_2 deficit irrigation based on the replacement of 70% of the previous week estimated ET_c from pea-size berries to veraison.

lately there is a expanding interest in the development of effective water management strategies, designed to take into account the sequences of wet and dry periods, with the ultimate goal of maintaining yield and optimize the grape composition.

In 2009, the SWC, measured in the row between two vines at 0.4–0.5 m from the trunk, showed fast water depletion of the topsoil (0–0.3 m) from DOY 120 to DOY 151. The topsoil was replenished by frequent and abundant rainfalls, which maintained SWC near the FWC threshold until DOY 188, when a dry period started (Fig. 4). The SWC decreased for the high vine water demand due to fully developed canopy with high evaporative demand. In less than 15 days, the uppermost layers, to 0.6 m in depth along the row under the drip line, reached the WP threshold (DOY 204). Drip-irrigation on DOY 205 increased SWC, which gave rise to values close to the FWC threshold in the I_2 treatment on DOY 212. Thereafter, SWC decreased quickly in the first 0.6 m of depth along the row in between two adjacent vines, again reaching values close to WP on DOY 225. However, a certain degree of water availability during berry ripening (i.e., from DOY 210 to DOY 252) was maintained in the deep soil layer of both the DI and the non-irrigated I_0 treatment. A substantial increase of SWC in the top 0.6 m of soil under the canopy was recorded only late in the season from DOY 327 following autumn rains (Fig. 4).

3.3. Effects of DI on shoot development and vine size

Shoot number per vine averaged 8 in the first 2 years of the trial (2007 and 2008) and it was increased to 12–13 in 2009, to improve vine yield and target the winery production objectives (Table 2). In this last trial year (2009), regardless of treatment, the abundant spring precipitation led to vigorous shoot growth (67–80 g), almost double the 2007 season (35–42 g). Irrigation did not impact cane weight, suggesting that low water volume application does not reduce shoot vigor in comparison to I_0. Additionally, vine size, indexed as one-year-old cane pruning weight, was varied greatly among the different seasons and was influenced in I_1 treatment in 2008, while in the wettest 2009 season I_0 and I_1 showed similar values higher than I_2.

3.4. Effects of DI on yield and grape composition

The number of clusters per vine was lower in the first two seasons (10–13 units) when compared to 2009 (16–17 units) (Table 3). There was no impact of irrigation on the number of clusters per vine or the number of berries per cluster during the three experimental years. The wettest season (2009) resulted in the highest yield per vine, while the driest one (2007) led to the lowest yield per vine (Table 3). Water supplied by irrigation in 2007 led to significant improvement in grapevine yield, increasing from 2.5 kg of I_0 to 3.8–3.5 kg of I_1 and I_2 due to increased cluster and berry weight (Table 3). Thus, DI treatments, when compared to I_0

Table 2
Vegetative performance of 'Sangiovese' grapevines grown under different irrigation regimes in 2007–2009. I_0, non-irrigated, rain-fed; I_1 and I_2 regulated deficit irrigation respectively based on the restitution of 33% and 70% of the previous week ET_c during the period between pea-size berries(4–5 mm) and full veraison.

Parameters[x]	2007			2008			2009		
	I_0	I_1	I_2	I_0	I_1	I_2	I_0	I_1	I_2
Cane (no/vine)	7.2b	8.5a	6.8b	8.3a	8.5a	8.2a	12.6a	12.9a	12.5a
Cane wt (g)	41a	35a	42a	66ab	79a	64b	80a	80a	67b
Pruning wt(kg/vine)	0.29a	0.28a	0.27a	0.54b	0.66a	0.52b	0.95a	1.02a	0.81b

[x] Within rows and years, means followed by the same letter are not different at $P = 0.05$ (Student–Newman–Keuls test).

Table 3
Yield and vine components of 'Sangiovese' grown under different irrigation regimes in 2007–2009. I_0, non-irrigated, rain-fed; I_1 and I_2 regulated deficit irrigation respectively based on the restitution of 33% and 70% of the previous week ET_c from pea-size (4–5 mm) berries to veraison phenological stages.

Parameters[x]	2007			2008			2009		
	I_0	I_1	I_2	I_0	I_1	I_2	I_0	I_1	I_2
Clusters (no/vine)	10.1a	12.2a	11.2a	13.1a	11.3a	12.2a	18.0a	16.2a	17.0a
Yield (kg/vine)	2.5b	3.8a	3.5a	4.7b	5.0a	5.0a	7.2a	7.5a	7.0a
Cluster wt (g)	262b	315a	316a	361b	451a	410a	453a	469a	415a
Berry wt (g) at harvest	1.55b	1.81a	1.73a	1.88b	2.15a	2.11a	2.57a	2.47a	2.55a
Berries (no/cluster)	170a	174a	181a	192a	209a	194a	176a	188a	163a
Yield-to-pruning wt ratio (kg/kg)	8.4b	13.5a	12.8a	8.6a	7.6a	9.6a	7.7a	7.4a	8.6a

[x] Within rows and years, means followed by the same letter are not different at $P = 0.05$ (Student–Newman–Keuls test).

rain-fed, increased yield per vine of about +40–50% in 2007, while in 2008 the increase was only +6% (Table 3). Regardless of vintage, the comparison of I_1 and I_2 treatments did not show significant differences in vine yield or on yield components (Table 3). Moreover, in comparison of I_2 and I_1 treatment allowed a considerable water saving for the winery, from a minimum of 15 mm in 2009 to a maximum of 34 mm in 2008.

Berry weight responded to irrigation only in the first two years of the experiment, which featured more water stress than 2009 when no treatment effect was observed. As compared to I_0, I_1 and I_2 vines showed an increase of berry weight averaging +14% in 2007 and +11% in 2008 (Table 3). The ratio between yield and one year-old pruning weight was not influenced by the irrigation regimes in 2008 and 2009, while in the driest 2007 season the two DI treatments led to a significant increase in that ratio compared to the I_0 treatment due to higher vine yield.

In the 2007, fruit from I_0 vines had the highest TSS concentration and the lowest TA in comparison to 2008 and 2009, whereas a significant increase of anthocyanins and total phenols content (+82–94%) and YAN (+26–33%) was found in I_0 respect to DI treatments in 2007 season (Table 4). In fact, both DI treatments significantly lowered TSS concentration in the driest season (about 3.4 °Brix), as well as anthocyanins (an average of 137 mg/kg), total phenols (504 mg/kg) and YAN (an average of 32 mg/L), whereas malic acid concentration increased (an average of 0.34 g/L) (Table 4).

Titratable acidity and must pH were not influenced by DI treatments in any year, while other parameters were affected differently by irrigation supply, depending of the season. Indeed, no statistical differences were found in TSS, total phenols and YAN either 2008 or 2009 (Table 4). In 2008, DI treatments showed an increase of malic and tartaric acid concentration, while grape anthocyanins were significantly reduced only in I_2 grapes (−34%) when compared to control I_0. DI did not affect grape composition in 2009, when the increased number of retained shoots per vine and the abundant rainfall resulted in a yield higher than 7 kg of grapes per vine, corresponding to 23.3 t/ha. In 2009, the low TSS (19.3–20.1 °Brix) coupled with the high content of titratable acidity (7.0–7.5 g/L) and malic and tartaric acids, suggested a delay of the berry ripening process.

4. Discussion

Based on the climatic data, the three seasons (2007, 2008, and 2009) can be defined as seasons characterized by severe, mild, and low water stress, respectively. Vegetative and reproductive responses of the high-yielding black-berried grapevine variety 'Sangiovese' were significantly impacted by water availability during the seasons.

As compared to the driest 2007 season, 2008 and 2009 significantly increased vine yield as well as higher TSS, rather than increased anthocyanins and phenols content in the grape. These

Table 4
Grape composition at harvest recorded on 'Sangiovese' grapevines grown under different irrigation regimes in 2007–2009. I_0, non-irrigated, rain-fed; I_1 and I_2 regulated deficit irrigation respectively based on the restitution of 33% and 70% of the previous week ET_c from pea-size berries to veraison phenological stages.

Parameters[x]	2007			2008			2009		
	I_0	I_1	I_2	I_0	I_1	I_2	I_0	I_1	I_2
Soluble solids (°Brix)	25.7a	21.9b	22.6b	24.1a	24.0a	23.6a	19.4a	19.3a	20.1a
Titratable acidity (g/L)	5.8a	6.0a	6.0a	6.0a	6.3a	6.2a	7.1a	7.5a	7.0a
Must pH	3.46a	3.38a	3.40a	3.38a	3.40a	3.37a	3.26a	3.25a	3.27a
Tartaric acid (g/L)	7.6a	7.2a	7.4a	4.7b	5.7a	5.2ab	8.0a	8.6a	8.2a
Malic acid (g/L)	1.29b	1.55a	1.70a	1.04c	1.62a	1.31b	2.55a	2.69a	2.42a
Anthocyanins (mg/kg)	538a	383b	419ab	680a	681a	447b	404b	477ab	516a
Total phenols (mg/kg)	2519a	1969b	2061b	1301a	1436a	1357a	1378a	1324a	1202a
YAN (mg/L)[y]	117a	77b	93b	93a	107a	88a	133a	130a	111a

[x] Within rows and years, means followed by the same letter are not different at $P = 0.05$ (Student–Newman–Keuls test).

[y] Yeast assimilable nitrogen.

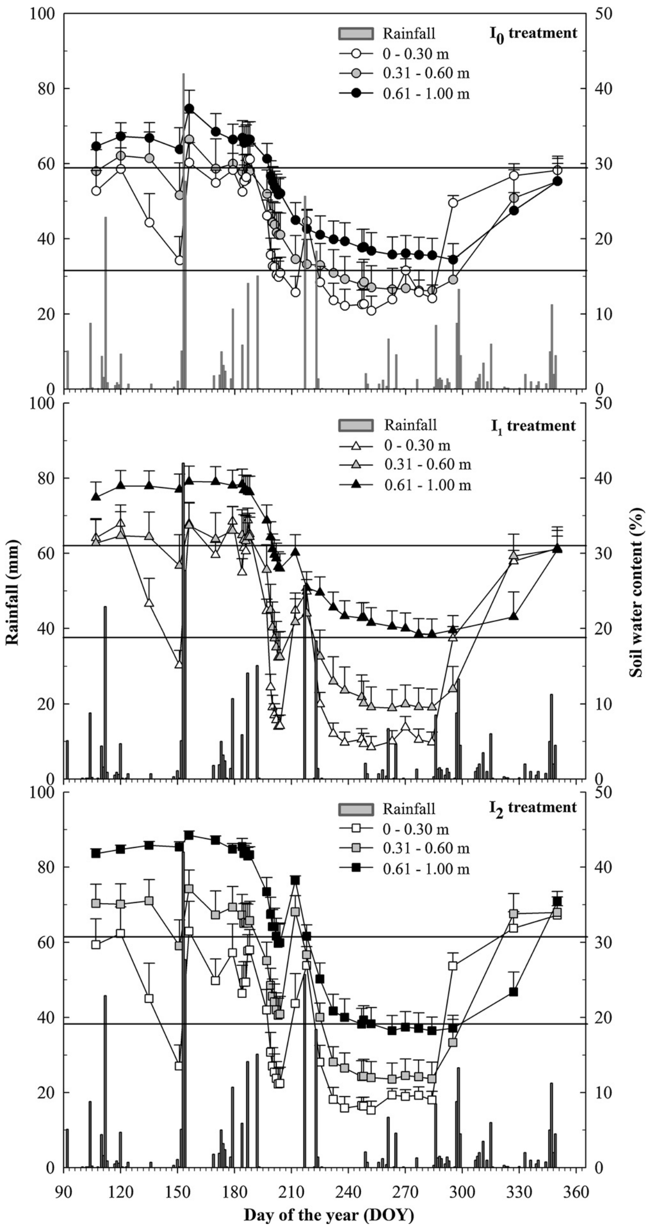

Fig. 4. Evolution of rainfall (mm) and soil water content (% H_2O) measured by Diviner 2000® portable capacitance probe in the first meter of depth under the dripping line between two emitters 0.6 m spaced. Data were taken on 2009 season at 0.4–0.5 m distance from the vine trunk in plots under different irrigation regimes since 2007. I_0, non-irrigated, rain-fed; I_1 and I_2 deficit irrigation respectively based on the replacement of 33% and 70% of the previous week estimated ET_c from pea-size (4–5 mm berry diameter) to veraison. Each point is the mean of two measurements with the standard error taken at different depths in two blocks. Area between field water capacity and wilting point is marked by lines. Bud burst, anthesis, veraison and harvest occurred respectively on day of year (DOY) 95, 140, 210 and 252.

results agree with those obtained by Santos et al. (2005) and Salón et al. (2005) and are mainly related to reduced cluster and berry weight, since the number of berries per cluster was not impacted. The reduced soil water and high temperatures during berry development might have reduced cell expansion and increased water loss by the berry via a higher rate of both berry transpiration and dehydration (Greenspan et al., 1994). As already reported in literature, the level of irrigation applied did not modify cluster number, which was similar for all treatments and it was primarily linked to the number of nodes retained per vine at pruning (Kliewer et al., 1983; Salón et al., 2005).

As compared to I_1 and I_2 DI treatments, the significant increases in the I_0 vines of TSS, anthocyanins, and phenolics found in 2007 were not related to cluster exposure. Mean cane weight and, likely, canopy density were not influenced by irrigation and basic fruit characteristics were ascribed to low yield per vine (2.5 kg or 8.3 t/ha). According to Bravdo et al. (1985), the yield-to-pruning weight ratio showed by I_0 vines (8.4) indicates good vine balance, where as the values of this index for the I_1 and I_2 vines (13.5 and 12.8 respectively) seems to indicate over-cropping conditions. High crop levels were not adequately supported by vegetative growth with average cane weight at about 40 g in 2007. In that season, both I_1 and I_2 DI treatments were able to avoid reduction in vine yield and to maintain target soluble solids concentration projecting alcohol potential of about 12.1% and 12.5%, respectively, against 14.5% calculated for wines produced with I_0 vines. Therefore, in the 2007 dry season, the results concerning the technological maturity reached in I_1 and I_2 vines are in line with recent consumer preferences for wine with moderate alcohol content (Borrelli and Raia, 2008). In seasons with moderate and slight water stress (2008 and 2009) respectively, neither I_1 nor I_2 DI irrigation treatments modified vine yield and the ripening processes. Furthermore, soluble solids, anthocyanins and phenolics contents in the grapes were also unaffected. In the wet season (2009), grapes failed to reach target TSS levels because of an early harvest necessitated by fungal disease pressure coupled with an abundant yield (27 t/ha). At harvest, TSS figures ranged from 19.3 to 20.1%, corresponding to wine alcohol concentrations lower than 10.8% by volume. The high rainfall amount occurring between anthesis and pre-veraison stage (about 312 mm from DOY 144 to DOY 192, Fig. 3), increased both berry and cluster weight. The higher leaf Pn demonstrated by both I_1 and I_2 DI vines during the last 40 days before harvest in 2009 did not improve yield quantity or quality as compared to non-irrigated vines. We speculated that the increased amount of carbohydrates produced by the leaves was likely used for canopy growth and/or root expansion. The analysis of carbon isotope discrimination a valid method of estimating water use efficiency (WUE) and can be used to study the plant–environment interactions and to identify genotypes tolerant/resistant to water shortages (Farquhar et al., 1989). Therefore, in this trial, data on carbon isotope discrimination indicated lower WUE in I_0 treatment at node 15 suggesting better regulation of stomata behavior in I_2 vines. In addition, water reserves in the deepest soil layers may have played a crucial role in limiting stress conditions of I_0 vines in the last 40 days before harvest.

Although the values of TA was uninfected by irrigation treatments, the tartaric and malic acid showed values fundamentally lower in I_0 treatment in both warmest seasons (2007–2008), probably due to the degradation of the acids that was increased with higher berry temperatures, due to summer drought, which occurred during the cluster ripening in those seasons. It is in accordance with other recent studies with other varieties or conditions, where the higher respiratory rate malic acid enhanced by higher temperatures reached the clusters of the vine stressed, more

Table 5
Regression equations between variables used to monitor vineyard water status and grapevine physiological performance. Data refer to all tested treatments in 2009 season. Coefficient of determination values (R^2) are also given.

x	y	Regression equation	R^2
Ψ_{stem} (MPa)	Ψ_l (MPa)	$y = -0.26 + 0.881x$	0.90
SWC (%)	Ψ_l (MPa)	$y = -1.39 + 0.026x$	0.80
SWC (%)	Ψ_{stem} (MPa)	$y = -1.25 + 0.028x$	0.81
SWC (%)	Pn ($\mu mol\ m^{-2}\ s^{-1}$)	$y = 7.86 + 0.137x$	0.69
Ψ_l (−MPa)	Pn ($\mu mol\ m^{-2}\ s^{-1}$)	y = 14.7 + 4.61x	0.68
Ψ_{stem} (−MPa)	Pn ($\mu mol\ m^{-2}\ s^{-1}$)	y = 13.5 + 4.02x	0.57

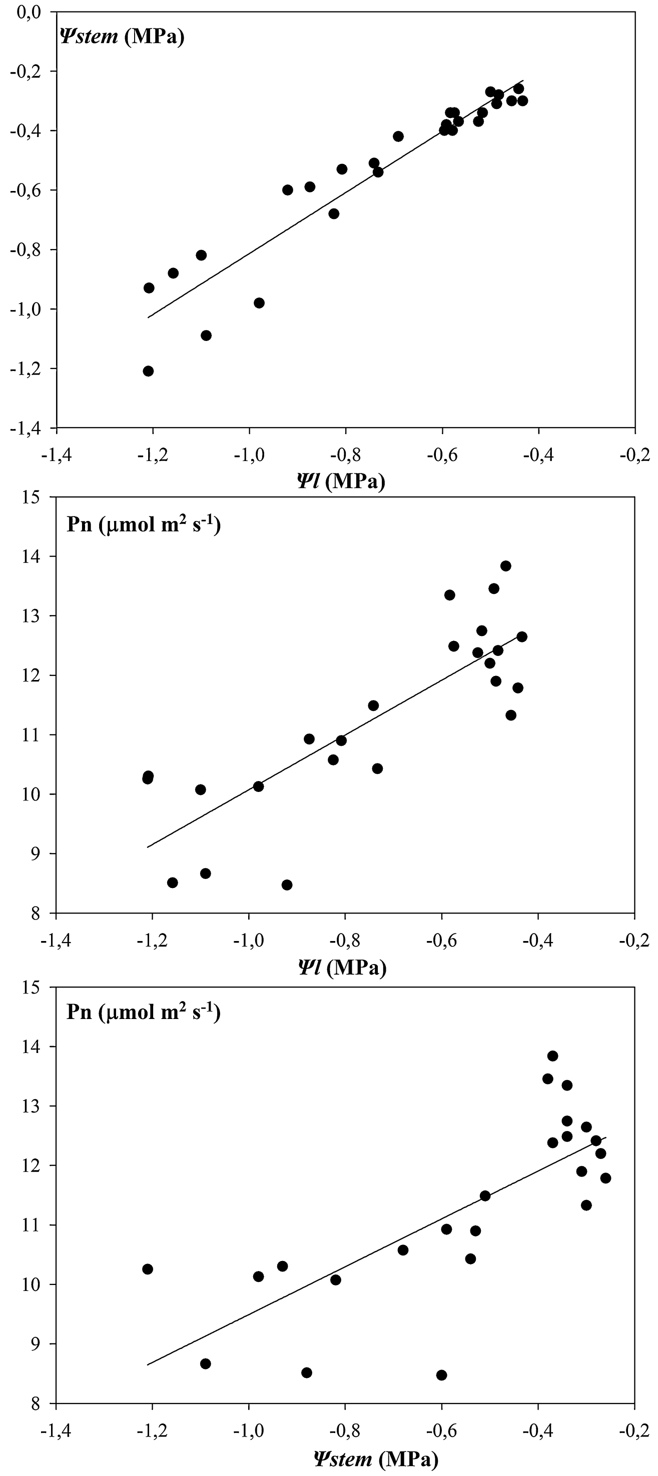

Fig. 5. Relationship between midday stem (Ψ_{stem}) and leaf (Ψ_l) water potential and net photosynthesis (Pn) in 'Sangiovese' vines grown under different irrigation regimes in 2009 season. I_0, non-irrigated, rain-fed I_1 and I_2 deficit irrigation respectively based on the replacement of 33% and 70% of the previous week estimated ET_c from pea-size berries to veraison. Each values is an individual leaf replicate.

exposed to sunlight maybe as a result of minor vegetative development (De Souza et al., 2005; Spayd et al., 2002).

Of concern is the reduction of YAN in grapes from I_1 and I_2 vines in the 2007 season and potentially inducing sluggish or stuck fermentations. This agrees with Keller (2005) and suggests nitrogen application to the must at a rate that precludes a shortage below recommended thresholds. In 2007 and 2008, I_2 vines had lower berry anthocyanin concentrations and the potential for low color in the finished wine. This is especially significant for the 'Sangiovese' cultivar because typically suffers reduced ability to accumulate anthocyanins compared to other black-berried grapevine cultivars (Mattivi et al., 2002; Palliotti et al., 2011). In Italy, the 'Sangiovese' cultivar is grown on nearly 70.000 ha (representing about 10% of total Italian vineyard acreage) and it is used to produce both ultra-premium wine (e.g., Brunello di Montalcino, Chianti, and Nobile di Montepulciano) as well as table wines. In the latter case, in order to insure profitability it is essential to obtain a sufficient yield per hectare (>11–12 t) while maintaining an adequate quality grape composition. In the Central Italian mesoclimates, the management of vine water stress is a recommended solution.

SWC measured underneath the canopy in the first meter of soil depth showed a strong linear correlation with Ψ_l and Ψ_{stem} (r^2 higher than 0.80) and a positive linear correlation with Pn (r^2 of 0.69), thereby offering a sensitive tool for defining grapevine water status at least in moderate dry seasons. A strong correlation between Ψ_l and Ψ_{stem} was also confirmed, as previously reported (Williams and Araujo, 2002; Salón et al., 2005), there was also a convincing linear relationship between these two parameters and Pn values with r^2 ranging from 0.57 to 0.68 for Ψ_{stem} and Ψ_l, respectively (Table 5; Fig. 5). The irrigation increased Pn, Ψ_l, Ψ_{stem}, and SWC and strict linear correlations were found among those parameters suggesting the possibility of using them as reliable indicators of grapevine water status. Therefore, the estimated water status of the vine should reflect the photosynthetic capacity, and vice versa, as well as the soil water availability and the amounts of water to be applied with irrigation.

5. Conclusions

Grape growing in Central Italy has several environmental challenges primarily related to a high year-to-year within season variability in rainfall. Heat accumulation between bud-burst and harvest does not vary greatly. Under these conditions, it is difficult to achieve consistent technological fruit maturity. Dry seasons, like 2007, greatly reduce the yield of 'Sangiovese' vines (lower than 8 t/ha) and, consequently, induce a significant increase in TSS in the grapes (25.7 °Brix). In this case the DI strategy, with 33% of estimated ET_c water supplied from berry pea-size to veraison, was effective in improving vine yield (up to 12 t/ha) leading to lower and improved TSS (about 22 °Brix) and satisfactory anthocyanins and total phenolics, while saving 25 mm of water as compared to the I_2 treatment. It would seem logical, therefore, to conclude that the DI strategy could be used in particularly challenging seasons to reduce berry sugar accumulation, thus allowing the production of wines less rich in alcohol and more friendly for today's consumers.

In Central Italy, however, in seasons like 2008 and 2009 characterized by moderate water stress, irrigation practices do not seem to be necessary, with the exception of vineyards established in sandy soils with low water retention capacity. It is also noteworthy that, regardless of season and yield level, the DI treatment with 70% of estimated ET_c replacement from berry pea size and veraison did not improve any of the parameters measured in this experiment, when compared to the DI treatment with 33% of estimated ET_c. Finally, midday Ψ_l and Ψ_{stem} were strongly correlated with SWC in the first meter of soil depth below the vine canopy using a Diviner 2000®. Thus, SWC, easily monitored in the field by a capacitance probe, is a good indicator of vine water status. SWC and midday Ψ_l were positively correlated to leaf Pn. Both of these parameters can be monitored in the field by capacitance probe and pressure chamber and can be used as effective indicators of grapevine water status and photosynthetic performance.

Acknowledgements

This research was supported by Regione Marche (LR37/1999 Progetto 18: microirrigazione). We appreciate the assistance of Terre Cortesi Moncaro Winery (Montecarotto, Italy) for providing the commercial vineyard used in this research and for helping in vineyard maintenance. We thank A. Green for critical reading of the manuscript.

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