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Leaf water potential and sap flow as indicators of water stress in Crimson 'seedless' grapevines under different irrigation strategies

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Abstract *Vitis vinifera* L. cv. Crimson Seedless is a late season red table grape developed in 1989, with a high market value and increasingly cultivated under protected environments to extend the availability of seedless table grapes into the late fall. The purpose of this work was to evaluate leaf water potential and sap flow as indicators of water stress in Crimson Seedless vines under standard and reduced irrigation strategy, consisting of 70 % of the standard irrigation depth. Additionally, two sub-treatments were applied, consisting of normal irrigation throughout the growing season and a short irrigation induced stress period between *veraison* and harvest. Leaf water potential measurements coherently signaled crop-available water variations caused by different irrigation treatments, suggesting that this plant-based method can be reliably used to identify water-stress conditions. The use of sap flow density data to establish a ratio based on a reference 'well irrigated vine' and less irrigated vines can potentially be used to signal differences in the transpiration rates, which may be suitable for improving irrigation management strategies while preventing undesirable levels of water stress. Although all four irrigation strategies resulted in the production of quality table grapes, significant differences ($p \leq 0.05$) were found in both berry weight and sugar content between the standard irrigation and reduced irrigation treatments. Reduced irrigation increased slightly the average berry size as well as sugar content and technical maturity index. The 2-week irrigation stress period had a negative effect on these parameters.

Keywords Water · Granier · Table grapes · Transpiration · Stomatal conductance · Total soluble solids

1 Introduction

Grapevine (*Vitis vinifera L.)* is the most widely cultivated and economically important fruit crop in the world (Cunha et al. 2013). Grapevines are well adapted to the Mediterranean climate which has a long growing season, high summer temperatures, low humidity, a rainfall-free ripening season, and mild winter temperatures (Mencarelli et al. 2005). Table grape vineyards generally require more intensive irrigation than wine grapes due to the use of training systems designed to accommodate large leaf areas for higher production (Silva-Contreras et al. 2012), thus resulting in higher canopy water loss. The

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importance of irrigation management as a key factor for the successful cultivation of table grapevines prompts the need for new research approaches to improve water management, aiming not only at environmentally sustainable agricultural practices but also at restraining water-related production costs (Martínez de Toda et al. 2010).

The Crimson Seedless cultivar, is a late-season red table grape developed in 1989 at the USDA Fruit Genetics and Breeding Research Unit in Fresno, California producing medium to large cylindrical seedless berries. The organoleptic quality of table grapes depends mainly on the sugar content, organic acid content and the balance between them (Muñoz-Robredo et al. 2011). Total soluble solids (TSS), and total titratable acidity (TTA), are the most common metabolites associated with the grape flavor. TSS is a measure of fruit sweetness and is an indicator of grape ripeness, while most of the commercial table grape varieties are considered mature when TSS ranges from 15° to 18° Brix (Giacosa et al. 2014).

Studies have shown that by managing the vineyard as a protected cultivation, that is, by covering the top and the lateral belts of the vineyard with transparent plastic films, it is possible to advance the harvest date by inducing a precocious bud break (Novello and Palma 2008) and thus achieving higher market value.

Trunk girdling, where a thin strip of bark and the subtending cambium layer is removed all the way around the trunk, can potentially increase the berry size of Crimson seedless grape varieties by as much as 30 % (Dokoozlian et al. 1995). This practice is done each year after full bloom (fruit set) in late May and temporarily disrupts the downward flow of carbohydrates and hormones through the phloem (inner bark).

These management practices used to enhance fruit quality and control harvest timings introduce a layer of complexity to the study of the water transport dynamics in the soil-plant-atmosphere continuum (SPAC), affecting plant water use dynamics and thus, displacing crop field status out of the standard conditions required to achieve efficient irrigation management with the classic water balance approach. Researchers have expressed difficulty in applying the classical FAO 56 methodology (Allen et al. 1998) for estimating crop water needs, as this has overestimated the ETc by up to 36 % (Jairmain et al. 2007; Er-Raki et al. 2013), requiring local calibration of the crop coefficient (kc) values.

Plant-based and soil-based measures are the most common methods used as indicators of plant water status (Hsiao 1973; Rana et al. 2004). The pressure chamber (Scholander et al. 1965) is a reliable plant-based method for determining the water status of grapevines, but it requires specialized personnel and in situ measurement, making it unsuitable for feeding automatic real-time data acquisition systems. Currently, the most widely used technique to monitor vine water status in commercial vineyards is midday leaf water potential (Ψ_{leaf}), and to a lesser extent, midday stem water potential (Ψ_{stem}) (Baeza et al. 2007). Midday measurements of leaf water potential and stomatal conductance (g_s) were found to be highly correlated with daily water use of Thompson Seedless grapevines (Williams et al. 2012). Stomatal conductance can also be used to measure vine water status as stomata of grapevine leaves respond to atmospheric evaporative demand and changes in soil water.

Sap flow measurement methodologies have attracted high interest and are widely studied by researchers, allowing high temporal resolution data of water uptake through the stem, which is directly related to transpiration (Jones 2004; Cifre et al. 2005). Braun and Schmid (1999), verified that grapevine stems do not develop heartwood, thus, from a practical standpoint, the whole stem section area excluding bark can be considered as sapwood, simplifying the implementation of sap flow methodology in vines and minimizing errors associated with establishing the effective sap conductive stem section often found on other woody crops. The Granier method (Granier 1985) is one of the simplest methodologies to measure sap flow, requiring only two cylindrical probes inserted radially into the stem allowing the computation of sap flow using the temperature difference between a heated and un-heated probe. The major drawback of this methodology is the requirement of a calibration procedure using the temperature difference (dT) between the heated and unheated probes when sap flow is zero (dT_M). This is achieved by assuming that sap flow is negligible at nighttime, thus considering dT equivalent to dT_M during the night until predawn.

The potential of sap flow measurements for scheduling high frequency irrigation in vineyards was investigated by Fernández et al. (2008), who compared sap flow in target vines in field conditions with reference well-irrigated vines. This approach yielded suitable results and can be considered a useful tool for irrigation scheduling. Fernández et al. (2008) also noted that full irrigation may not be required to maximize fruit yield, stating that in grapevines deficit irrigation can produce better fruit when compared to full irrigation treatment.

The purpose of this study was to evaluate leaf water potential and sap flow as indicators of water stress in Crimson Seedless grapevines under different irrigation strategies. Additionally, the physiological response of the Crimson vine to reduced irrigation regime as well as a short stress period both in terms of transpiration, berry quality and precocity were studied. The experimental irrigation treatments were designed to induce only light to mild water deficit conditions by applying small changes to the irrigation practices, combining treatments with a continuous irrigation reduction during the entire season and sub-treatments where water stress was applied at a selected stage.

2 Materials and methods

2.1 Experimental description

This study was conducted in 2014 at *Herdade Vale da Rosa* located in Ferreira do Alentejo, south Alentejo, Portugal (38°04′56.94″N, 8°04′14.98″W, elev. 150 m a.s.l.) at a Crimson Seedless cultivar (*Vitis vinifera* L.) field planted in 2006. The 230 ha vineyard is planted in a square pattern of 3 × 3 m (1111 plants per hectare) with North–South oriented rows. The trunks are guided by vertical metal poles with the fruit-bearing vine branches, canopy and dripper lines supported by a robust steel wire trellis 2 m above the soil. The standard irrigation arrangement consists of drip lines placed at mid-row, equipped with 4 L h^{-1} pressure compensating drippers spaced 1 m apart.

The lines are covered by plastic film screen and hail-bird barrier net during most of the crop cycle. The climate is Mediterranean with an average 600 mm of annual rainfall and 3000 h of annual sunshine. The Köppen-Geiger climate classification of the study area corresponds to a *Csa*—Temperate with a dry-summer. Local climate combines winter chilling period (dormant period) and a long growing season that can be dry during the summer. The soil of the study area has a clay texture (40 % clay, 30 % silt, 30 % sand) with 0.92 % organic matter content (Nóbrega 2013). Pilot holes showed that the soil is highly compacted with a hardpan layer located at approximately 0.5 m depth.

During the experiment, the vines were maintained according to the standard production procedures in terms of pruning, crop load adjustment and fertilization. Irrigation management criteria followed by the *Vale da Rosa* relies on soil moisture status analysis, empirical field observation of vine stages and water balance, and thus is not totally tied to evapotranspiration (ETc). Potential evapotranspiration (ETo) was computed from onsite weather station data following the FAO 56 methodology (Allen et al. 1998), and then irrigation for each given vine stage is scheduled to meet ETc, allowing 2–3 days between irrigation events depending on soil moisture status and observed grape development status. Two main irrigation treatments were implemented (Table 1): 100 % or full irrigation (T100) where the irrigation rate was kept according to the usual practice of the site manager, and 70 % irrigation (T70) which was achieved by replacing the existing drippers (4.0 L h^{-1}) with lower output drippers (2.8 L h^{-1}). Two sub-treatments were implemented, consisting of 3 rows with 7 vines each, where ST0 corresponds to the regular farmeŕs irrigation scheme and ST1 to irrigation induced stress by turning off irrigation for a 15-day period in July after the start of *veraison* (change of color of the grape berries). Various works have studied the influence of a moderate stress during *veraison* on yield and quality of grapes (Ginestar et al. 1998, Shellie 2006, Faci et al. 2014), but not on the precocity of the fruit, which is a fundamental criterion for the market value of the crop. To minimize border effect, only the central vines in the central row were evaluated. In each sub-treatment, water flow on central dripper line was monitored with an electromagnetic pulse flow meter (Regaber S.A., Spain) connected to a CR1000 datalogger (Campbell Scientific Ltd.).

2.2 Micro meteorological data collection

Microclimate variables air temperature T (°C) and air relative humidity RH (%) were measured at 30 min intervals using a LM35 temperature sensor (National Semiconductor Corp., USA), and a HIH4602 hygrometer (Honeywell International Inc., USA). The sensors were assembled in a custom-made ventilated plastic shield placed below canopy to avoid errors induced by

Table 1 Irrigation treatments and sub-treatments implemented at the *Vale da Rosa* and period (day of year, DOY) of irrigation-induced stress periods

Plot	Dripper output (L h^{-1})	Period of irrigation induced stress
T100 ST0	4	None
T100 ST1	4	17 July–31 July, DOY (198–212)
T70 ST0	2.8	None
T70 ST1	2.8	17 July–31 July, DOY (198–212)

direct solar radiation. Vapor pressure deficit, VPD (kPa) was determined as the e_s (kPa)−e (kPa) difference between saturation vapor pressure (e_s) and partial vapor pressure (e) computed from air temperature T (°C) and relative humidity RH (%) using Eq. 1 and 2:

$$e_s = 0.61 \exp\left(\frac{17.27}{T + 237.3}\right) \tag{1}$$

$$e = e_s(HR/100) \tag{2}$$

Broadband solar radiation was measured using two SKS1110 pyranometers (Skye instruments Ltd., UK) placed in zenith angle to measure: 1) clear sky above the canopy and plastic film cover; and 2) an intermediate position below the plastic cover, but above the canopy. Photosynthetically active radiation (PAR) from the 400–700 nm range wavelengths were measured with two PAR sensors SQ-215 (Apogee Instruments Inc., USA) installed in analogous positions to the pyranometers. A third PAR sensor SQ-326 (Apogee Instruments Inc., USA) consisting of an array of 6 inline 0.5 m bar mounted sensors was placed under-canopy, measuring average PAR traversing the canopy. Both solar radiation and PAR sensors data was collected at 30 min intervals.

2.3 Sap flow measurements

Xylem sap flow was measured in a representative vine of each sub-treatment (a total of 4 vines) by thermal dissipation also known as Granier's method (Granier 1985) with automatic data collection implemented using Campbell CR1000 dataloggers. Granier probes have two main components: an upstream reference needle containing a copper/constantan thermocouple (T-type) and a downstream needle housing both a second thermocouple and a *nichrome* wire heater. The constantan ends of the two thermocouples on both needles are wired to allow the measurement of the temperature difference between the thermocouples with a single voltage reading at their copper ends.

When inserted in the xylem, the downstream needle is heated using a constant power supply (~0.2 W) while the upstream needle measures the reference temperature of the xylem tissue. Sap flow density (F_s) ($cm^3\ cm^{-2}\ h^{-1}$) is therefore estimated by using the temperature difference between the heated needle and the reference needle.

Granier probe needles were inserted radially in bark cleared stem 50 mm vertically apart in 30 mm deep holes made with a 1.8 mm diameter drill. Prior to insertion, the probes were dipped in thermal silicon grease sealing possible voids between the xylem and the probe. An aluminum foil collar was placed around the probe installation section of the stem to minimize the chance of direct sunlight-induced temperature gradients.

Readings of the sap flow probes corresponding to the thermocouple 30 min average of sample readings of temperature differentials were stored and used for continuous computation of plant transpiration. Collected data was then processed by a spreadsheet to compute the stem sap flow density, F_s ($cm^3\ cm^{-2}\ h^{-1}$) using the following empirical equation:

$$F_s = 0.0119K^{1.231} \times 3600 \tag{3}$$

The K coefficient is a dimensionless flow index coefficient given by Eq. 4, where dT is the actual temperature difference (°C) between the upstream unheated (lower) needle and the downstream (upper) heated needle and dT_M is the temperature difference (°C) at night in zero or minimum sap flow conditions ($Fs \approx 0$).

$$K = (dT_M - dT)/dT \tag{4}$$

The difference in temperature dT between the two needles in the Granier probe is dependent on sap velocity in the stem dT_M and was considered equal to dT: ($dT_M = dT$) when no sap is flowing (between 11:00 pm and 4:00 am). Hourly sap flow, F (L h^{-1}) can be estimated from the area of the stem A (cm^2) without bark at the position of the heated needle:

$$F = F_s \times A \tag{5}$$

Accumulated daily sap flow, F_{daily} (L $vine^{-1}$ day^{-1}), equivalent to daily vine transpiration T, was computed by estimating the area below the resulting F curve by integration.

To analyze short-term vine transpiration response to the irrigation stress and resume in ST1 sub-treatments in both T100 and T70, two consecutive 10-day periods were considered encompassing: (i) the 10 day-period of irrigation stress just before irrigation resume in ST1 between 22 July and 1 August (DOY 203–213); and (ii) the first 10-day period after irrigation resumed between DOY 213 and 223. This analysis was performed using 30 min interval datasets of measured sap flow (cm^3 cm^2 h^{-1}) along with climate and atmospheric drivers of transpiration: Solar radiation (Wm^{-2}), PAR ($\mu mol\ m^{-2}\ s^{-1}$) and VPD (kPa).

2.4 Physiological measurements

Sun-exposed (transpiring) leaf water potential, Ψ_{leaf} was measured on mature leaves from vine's outer canopy approximately at solar noon (12:00 pm to 13:30 pm), from seven different plants at each sub-treatment, using pressure chamber (Soil Moisture Equipment Corp., USA). Stomatal conductance (g_s) was measured regularly at solar noon with a steady-state Delta-T AP4 leaf porometer (Delta-T Devices Ltd., UK). Values of g_s were collected as the average of measurements on three unshaded leaves in each seven consecutive vines for each irrigation treatment.

2.5 Harvest berry qualitative analysis

Measurements of berry weight (g), total soluble solids, TSS (°brix), and total titratable acidity, TTA (% tartaric acid), were performed in a sample of 30 berries per irrigation sub-treatment, picking 6 berries from clusters of 5 different vines in the middle rows of each sub-treatment immediately before harvest, on day of year (DOY) 244. Total soluble solid (TSS) content in the juice was determined using hand refractometer (Atago Model ATC-1E, USA) and titratable acidity (TTA) measured using potentiometric method, in which berry juice was titrated with 0.1 N, NaOH solution. Studies have shown that the optimum harvesting moment is reached when the total soluble solid (TSS) approaches 22 percent (Mehmandoost et al. 2008). The technical maturity index (TMI) was estimated as the quotient TSS/TTA. Until optimum maturity point, TTS and TMI increase while TA is expected to decline (Almanza et al. 2010).

2.6 Statistical analysis

Statistical analysis of leaf water potential (Ψ_{leaf}) measurements were performed in a spreadsheet using two-tailed distribution *t* test, considering as the null hypothesis that the means of two populations are equal and a significance level of 5 % ($p \leq 0.05$).

The relationship between midday VPD and (Ψ_{leaf}) sap flow and also short term (30 min intervals) of sap flow versus measured climate variables (PAR, VPD, Solar Radiation) was estimated using the spreadsheet's linear regression and determination coefficient (R^2). The determination coefficient was used in this context to provide a measure of vine response (Ψ_{leaf} and sap flow) to climate driven factors under the different irrigation treatments, assuming that water deficit conditions will be signalled by a lower R^2 as vine drought defence mechanisms such as leaf stomatal closure are triggered.

The significance analysis ($p \leq 0.05$) of sampled chemical and textural berry parameters between irrigation treatments and the berry physical and chemical parameters was performed by a Tukey HSD test of multiple comparisons, using the R statistical software v.3.1.2 (R Core Team 2014) and the *Agricolae* R package v.1.2-1 (Mendiburu 2014).

3 Results

Potential evapotranspiration (ETo), as calculated by FAO 56 method (Allen et al. 1998) averaged 4.9 mm day^{-1} during the study period (DOY 170–270) and reached its highest value (8.9 mm day^{-1}) in mid August (Fig. 1). Rain contribution to soil water refill during the growing season was negligible with the exception of a rainfall of 24 mm recorded on DOY 261.

Between DOY 170 and 270, the accumulated irrigation volume was 1165.7 L per vine on T100 ST0 and 917.1 L per vine on T100 ST1. Irrigation in T70 was approximately 30 % lower than the T100 in each of the sub-treatments due to the proportionally lower output of the drippers. For both main treatments T100 and T70, the 2-week irrigation stress period in

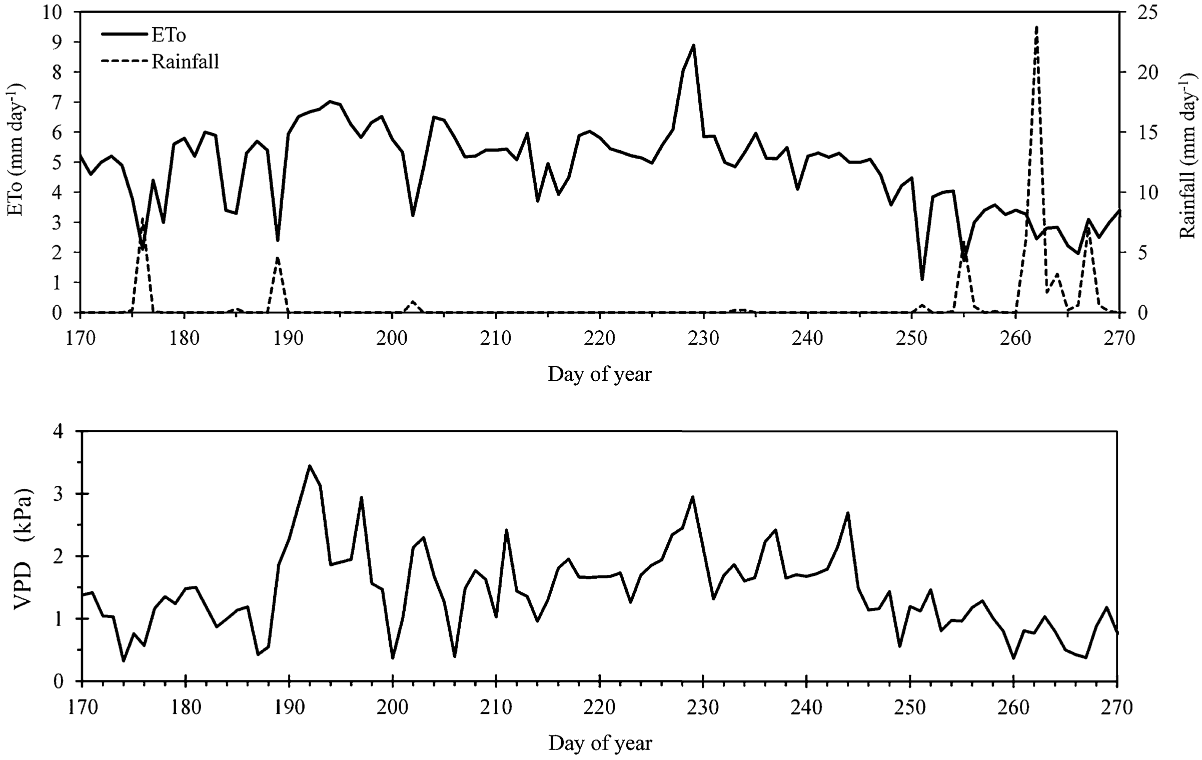


Fig. 1 Daily rainfall (mm day^{-1}), potential evapotranspiration ETo (mm day^{-1}) and midday daily VPD (kPa) between day of year 170 and 270

sub-treatment ST1 generated an accumulated reduction of 21.3 % in total application depth.

Figure 2 depicts the daily average broadband solar radiation (Wm^{-2}) above and below the vine cover and daily average photosynthetically active radiation, PAR (μmol m^{-2} s^{-1}), during the season. The broadband solar radiation reduction caused by the plastic cover, as measured by the two pyranometers, above and below the plastic film, was 28 % during the measurement period. In the same period, the average daily PAR reduction was 39.9 %, while the average PAR interception by vine's canopy, accounted as the difference between PAR below the plastic film and PAR below the canopy reached 96.8 %.

Midday Ψ_{leaf} declined throughout the season for all irrigation treatments (Fig. 3), with a sharp decrease of Ψ_{leaf} values to −1.6 MPa, immediately before harvest induced by irrigation cut-off. Comparing the two normally irrigated sub-treatments (ST0), vines under the reduced irrigation treatment (T70) were able to maintain their leaf water status at values similar to the vines under full irrigation (T100) until DOY 206, after which Ψ_{leaf} values become lower than the reference treatment. A 3-day consecutive irrigation event (DOY 204–206) caused a steep leaf water potential recovery in both treatments, with Ψ_{leaf} reaching values close to non water stressed early season conditions ($\Psi \approx -0.9$ MPa). The largest Ψ_{leaf} discrepancy between treatments T70 and T100 occurred later in the season (DOY 220–232) where a 0.2 MPa average difference was recorded.

Paired t-test on full sampled Ψ_{leaf} dataset between T70 and T100 indicate, that Ψ_{leaf} averages did not return significant differences between treatments until DOY 206 ($p = 0.829$), but from DOY 212 onwards, the difference between Ψ_{leaf} averages was significant ($p = 0.007$), considering a 5 % significance level.

Figure 4 shows the relationship between average solar noon Ψ_{leaf} and VPD between fruit set and pre-harvest (DOY 178 and 244). Daily VPD varied between 0.39 and 2.70 kPa at solar noon, and had a significant and negative correlation with Ψ_{leaf}. The treatment with full irrigation and no irrigation stress (T100 ST0) showed the highest determination

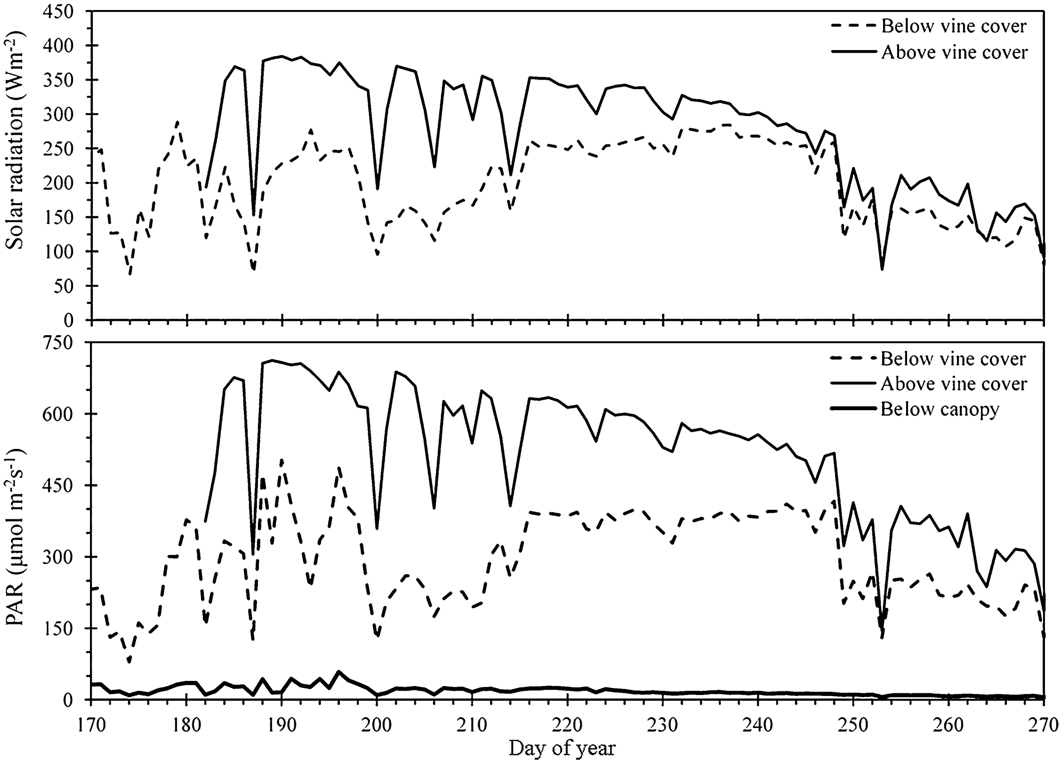


Fig. 2 Daily average broadband solar radiation measured by pyranometers (W m^{-2}) placed above and below the vinés plastic cover and photosynthetically active radiation PAR (µmol m^{-2} s^{-1}) measured above the vine cover, below vine cover, and below the vine's canopy

coefficient, $R^2 = 0.88$ when fitted with a linear function, followed by the treatment with reduced irrigation and no irrigation stress, (T70 ST0) $R^2 = 0.83$, showing that leaf water status responded directly to evaporative demand variations.

The irrigation stress in ST1 (17–31 of July) affected the relationship between average leaf water potential and vapor pressure deficit, leading to higher dispersion on the VPD versus Ψ_{leaf} plot, indicating that leaf water status becomes less responsive to VPD in case of a stress period. Additionally, the slope of the regression line changed from approximately $-3.2 \sim -3.9$ to $-1.4 \sim -1.1$, emphasizing the lack of responsiveness induced by the stress period.

Figure 5 shows sap flow density F_s ($cm^3\ cm^{-2}\ h^{-1}$) in vine stems, irrigation (L $vine^{-1}$ day^{-1}) and midday stomatal conductance (mmol m^{-2} s^{-1}) in T100 ST0 and ST1 sub-treatments (the sap flow probes used in the T100 plot failed between DOY 188 and 198). Sap flow responded very coherently to irrigation events, as these resulted in immediate peaks in the sap flow. After resuming irrigation in sub-treatment ST1 on DOY 213, both ST0 and ST1 were subject to equal irrigation and while the ST0 sub-treatment kept similar sap flow rates as in the previous interval, the sap flow in the ST1 sub-treatment increased by 70 % when compared to the previous period. The sudden raise of sap flow density observed on DOY 261 was likely due to the occurrence of the first significant rainfall event.

Similarly, Fig. 6 depicts the time-course of stem sap flow density, irrigation and stomatal conductance for the T70 ST0 and ST1 sub-treatments. Sap flow presented very similar values throughout the season, but during the irrigation stress period in treatment T70 ST1, sap flow decreased and reached a local minimum of 4.27 cm^3 cm^2 h^{-1} in DOY 210, while in the same period T70 ST0 kept sap flow densities within 6.46 and 10.30 cm^3 cm^2 h^{-1} range. Here also the stress period caused a partial closure of the stomata, as indicated by the important reduction in the midday g_s during that period.

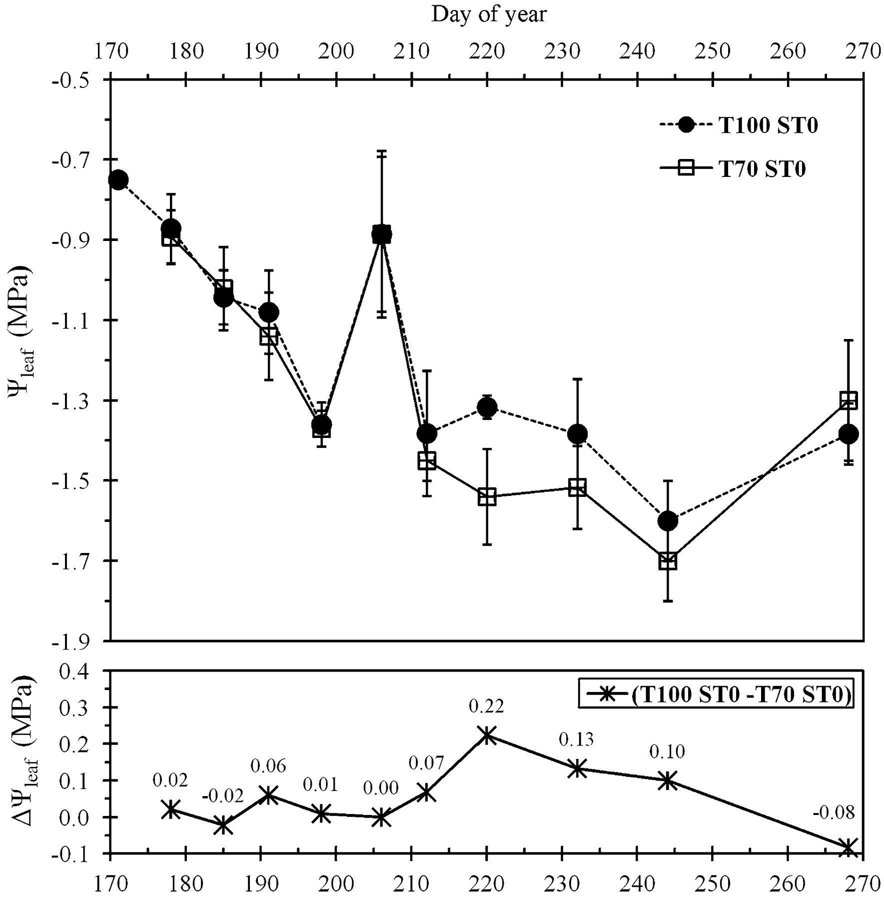


Fig. 3 *Top* Seasonal pattern of leaf Ψ (MPa) at solar noon for reference irrigation T100 and reduced irrigation T70, both under the regular farmeŕs irrigation scheme (ST0) taken from the average of seven adjacent vines of *Vitis vinifera* cv. Crimson Seedless in each plot. Error bars indicate sampled standard deviation. *Bottom* The difference in leaf water potential between the two treatments

Table 2 summarizes whole vine transpiration T (L vine^{-1} day^{-1}) expressed as equivalent to daily accumulated sap flow (F_{daily}), measured in the stem sapwood area of the sampled vines; and average daily irrigation for each sub-treatment during the irrigation period (DOY 183–253). Standard deviation of the resulting average daily values indicates that sap flow data was considerably noisier in the T100 plot than in T70, which indicates greater dependence of T on climatic factors, in fully irrigated treatments. The irrigation stress applied in the ST1 sub-treatments had a visible impact on the average daily transpiration in both the T100 and T70 treatments, although a more drastic decrease is observable in T100.

Figure 7 depicts the sap flow/climate response of T100 ST0 and ST1 in the last 10 days of irrigation stress in ST1; and Fig. 8 the 10-day period after irrigation was resumed. Similarly Figs. 9, 10 show the analogue correlation analysis between ST1 and ST0 for the reduced treatment (T70).

Figure 7 clearly shows that sap flow amplitude was limited to a lower range by the stress with an important increase in the slope of the regression line. The fully irrigated vines (T100 ST0) exhibit higher determination coefficients between the selected transpiration driver parameters and measured sap flow than the vines under irrigation stress (T100 ST1). Solar radiation datasets yielded the best overall linear regression determination coefficient with sap flow under the ST0 treatment ($R^2 = 0.80$), closely followed by the PAR fit, while the VPD generally showed to be a weaker model to explain sap flow variations.

After the resume of irrigation (Fig. 8), the linear fit between the selected transpiration driver parameters (PAR, VPD and solar radiation) and sap flow returned lower R^2, thus with higher dispersion than during the irrigation stress period. This indicates that the resume of irrigation in the T100 ST1 caused a rise in sap flow response that is greater than evaporative demand.

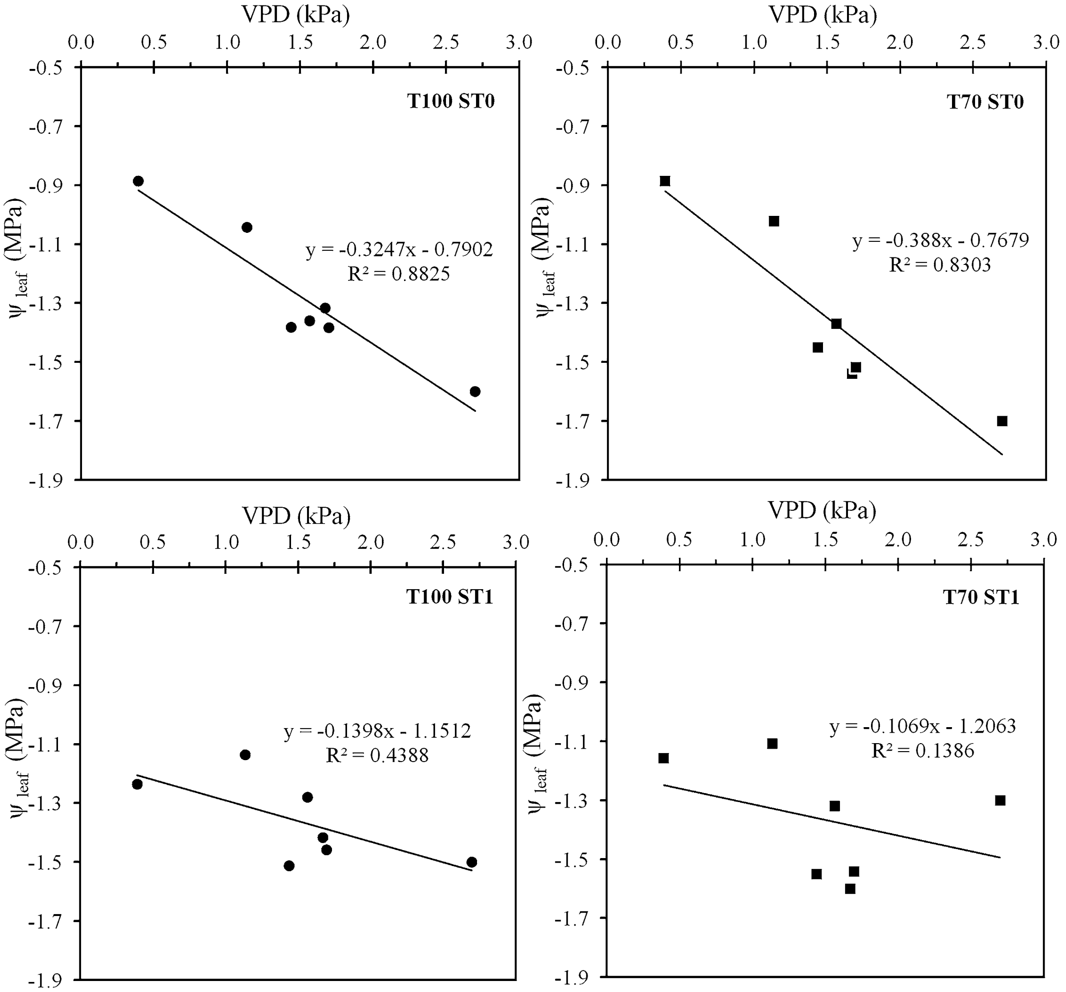


Fig. 4 Average midday leaf water potential Ψ_{leaf} (MPa) from fruit set to harvest represented in function of atmospheric vapor pressure deficit VPD (kPa) for *Vitis vinifera* cv. Crimson Seedless in T100 (*filled circle*) and T70 (*filled triangle*) for each sub treatment (ST0 and ST1)

In the reduced irrigation treatment (T70), the two corresponding 10-day period analysis (Figs. 9, 10), exhibited similar results to the fully irrigated vines (T100), in the correlation between sap flow and the transpiration driver parameters during the irrigation stress, with the non stressed treatment T70 ST0 yielding higher determination coefficients. However, after irrigation restart in T70 ST1 (Fig. 10) the linear fit between sap flow and the transpiration driver parameters returned similar R^2 values to T70 ST0 indicating that within the 10 days after irrigation restart, the vines subject to irrigation stress returned to normal sap flow patterns in response to transpiration driver parameters. This suggests that well irrigated vines under T100 treatment and reduced irrigation (V70), responded differently to the irrigation stress periods, with reduced irrigation vines being able to recover their normal sap flow response more rapidly after drought conditions.

In large commercial vineyards it might be desirable to use a set of well irrigated vines as reference for evaluating irrigation quality in the main areas of the vineyard. To evaluate this possibility, the T100 ST0 which represents the usual irrigation management of the *Vale da Rosa*, was used as the 'well irrigated' reference, and sap flow was calculated as a dimensionless ratio ($F_{sT100ST0}/F_{sx}$), where F_{sx} represents the sap flow in the different sub-treatments. The sap flow

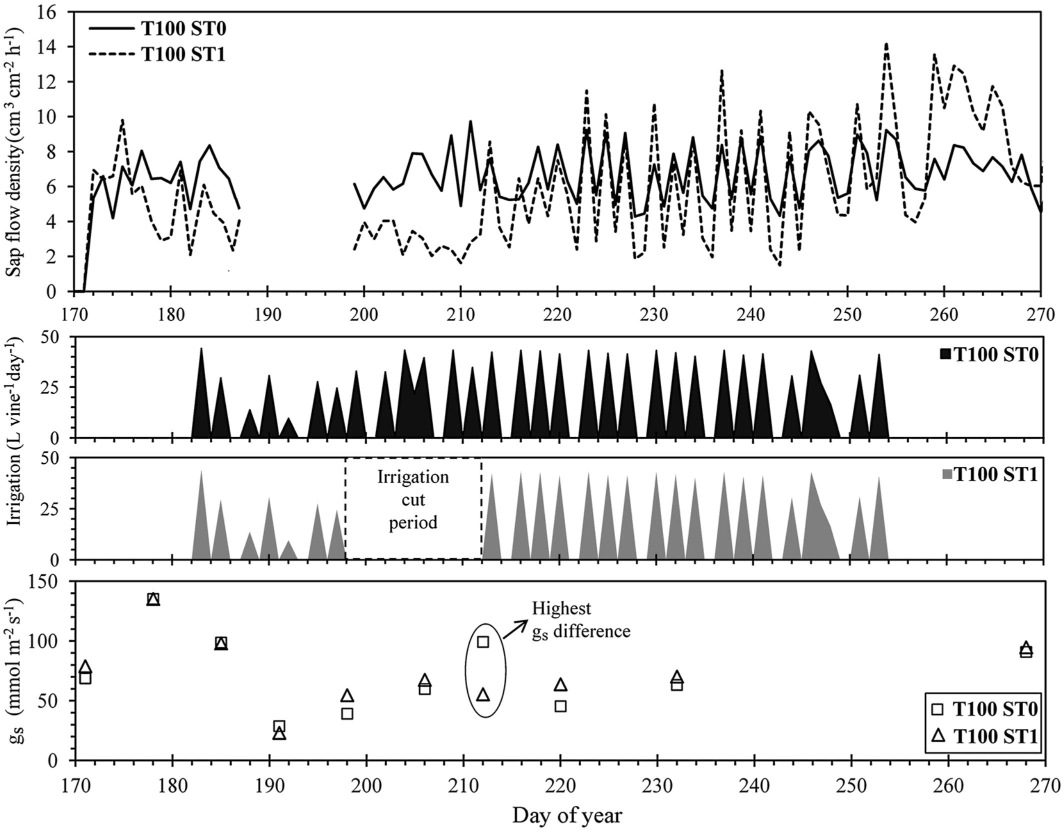


Fig. 5 Time-course of daily average sap flow density (cm^3 cm^2 h^{-1}); daily irrigation (L vine^{-1} day^{-1}) for sub-treatments ST1 (with irrigation cut) and ST0 (no irrigation cuts); and midday stomatal conductance g$_s$ (mmol m^{-2} s^{-1}) on both sunlit and shadowed leaves of *Vitis vinifera* cv. Crimson Seedless in treatment T100

ratio was computed from average daily sap flow density (cm^3 cm^2 h^{-1}) in 30 min interval readings instead of daily vine transpiration (L day^{-1}), allowing a comparison independent of stem diameter differences between individual vines. The results are presented in Fig. 11, where the dashed line at value 1 of the vertical axis represents the reference treatment (T100 ST0), and values above the reference indicate sap flow densities lower than reference.

The T70 ST0 reduced irrigation treatment returned sap flow ratios distributed within a 0.5 band above and below the reference line suggesting that a 30 % reduction in irrigation caused little difference in the time course of sap flow during the season in comparison with the reference treatment vine.

The treatments with irrigation stress (ST1) clearly exhibited a different behaviour with sap flow ratios reaching persistently higher values than the reference vine. In the T70 ST1, sap flow had the highest ratios, most noticeably after the irrigation stress period (DOY 198–212). The time lag between the irrigation stress and the sap flow ratio increase indicates that the soil water reserve was enough to maintain a sap flow rate similar to the reference vine until DOY 210 where the sap flow ratio of T70 ST1 reached a distinctly higher value of 2.28. The sap flow ratio of the T100 ST1 treatment returned the highest dispersion from the reference line, however it is possible to identify a similar sap flow ratio behaviour as the T70 ST1 with the irrigation stress causing a steep raise in the sap flow ratio.

Figure 12 depicts the postharvest mean and standard deviation of berry physical and chemical attributes, and the results of Tukeýs multiple comparison statistical significance test ($p \leq 0.05$) for each sub-treatment. The T70 ST0 treatment, despite high sample dispersion, featured the highest mean berry weight (7.48 g) followed by the reference treatment with 7.32 g. The 2-week stress period had a significant

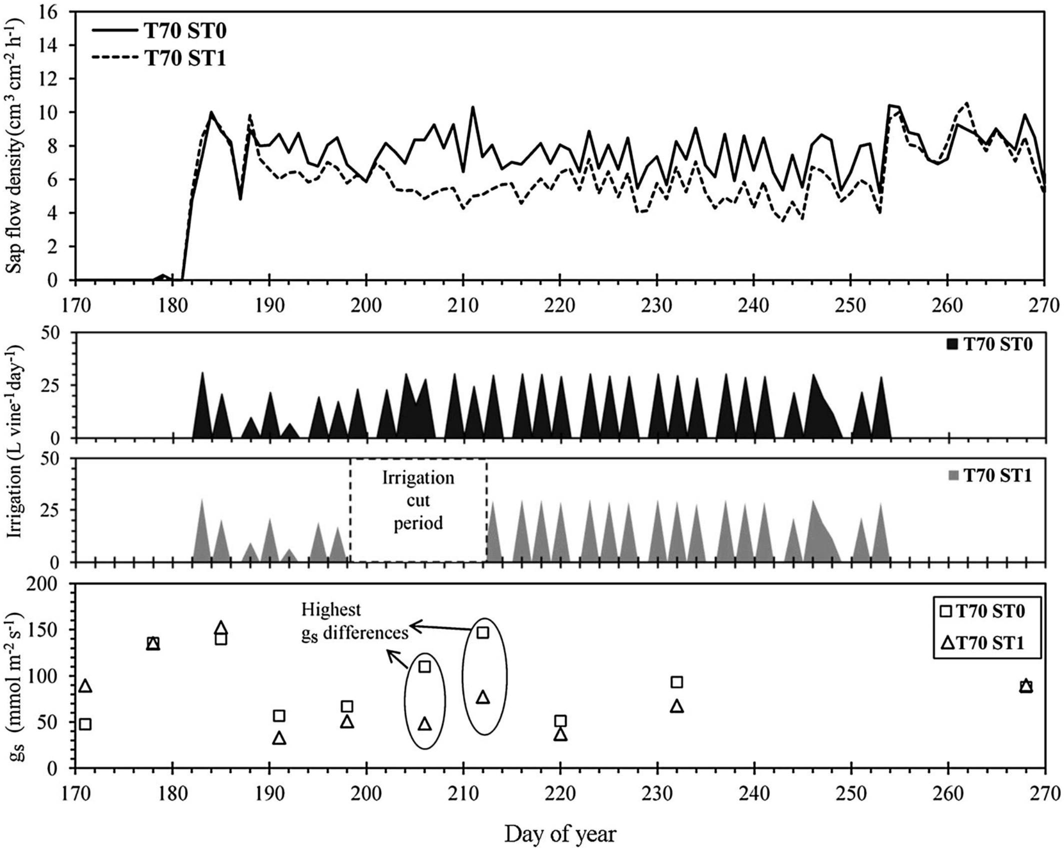


Fig. 6 Time-course of daily average sap flow density (cm^3 cm^2 h^{-1}); daily irrigation (L vine^{-1} day^{-1}) for sub-treatments ST1 (with irrigation cut) and ST0 (no irrigation cuts); and midday stomatal conductance g_s (mmol m^{-2} s^{-1}) on both sunlit and shadowed leaves of *Vitis vinifera* cv. Crimson Seedless in treatment T70

Table 2 Summary stem sapwood area on the monitored vines (cm^2); maximum, average and standard deviation of average daily transpiration T (L vine^{-1} day^{-1}) estimated from sap flow measurements on the monitored vines; and season's average daily irrigation (L vine^{-1} day^{-1}) between DOY 183 and 253 for each treatment

	T100 ST0	T100 ST1	T70 ST0	T70 ST1
Stem sapwood area (cm^2)	45.84	37.13	32.15	31.51
T, Maximum (L vine^{-1} day^{-1})	10.70	11.25	7.79	7.58
T Average (L vine^{-1} day^{-1})	7.31	4.46	5.66	4.47
T Standard deviation (L vine^{-1} day^{-1})	1.73	2.73	0.89	0.98
Irrigation (L vine^{-1} day^{-1})	16.4	12.9	11.5	9.0

($p \leq 0.05$) effect on the berry weight, indicating the detrimental effect of reduced irrigation combined with a period of water stress. In the case of full irrigation, the stress period did not have any significant effect on the berry weight.

Vines under reduced irrigation (T70 ST0 and T70 ST1) yielded grapes with higher mean sugar content (18.22° and 17.80° Brix) than the vines under full irrigation (17.23° and 15.85° Brix). In terms of the stress period, the normally irrigated vines, under sub-treatments ST0 returned berries with higher sugar content than vines subjected to irrigation stress (ST1).

Reduced irrigation also resulted in lower percentage of tartaric acid, which combined with higher sugar content results in higher technical maturity index (TTS/TTA).

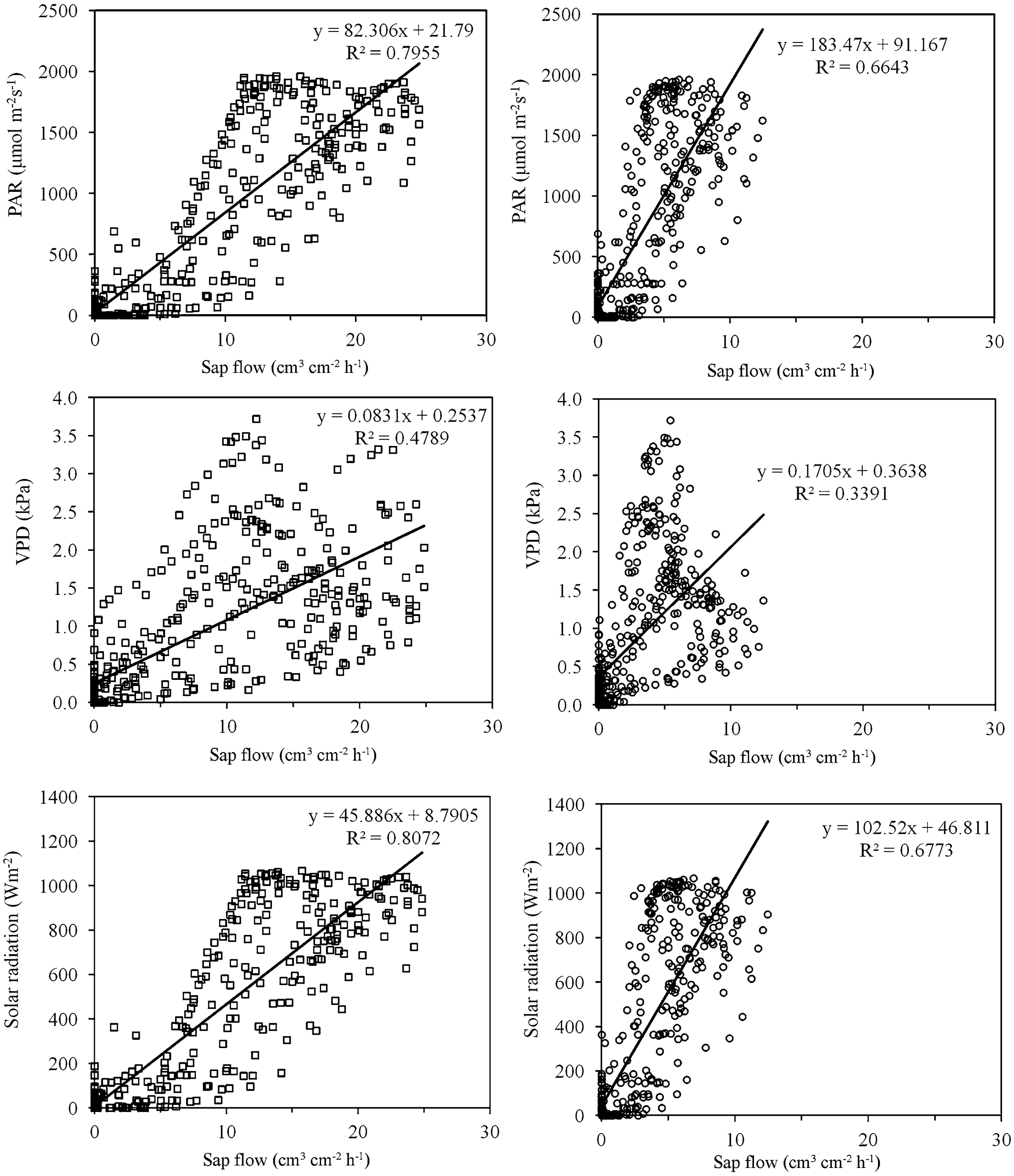


Fig. 7 Correlation between sap flow of *Vitis vinifera* cv. Crimson Seedless and climate variables PAR, VPD and Solar radiation for T100 ST0 (*square*, *left*) and T100 ST1 (*circle*, *right*) during the last 10 days of irrigation cut in sub-treatment ST1 (DOY 203–213)

4 Discussion

The plastic film resulted in a 28 % reduction of broadband solar radiation, and 39.9 % of PAR. These results are in agreement with those of Rana et al. (2004) who observed a 25 % lower PAR in a vineyard under plastic cover, when compared to uncovered vineyard. The percentage of interception of solar radiation by the plastic cover decreased along the season, which is probably caused by the gradual change in the solar zenith angle. This can be a very interesting advantage of using this type of covering for the production of Crimson Seedless grapes which is challenging in terms of obtaining adequate fruit colour

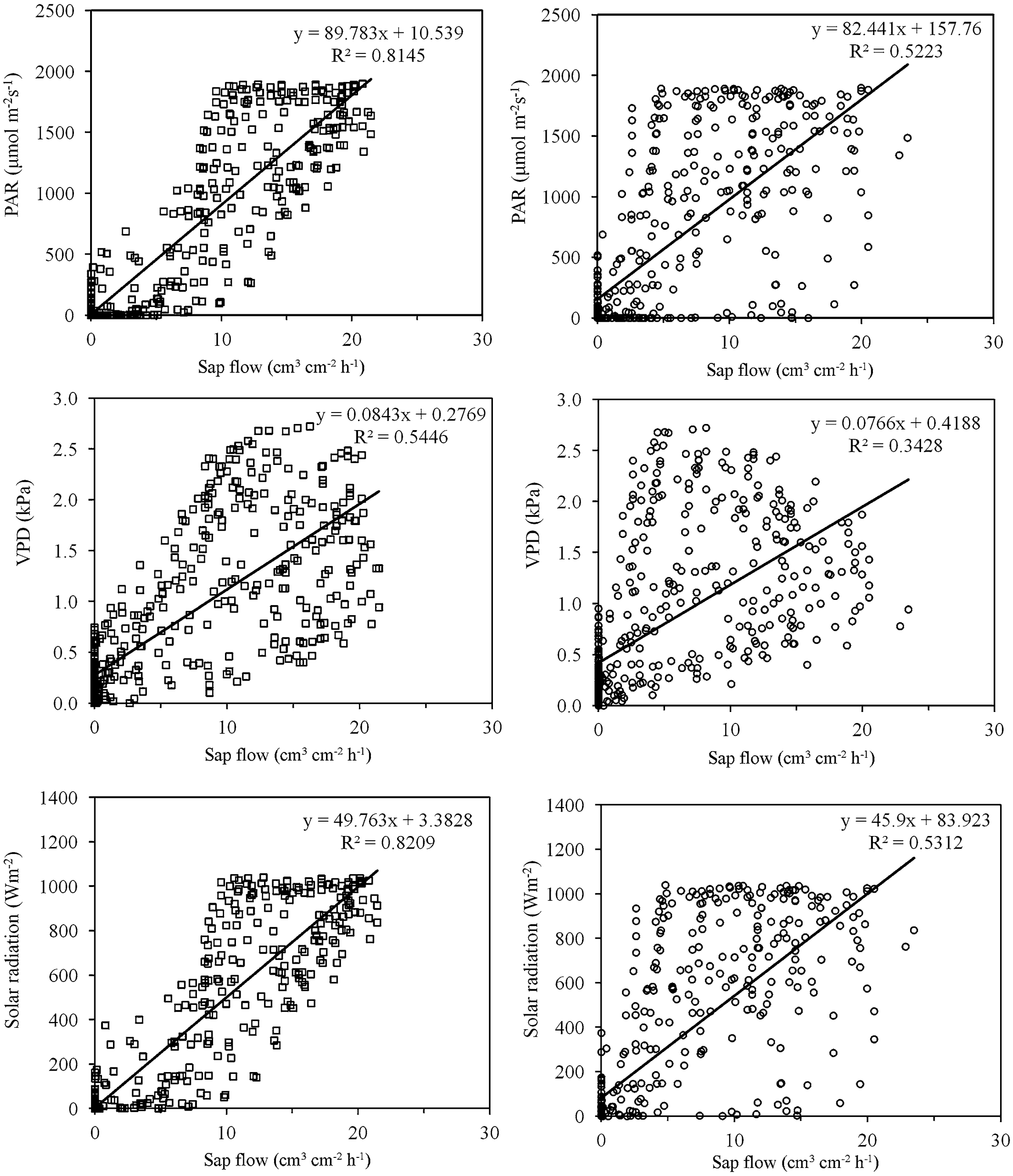


Fig. 8 Correlation between sap flow of *Vitis vinifera* cv. Crimson Seedless and climate variables PAR, VPD and Solar radiation for T100 ST0 (*square*) and T100 ST1 (*circle*) during the 10 days after irrigation restart in treatment ST1, (DOY 213–223)

for harvest, as it actually provides an increase in incident solar radiation as the berries approach maturation.

The results showed that Crimson Seedless vines under the reduced irrigation treatment were able to maintain their leaf water status at values similar to the vines under full irrigation until DOY 206, after which Ψ_{leaf} values become lower than the reference treatment. Based on their water potential behaviour in response to water stress, grapevine cultivars have been classified as isohydric or anisohydric (Vandeleur et al. 2009). Isohydric cultivars are those that keep their leaf water potential above a certain threshold regardless of soil water availability or atmospheric water demand. In isohydric grapevines, leaf water potential rarely drops below −1.5 MPa. Some *Vitis*

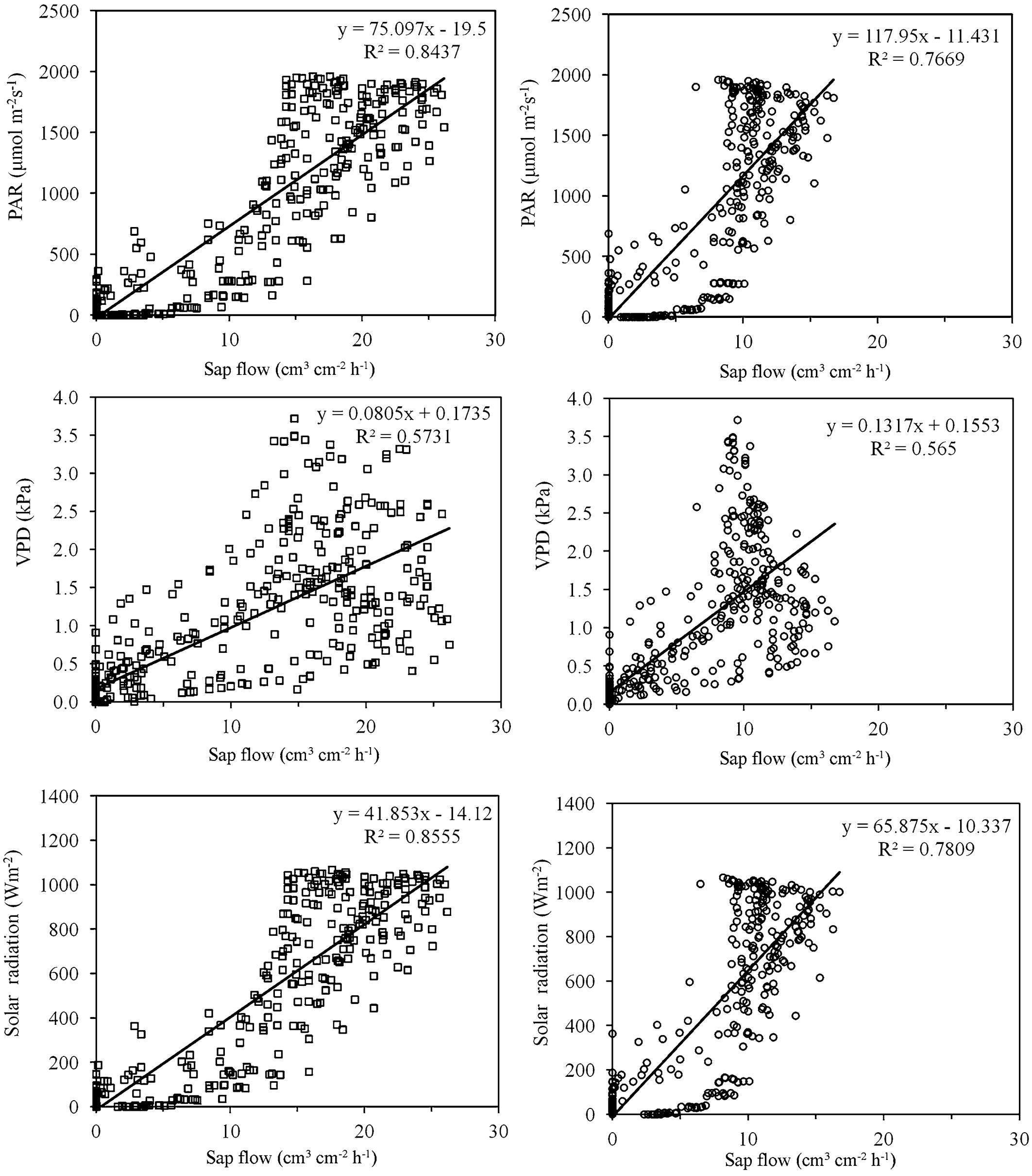


Fig. 9 Correlation between sap flow of *Vitis vinifera* cv. Crimson Seedless and climate variables PAR, VPD and Solar radiation for T70 ST0 (*square*) and T70 ST1 (*circle*) during the last 10 days before irrigation restart in treatment ST1 (DOY 203–213)

vinifera L. cultivars of contrasting genetic origin show very different responses of Ψ_{leaf} during water stress (Schultz 2003) and it has been shown that anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour (Pou et al. 2012; Tardieu and Simonneau 1998). The results obtained in this work indicate that Crimson Seedless behaves initially as an isohydric cultivar and then after DOY 206, as an anisohydric variety whose Ψ_{leaf} is lower in drought than in watered plants. Some other cultivars, such as Pinot Noir behave as anisohydric when water stress is applied pre-*veraison* and as isohydric when it is applied post-*veraison* (Poni et al. 1993).

A good linear relationship ($R^2 = 0.88$) was found between midday VPD and ψ_{leaf} in the grower's normal irrigation scheme. Thus, it can be concluded that under full irrigation, leaf water status, as measured by

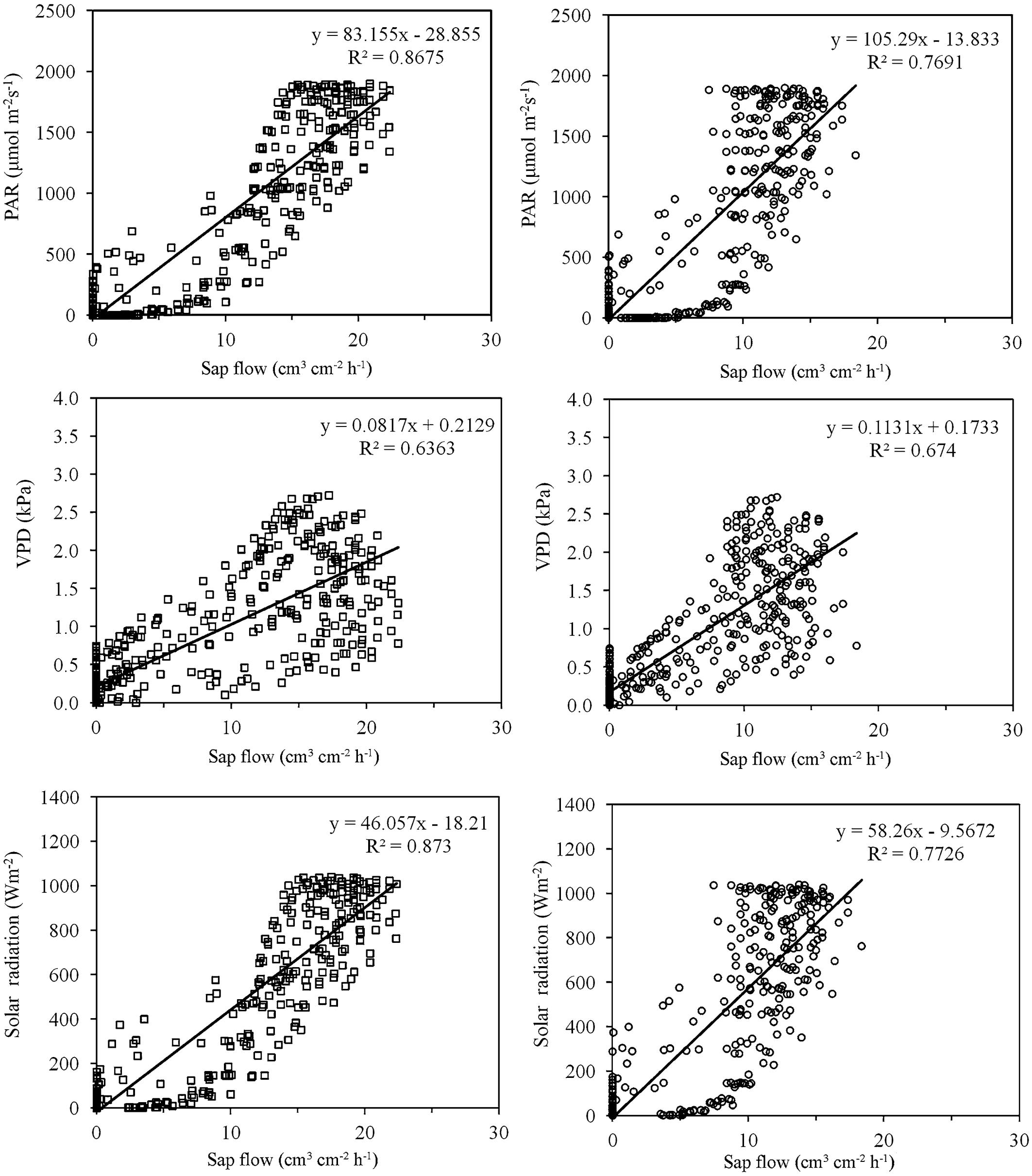


Fig. 10 Correlation between sap flow of *Vitis vinifera* cv. Crimson Seedless and climate variables PAR, VPD and Solar radiation for T70 ST0 (*square*) and T70 ST1 (*circle*) during the 10 days after irrigation restart in treatment ST1 (DOY 213–223)

midday leaf water potential responds directly to evaporative demand variations. Williams and Baeza (2007) found a similar relationship in a study focusing on four varieties and five different sites but using stem water potential instead of Ψ_{leaf}. The normal physiological response of the vine to VPD was little affected by a sustained water stress (30 % reduction). However, when a period of post-*veraison* irrigation stress was applied, the relationship between average leaf water potential and vapor pressure deficit were noticeably affected, indicating physiological response to water deficit, and lower responsiveness of the vines to VPD after a period of stress.

After resuming irrigation in sub-treatment T100 ST1 on DOY 213, the sap flow increased by 70 % when compared to the previous period. The resume of irrigation caused a rise in sap flow response that is not only due to climate driven effects but also possibly to

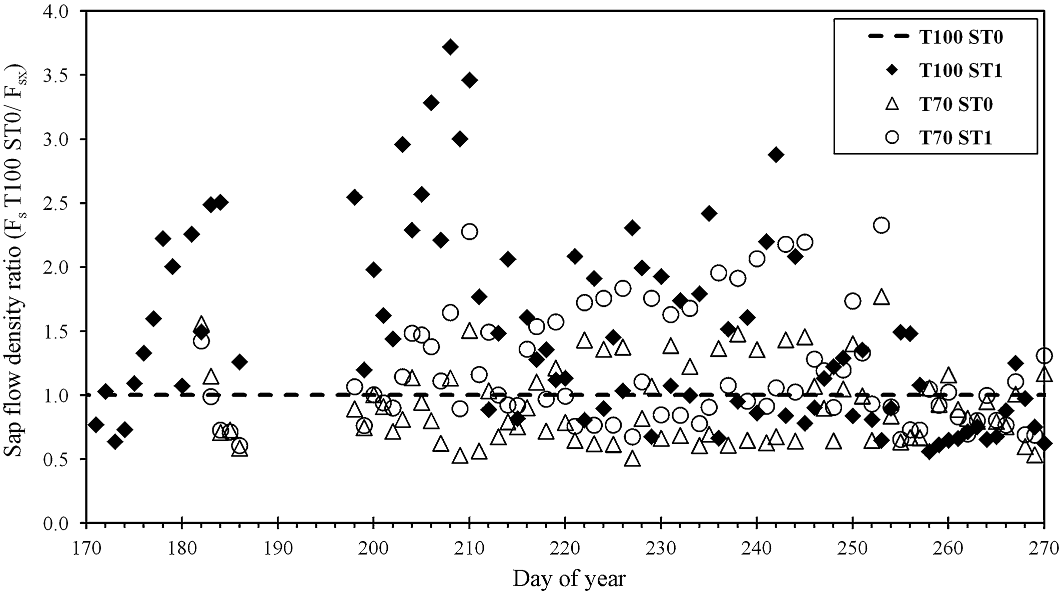


Fig. 11 Time-course of daily average sap flow density ratios (Fs T100 ST0/Fsx) where Fsx irrigation treatments are T100 ST1, T70 ST0 and T70 ST1, using T100 ST0 as reference for 'well irrigated conditions'

the xylem vessel refilling. This effect of sudden sap flow rise after resuming irrigation indicates that vines under mild water deficit, when re-supplied with water can promptly increase their sap flow rate to allow faster recovery of plant water potential. Gómez-del-Campo et al. (2007) also found that 3 days after rewatering leaf conductance and transpiration were significantly higher in vines previously subjected to water stress. Net photosynthesis was also significantly higher in water-stressed vines 5 days after rewatering.

The midday g_s exhibited a higher difference between irrigated and non irrigated vines during the irrigation stress periods, signaling that the stomata had partially closed to control leaf water loss, thus reducing transpiration.

As in the case of midday leaf water potential, sap flow data was considerably noisier in the T100 plot than in T70, which indicates greater dependence of transpiration on climatic factors, in fully irrigated treatments. The irrigation stress applied in the ST1 sub-treatments had a visible impact on the average daily transpiration in both treatments, although a more drastic decrease is observable in T100.

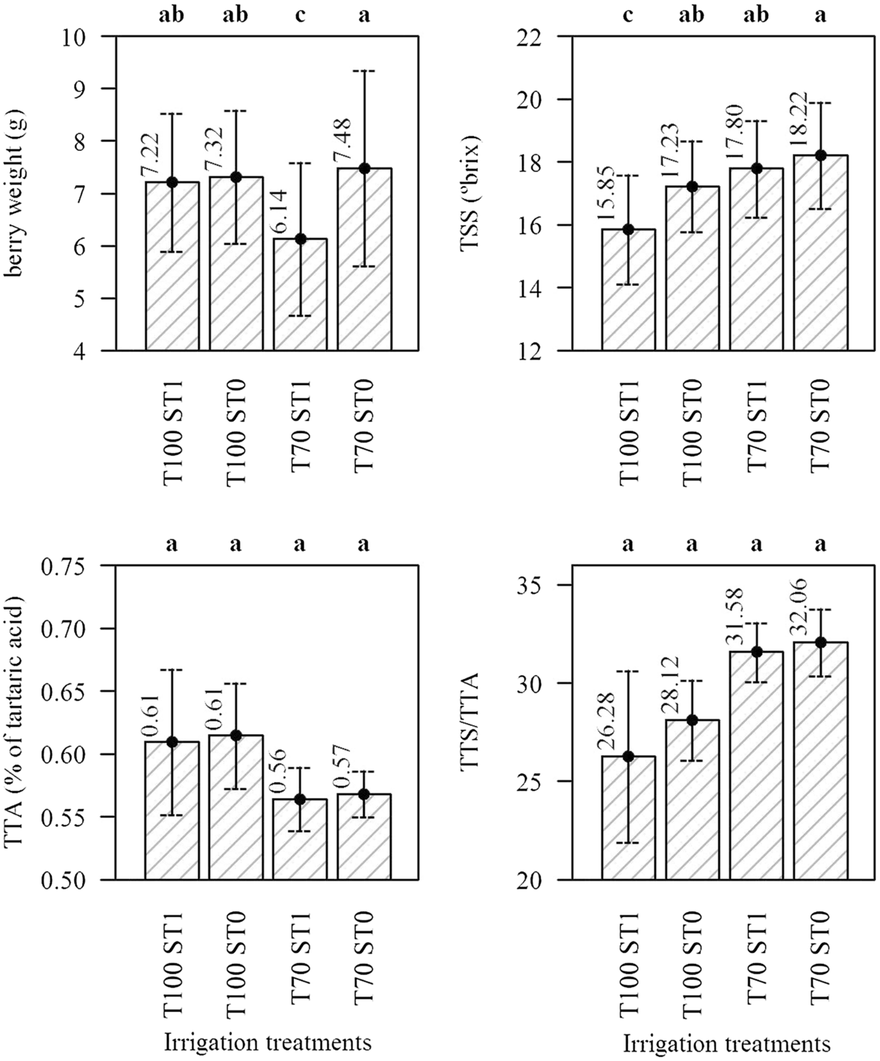
In T70 the scarce difference in transpiration between sub-treatments ST0 and ST1 suggest that due to the reduced irrigation treatment, transpiration was already being limited by lower soil water content, thus, below its potential maximum, therefore the irrigation stress applied in ST1 had a less significant impact.

It is well known that increased leaf and fruit exposure to sunlight generally improves vine yield, and berry composition (Carbonneau 1995). In all treatments, the correlation of sap flow with solar radiation was slightly higher than with PAR, which was probably due to the influence of total solar energy on transpiration.

Post-harvest analysis showed that a continuous 30 % reduced irrigation (T70 ST0) resulted in no significant ($p \leq 0.05$) mean differences in berry size when compared with vines under the grower's normal irrigation scheme (T100 ST0). But the reduced irrigation treatment yielded grapes with higher mean sugar content (18.22° and 17.80° Brix) than full irrigation (17.23° and 15.85° Brix). Reduced irrigation also resulted in lower percentage of tartaric acid, which combined with higher sugar content resulted in higher technical maturity index (TTS/TTA). This indicates faster maturity of these grapes due to the reduced irrigation treatment, and thus the advantage of implementing a stricter control over irrigation.

Irrigation sub-treatments with stress periods (ST1), showed significant decrease in mean berry weight, and

Fig. 12 Post-harvest berry physical and chemical mean attributes of *Vitis vinifera* cv. Crimson Seedless for all irrigation treatments: berry weight (g), Total soluble solids (TSS), Total titrable acidity (TTA) and Technical maturity index (TMI) represented as the TTS/TTA quotient. Mean attribute values (represented above *bars*). *Distinct letters* represented in *bold* on *top* of each chart's box indicate significantly different means according to Tukey's HSD test (p ≤ 0.05). Error *bars* indicate standard deviation



thus in marketable fruit. In terms of chemical attributes, these sub-treatments resulted in a reduction in TTS and an increase in TTA. Thus the stress period resulted actually in reduction in the technical maturity index and a delay in the maturity and did not fulfil the proposed objective. These results indicate the need to further parameterize and detail irrigation management criteria influencing application rate and stress periods to infer possible techniques to balance fruit quality and control harvest timings while applying water saving practices.

The sap flow dimensionless density ratio showed the ability to signal differences in the transpiration rates between vines with different irrigation treatments, where vines under irrigation reductions or stress contrasted with reference 'well irrigated' vines. Although further research is necessary to find the allowable level of irrigation reduction at non-critical stages of vine growth, the use of sap flow ratio between well irrigated reference vines and vines under reduced irrigation can potentially contribute to water savings, triggering irrigation events only at a previously defined critical threshold.

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