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Assessment of the Type of Deficit Irrigation Applied during Berry Development in 'Crimson Seedless' Table Grapes

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Abstract: This work assessed the effects of the sustained (during the whole berry growth) and regulated (at post-veraison) practices of deficit irrigation on water relations, yield components and berry quality in a commercial vineyard of 'Crimson Seedless' table grapes. For this, five irrigation treatments were established during a complete irrigation season (from April to October): (i) Control (CTL) irrigated to 110% crop evapotranspiration (ET_c); (ii) Regulated Deficit Irrigation (RDI) irrigated at 50% of CTL during the non-critical period of post-veraison; (iii) Sustained Deficit Irrigation (SDI), irrigated at 50% of CTL throughout the entire berry growing season; (iv) Partial Root-Zone Drying (PRD), irrigated similar to RDI but alternating the irrigation applied on the dry side every 10–14 days; (v) Sustained Partial Root-Zone Drying (SPRD), irrigated as SDI but alternating the irrigation on the dry side every 10–14 days. RDI and PRD received 24% and 28% less water than CTL, respectively. These reductions were higher in SDI and SPRD (65% and 53%, respectively). Total yield was not affected by any DI strategy. Only significantly lower productive values were observed in the weight and height of the berries as compared to CTL. However, the color parameters evaluated increased in all the DI treatments, being slightly higher in SDI and SPRD as compared with RDI and PRD. In addition, total soluble solids (TSS) were significantly higher in SDI, compared to other irrigated counterparts. Our findings showed that the application of water deficit during the entire period of berry growth using SDI and SPRD can be considered for irrigation scheduling in 'Crimson Seedless' table grapes when the aim is to solve the trouble of insufficient reddish color of the berries.

Keywords: berry color; Partial Root-zone Drying (PRD); Regulated Deficit Irrigation (RDI); Sustained Deficit Irrigation (SDI); veraison; *Vitis vinifera*; water use efficiency

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1. Introduction

Europe and Asia (mainly China) are the major grape producers, with approximately 40% of worldwide production. Spain is one of the most important producers of table grape cultivars in Europe, and the Region of Murcia is the largest in Spain, with 210,105 t cultivated per year, representing 65% of the production in Spain [1]. Indeed, the observed amount is mainly due to the establishment of new seedless varieties which have increased consumer acceptance [2–6].

The annual growth of vines is frequently described using the following stages: (1) budburst; (2) flower cluster initiation; (3) flowering; (4) fruit set; (5) berry development; (6) harvest; (7) dormancy [7]. Moreover, in red-purple cultivars, such as 'Crimson Seedless', the 'veraison' period takes place when the berry color changes and the ripening process begins [2,3,6] between stages (5) and (6). Recent reports on this cultivar have demonstrated that the period from the onset of berry color-change to harvest, known as 'post-veraison', is not sensitive to deficit irrigation (DI) [2–6,8]. Temnani et al. [6] confirmed, in a 3-year experiment, that post-veraison was a non-critical period for planning DI strategies in 'Crimson Seedless' table grapes. The authors reported irrigation water savings of more than 50%

during this period, and 30% over the entire crop cycle, with respect to well-irrigated vines. Indeed, reductions in midday stem water potential of 0.2 MPa during pre-veraison promote reductions in berry growth, whereas reductions of 0.3 MPa post-veraison did not have any negative effect on yield components or berry quality [4,5]. Moreover, the application of DI during post-veraison can improve berry quality in 'Crimson Seedless' table grapes through an increase in the red-berry color (regarding intensity and uniformity) which represents the most important drawback of this cultivar in terms of marketability [2–6,8]. In table grapes, Luquet et al. [9] indicated that DI applications could affect the returns of investment, by improving fruit quality due to the increasing sale prices for grapes.

In table grapes, the most common methods utilized for applying deficit irrigation to save water, increase water use efficiency, and manipulate vegetative and reproductive growth are regulated deficit irrigation (RDI) [10] and partial root zone drying (PRD) [11]. RDI is based on imposing water deficit during the ripening stages, with minimal effects on yield and quality, known as non-critical periods which, in the case of 'Crimson Seedless', coincides with post-veraison. On the other hand, PRD is the deliberate wetting and drying of alternate sites of the root zone so that the production of specific root-sourced chemical signals (e.g., abscisic acid) are optimized, inducing partial stomatal closure and thereby increasing water use efficiency [12,13]. Other studies on the same experimental plot, on the comparison of PRD and RDI applied in post-veraison, when both received the same irrigation, revealed that PRD enhanced berry coloration and health-promoting bioactive compounds [5], although it also has a worse post-harvest performance due to higher berry shattering [4] than RDI. El-Ansary et al. [14] compared the effects of moderate (irrigation 2 days after soil water potential reached −15 kPa) and severe post-veraison RDI (irrigation 4 days after soil water potential reached −15 kPa) with respect to Control vines, on the quality of 'Muscat of Alexandria' table grapes. They found that the moderate RDI had no effects on berry weight or juice quality at harvest. Nonetheless, the severe RDI decreased berry size, firmness, and acidity, and increased total soluble solids of the berries. Nevertheless, until now, no information has been found in the literature about the comparison between RDI and PRD when they are applied during the entire berry growth period, between stages (4) and (6), and only during post-veraison, corresponding with the non-critical period in 'Crimson Seedless' grapes, and neither the possible involvement in the berry color nor yield components. In this sense, the main goal of this work was to compare the effects of the DI treatments that were applied: (i) regulated: at post-veraison and (ii) sustained: during the entire berry growth period, with a different field experimental design based on one single-line of emitters (RDI management) and two-lines of emitters (PRD management). All DI treatments were compared with a Control treatment that received the full vine water needs throughout the irrigation season. Furthermore, the findings established guidelines for table grape farmers for how to proceed with similar Mediterranean experimental conditions.

2. Materials and Methods

2.1. Experimental Site

This research was carried out in a commercial table grape (*Vitis vinifera* L.) 'Crimson Seedless' orchard located in Southeastern Spain (38°15′ N; 1°33′ W), during the irrigation season (from April to October). The vines (14-years old) were grafted onto 1103-Paulsen rootstock and spaced at 4 × 4 m. The orchard was managed according to standard cultural practices of the commercial orchard in terms of fertilization, girdling, berry thinning, and winter pruning. The soil texture class was silty clay loam (37% clay, 46% silt, and 17% sand) with a bulk density of 1.25 g cm^{-3}, 2.1% organic matter content, and soil pH of 8.6. Field capacity and wilting point were reached at 0.34 and 0.18 m^3 m^{-3}. Below 60 cm, the substrate was mainly hard clay. The climate of the area was Mediterranean semiarid, with hot dry summers (maximum air temperature 39 °C) and mild winters (average temperature 8 °C). Average annual rainfall and reference evapotranspiration (ET_0) for the last five years amounted to 250 and 1320 mm, respectively. More details about the experimental site, soil characteristics, climate parameters, fertilization, and cultural practices are described in [15].

2.2. Irrigation Treatments

In this work, both RDI and PRD strategies were applied at two different times during a complete growing season (April to October): (1) at post-veraison (June to October); (2) during the entire berry growth period (including pre- and post-veraison, from April to October). Therefore, five irrigation treatments were imposed: (i) a Control (CTL), irrigated to 110% crop evapotranspiration (ET_c) throughout the entire season; (ii) Regulated Deficit Irrigation (RDI), irrigated at 50% of CTL during the non-critical period of post-veraison, (iii) Sustained Deficit Irrigation (SDI), irrigated at 50% of CTL throughout the entire berry growing season; (iv) Partial Root-zone Drying (PRD), irrigated similar to RDI, but alternating the irrigation applied on the dry side every 10–14 days when 75% of the soil field capacity (~34%, determined as gravimetric sampling) was reached in the dry root-zone; and (v) Sustained Partial Root-zone Drying (SPRD), irrigated as SDI but alternating and applying the same threshold criteria on the dry-zone than PRD. In CTL, RDI, and SDI the irrigation system consisted of one drip line in each vine row, with four self-compensating drippers (4 L h^{-1}) 0.50 m apart, while in the PRD and SPRD treatments, two drip lines with two drippers (4 L h^{-1}) were used per vine on each side of the root system. The volume of water applied in each irrigation treatment was measured by in-line water meters with digital output pulses by installing one per replication (n = 4 per treatment). Crop evapotranspiration ($ET_c = ET_0 \times K_c$) was estimated using crop coefficients (K_c) based on Williams and Ayars [16], varying from 0.2 to 0.8 according to the phenological stage, whereas reference crop evapotranspiration (ET_0) was calculated with the Penman Montheith-FAO method [17], with daily climatic data recorded by an automatic weather station of the Servicio de Información Agraria de Murcia, located 8.5 km from the experimental plot [18]. Climatic data was computed as an average of the 7 previous days.

More details of the experimental design, water characteristics, field conditions, crop coefficient (K_c), and methodology used to calculated ET_c has been previously described in Conesa et al. [4,15].

2.3. Soil and Vine Water Relations

Soil volumetric water content (θ_v) was measured from 10 cm down to a maximum depth of 100 cm, every 10 cm, with a frequency domain reflectometry (FDR) portable probe model Diviner 2000® (Sentek Pty. Ltd., Adelaide, Australia). As 90% of active roots were located within a depth from 20 to 50 cm, this profile was used to obtain θ_v. One access tube per replicate (n = 3 per treatment) was installed within the dripper wetting area on randomly selected vines. In the PRD treatment, two FDR probes on both sides of the root system were also installed per replicate (n = 6 per treatment). Measurements were taken every fortnight between 10:00 and 12:00 h GTM during the experimental period. Relative extractable water content (REW) for each irrigation treatment was also calculated from the summed θ_v with the equation defined by Granier [19]:

$$\text{REW} = (R - R_{MIN})/(R_{MAX} - R_{MIN}), \tag{1}$$

where R (%) is the actual soil water content, R_{MIN} (%) is the minimum soil water content measured in dr y conditions, and R_{MAX} (%) is the maximum soil water content obtained in each probe. The values of R_{MIN} and R_{MAX} were 14% and 41%, respectively.

Midday stem water potential (Ψ_{stem}) was monitored every fortnight with a pressure chamber Model 3000 (Soil Moisture Equipment, Santa Barbara, CA, USA) on at least two leaves per replicate (n = 8 per irrigation treatment) according to Hsiao [20]. Leaves were randomly selected from the shoots below the canopy and enclosed in an aluminized plastic bag two hours before measurement and placed in the chamber within 20 s after collection. The water stress accumulated by the crop was estimated with the water stress integral ($S\Psi_{\text{stem}}$) using the equation defined by Myers [21]:

$$S\Psi_{\text{stem}}(MPa \cdot \text{day}) = \sum(\Psi_{i,i+1} - \Psi_c)n, \tag{2}$$

where $\sum$ is the sum of the numbers of Ψ_{stem} measurements, $\Psi_{i,i+1}$ is the mean Ψ_{stem} for any measurement i and i + 1, Ψ_c is the maximum Ψ_{stem} value measured during the experiment, and n is the number of days in the interval. Values were normalized to CTL treatment. $S\Psi_{\text{stem}}$ obtained is the sum of Ψ_{stem} values from the whole berry growth.

Gas exchange parameters were taken every two weeks between 09.00 and 11.30 h in daylight hours from two mature leaves exposed to the sun (n = 6 per irrigation treatment). Net photosynthesis (P_n, µmol $m^2 \cdot s^{-1}$) and stomatal conductance (g_s, mmol $m^2 \cdot s^{-1}$) were measured at a photosynthetic photon flux density (PPFD) $\approx$ 1500 µmol $m^2 \cdot s^{-1}$, near constant ambient CO_2 concentration (Ca $\approx$ 350 µmol mol^{-1}) and leaf temperature ($T_{\text{leaf}} \approx 30$ °C) with a portable gas exchange system CIRAS-2 (PP Systems, Hitchin, Hertfordshire, UK). Intrinsic water use efficiency (P_n/g_s) was calculated as the ratio between P_n and g_s (mmol mol^{-1})

2.4. Productive Traits

The dynamics of berry growth were obtained by changes in berry equatorial diameter using a digital caliper, model CD-15D (Mitutoyo, Japan), on 15 berries per replicate (n = 60 tagged berries per treatment). Total yield (average of three harvestable picks) and fertility (number of clusters per vine and average berry/clusters weight) were determined in all the experimental vines (n = 72 per treatment) at the time of commercial harvest, as described in detail in Conesa et al. [5]. The earliness was obtained considering the volume of yield harvested in each picking operation with respect to the total yield [8].

The trunk diameter was measured annually before harvest (first harvestable pick) with a tape measure on 18 vines per replicate (n = 72 vines per treatment) at a marked location approximately 0.3 m from the soil surface. The trunk cross-sectional area (TCSA) was estimated as being equivalent to that of a circle.

Irrigation water use efficiency (IWUE) was determined as the ratio between yield and total irrigation applied. Production Efficiency (PE, kg cm^{-2} of TCSA) and Crop Load Efficiency (CLE, clusters cm^{-2} of TCSA) were calculated as the ratio of total yield and the number of clusters per tree to the TCSA, respectively.

2.5. Berry Quality Traits

The quality parameters used in the present study were widely described in Conesa et al. [4,5]. Briefly, objective color parameters were recorded in samples of 15 berries per replicate (n = 60 berries per treatment) on three equidistant points of the equatorial zone, using a colorimeter, model CR-300 (Minolta, Osaka, Japan). Results were expressed in the CIELAB chromatic coordinates L* (lightness), a* (red-green component), and b* (blue-yellow component). From these values, the color parameters chrome or chromacity (C*), hue angle or tone (h°), and the color index of red grapes (CIRG) were calculated as:

$$\text{C}^* = \sqrt{(\text{a}^*)^2 + (\text{b}^*)^2} \tag{3}$$

$$\text{h}^\circ = \tan^{-1}(\text{b}^*/\text{a}^*) \tag{4}$$

$$\text{CIRG} = (180 - \text{h}^\circ)/(\text{C}^* + \text{L}^*) \tag{5}$$

Particularly, CIRG is a color index used for red grapes that allows an objective evaluation of the berries' external color and differentiates all red-variations in a more sensitive manner [22,23]. The subjective berry color was estimated from the observations of several panelists (5 men and 3 women; aged from 27–65), who subjectively classified the color of berries (expressed in percentage) following the 5-point categories from different levels of red-color and intensity. For a more detailed explanation, please see Conesa et al. [5]. Berry firmness was measured as the maximum force needed to break the berry skin in the equatorial zone using a texture analyzer, model LFRA 1500 (Middleboro, Brookfield, MA, USA), equipped with a cylindrical probe (4 mm diameter) travelling at a speed of 10 mm s^{-1} to break the berry skin by 5 mm.

For determining the quality parameters of berry juice, 100 berries per replicate (n = 400 per treatment) were pressed with a juicer, model MR-6500 (Braun, Krongber, Germany). In the berry juice, the total soluble solid content (TSS) was determined with a handheld refractometer (Atago N1, Tokyo, Japan) and expressed as °Brix. The titratable acidity (TA) was obtained by titrating 5 mL of juice with 0.1 n NaOH and expressed as g L^{-1} of tartaric acid. The maturity index (MI) was calculated as the TSS/TA ratio. pH and electrical conductivity (EC) at 25 °C were also determined with a Cyberscan instrument, model PCD-6500 (Nijkerk, The Netherlands).

2.6. Experimental Layout and Statistical Analysis

The experimental design was a completely randomized block design with four block-replicates per irrigation treatment. Each replicate consisted of three adjacent rows of vines with six vines per row. The four central vines of the central row were used for monitoring the 'Crimson Seedless' vines' water relations assessed, while the others served as guard vines. A one-way ANOVA was used for data analysis with the SPSS (v.9.1) program. A *post hoc* pairwise comparison between all the means was performed with Duncan's multiple range test at $p \leq 0.05$.

A principal component analysis (PCA) was performed to explore the variability between treatments, with the study of the correlations between total yield, water stress integral ($S\Psi_{stem}$), and the physical (L*, C*, h°, CIRG, firmness, weight, equatorial diameter, and height) and chemical (TSS, TA, MI, pH, and $EC_{25\,°C}$) berry traits, with the InfoStat software [24]. This PCA was performed on the mean values of each treatment replicate. Since the variables have different units of measurement, the data were previously standardized, and the correlation matrix r-Pearson was used.

3. Results

3.1. Irrigation Applied

During the irrigation season (from April to October), the volume of irrigation received by the CTL treatment was 7466 m^3 ha^{-1}, whereas the irrigation amount in the deficit irrigation treatments implied a mean water reduction of 24% (RDI), 28% (PRD), 65% (SDI), and 53% (SPRD), as compared with the CTL treatment, respectively (Figure 1). Therefore, the water reduction applied was moderate in the regulated irrigation practices of RDI and PRD when deficit was applied during the post-veraison period, and severe in the sustained irrigation practices of SDI and SPRD when deficit was applied during the entire period of berry growth. Considering that the price of water for the period was 0.36 € m^{-3} [25], the water reductions obtained in the deficit irrigation treatments resulted in water savings of 645, 752, 1747, and 1424 € ha for the RDI, PRD, SDI, and SPRD treatments, respectively. Moreover, the energy cost that was not quantified in this study should be added to the economic cost.

3.2. Seasonal Evolution of Plant–Soil Water Status

The agrometeorological conditions were typical for Mediterranean semi-arid zones, characterized by dry summers and mild-wet winters. During the experimental period, total rainfall amounted to 203.2 mm, which was irregularly distributed through the season, with September being the rainiest month. The ET_0 recorded a total of 1307 mm, with maximum values of 7.35 mm a day^{-1} in July and the minimum values of 0.54 mm a day^{-1} in September, which represents a strong seasonal water deficit (Figure 2A). Vapor pressure deficit (VPD) reached daily mean values within a range from −6.1 kPa in August to −0.9 kPa in October, respectively. Indeed, VPD followed a similar trend to the maximum temperature values. Considering that the ten-year seasonal average rainfall and ET_0 were 250 mm and 1320 mm, respectively (data not shown), the ET_0 accumulated in the experiment was approximately 90% of the total water needs, while the rainfall value was recorded as normal.

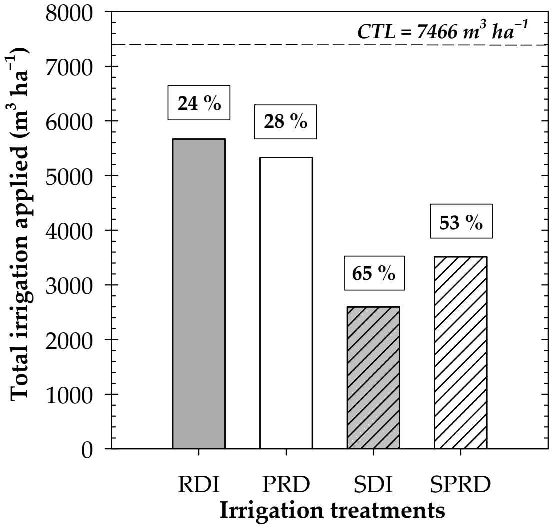

Figure 1. Total irrigation for each irrigation treatment in 'Crimson Seedless' table grapes. The dashed line indicates the amount received in the CTL (fully-irrigated) treatment. The percentages of the water saved in each deficit irrigation treatment: RDI and PRD (regulated deficit) and SPRD and SDI (sustained deficit) with respect to the CTL treatment are shown in boxes.

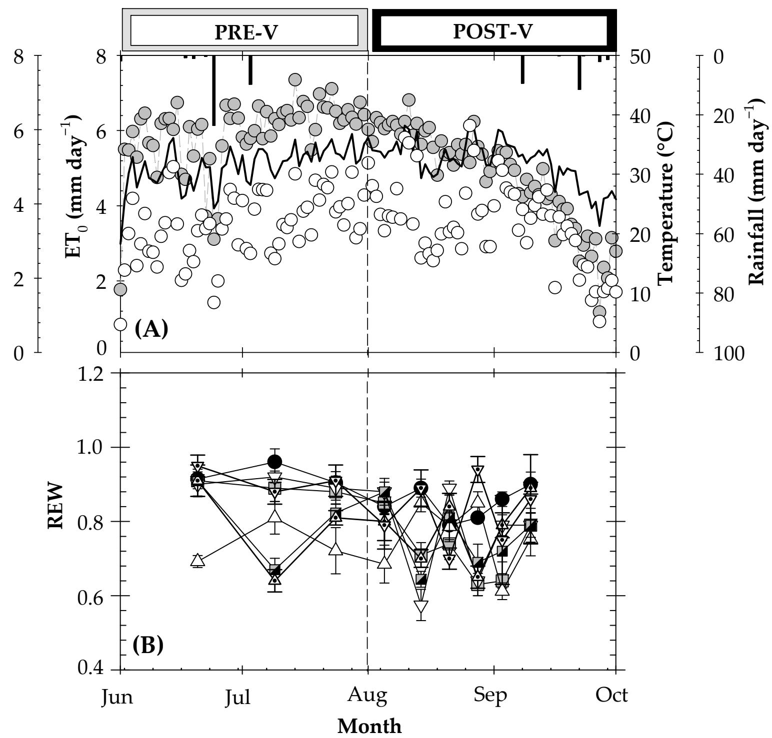

Figure 2. Seasonal evolution of: (**A**) reference evapotranspiration (ET_0;), mean temperature (T;), vapor pressure deficit (VPD; ○), rainfall ( ), and (**B**) relative extractable water (REW) in 'Crimson Seedless' grapes for each irrigation treatment: CTL ( ) or full-irrigated, regulated deficit treatments (RDC ( ), PRD right ( ), PRD left ( )), and sustained deficit treatments (SDI ( ), and SPRD right ( ) and SPRD left ( )). Means ± SE of n = 4 FDR probes. The statistical analysis in Figure (**B**) was omitted for better clarity. Vertical bars indicate the phenological periods of pre-veraison (PRE-V) and post-veraison (POST-V), respectively.

The seasonal relative extractable water (REW) values are depicted in Figure 2B. As expected, CTL, RDI and PRD treatments showed similar values, with a maximum of 0.9 during the pre-veraison period. However, the REW values in the SDI and SPRD treatments were significantly reduced compared with the CTL treatment. Particularly, SPRD presented alternating irrigation cycles during pre- and post-veraison periods at both halves of the root system (left and right side, respectively) due to the alternation of irrigation scheduling when 75% of field capacity values in the dry side were reached. Meanwhile, during post-veraison, REW values in RDI and PRD also decreased in post-veraison because of the soil deficit imposed. Thus, the REW values oscillated from approximately 0.6–0.8 in both deficit treatments, respectively (Figure 2B).

The dynamics of berry growth, from the equatorial diameter values measured, are shown in Figure 3A. The berries experienced an exponential growth until the middle of July, slowing down afterwards, and reaching the maximum growth at the end of August. During pre-veraison, no significant differences were observed between CTL and the deficit irrigation treatments. Meanwhile, the size of the berries in the SDI treatment suffered a strong decrease in its equatorial diameter in the post-veraison period, with a mean value of ≈17 mm, as compared with the ≈18 mm reached in the other irrigated treatments. However, the final berry size at harvest was similar in the all the deficit irrigation treatments with respect to the CTL treatment (Table 1).

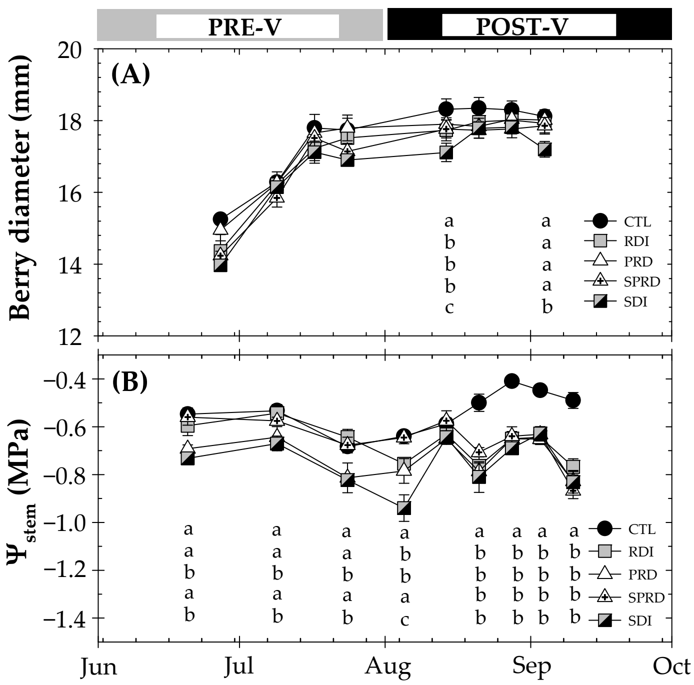

Figure 3. Seasonal evolution of (**A**) fruit diameter (means ± SE of n = 60 fruits per treatment) and (**B**) midday stem water potential (Ψ_{stem}; means ± SE of n = 8 leaves per treatment) in 'Crimson Seedless' table grapes for each irrigation treatment: CTL ( ) or full-irrigated, regulated deficit treatments (RDI ( ), PRD ( )), and sustained deficit treatments (SDI ( ) and SPRD ( )). Different letters indicate significant differences for each date and parameter, according to Duncan's multiple range test ($p \leq 0.05$). Vertical bars indicate the phenological periods of pre-veraison (PRE-V) and post-veraison (POST-V), respectively.

Regarding plant water status, which were estimated through Ψ_{stem} values, the CTL treatment averaged −0.59 and −0.51 MPa during the pre- and post-veraison periods, respectively (Figure 3B). Since the PRD and RDI treatments received the same amount of water as the CTL treatment at pre-veraison, no significant differences were detected between them in this period. In contrast, the SDI and SPRD showed the lowest Ψ_{stem} values, averaging −0.79 and −0.73 MPa, respectively. During post-veraison, there were significant reductions in the Ψ_{stem} values of approximately 0.19 and 0.17 MPa for the RDI and PRD treatments, as compared with the CTL treatment, respectively. Meanwhile, that difference was highest in the severe deficit treatments, with 0.20 and 0.25 MPa values in the SPRD and SDI less than the CTL treatment. Indeed, the minimum Ψ_{stem} value of −0.94 MPa was reached in the SDI treatment (Figure 3B).

Table 1. Yield, number of clusters per vine, mean weight of clusters, number of berries, mean weight of berries, and trunk-cross-sectional area (TCSA) at harvest, production efficiency (PE), and crop load efficiency (CLE) of 'Crimson Seedless' table grapes for CTL (full-irrigated) and deficit treatments: RDI and PRD (regulated deficit), and SDI and SPRD (sustained deficit).

Productivity Parameters	CTL	RDI	PRD	SDI	SPRD	ANOVA
Yield (kg vine^{-1})	55.00	53.33	54.67	61.67	64.67	n.s.
Number of clusters	106.00	82.83	90.61	91.5	104.78	n.s.
Mean weight of clusters (g)	549.30	636.08	630.47	674.17	620.33	n.s
Number of berries	96.23	121.43	131.91	134.72	126.25	n.s.
Mean weight of berries (g)	5.74 a	5.33 ab	4.82 b	4.99 b	4.90 b	*
TCSA (cm^2)	8.46	8.67	8.63	8.61	8.27	n.s.
PE (g cm^{-2})	153.82	162.57	157.86	139.61	127.88	n.s.
CLE (clusters cm^{-2})	12.53	9.55	10.50	10.63	12.67	n.s.

Values are means of n = 72 vines per treatment. Means within rows followed by a different letter were significantly different according to Duncan's multiple range testing ($p \leq 0.05$). * indicates significance at $p \leq 0.05$ and n.s.: not significant.

Similar results were found in the $S\Psi_{stem}$ values recorded (Figure 4). As expected, the SDI treatment reached the highest level of accumulated water deficit in the pre-veraison (10.56 MPa day) and post-veraison (14.72 MPa day) periods, respectively, followed by SPRD, with mean values of 8.67 MPa day in pre-veraison and 13.77 MPa day in post-veraison. During pre-veraison, the $S\Psi_{stem}$ levels obtained were similar between the CTL, RDI, and PRD treatments, in agreement with the absence of water stress. From the time of post-veraison, the $S\Psi_{stem}$ levels in the regulated RDI and PRD strategies were higher than the CTL treatment because of the soil deficit imposed at 50%.

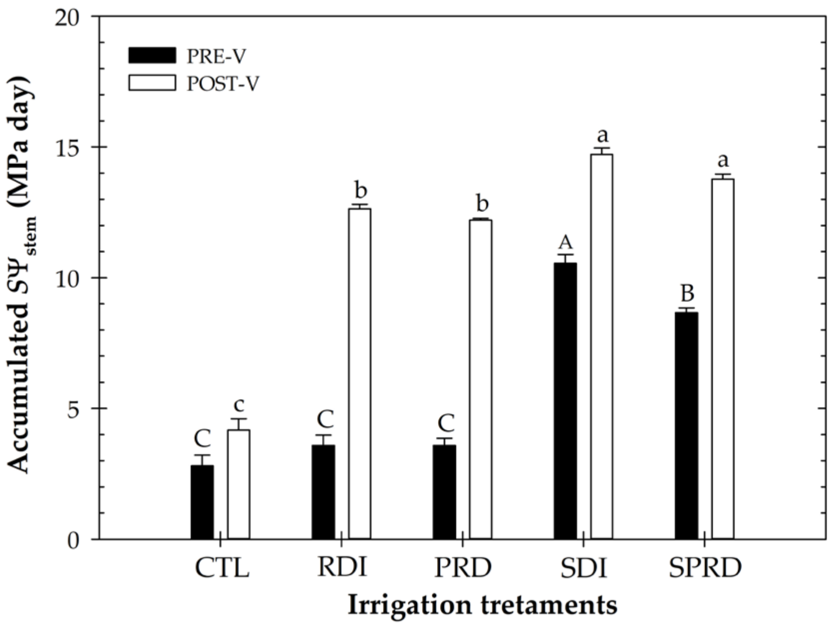

Figure 4. Accumulated water stress integral ($S\Psi_{stem}$) during the phenological periods of pre-veraison (PRE-V, black bars) and post-veraison (POST-V, white bars) of 'Crimson Seedless' table grapes for each irrigation treatment: CTL (full-irrigated), RDI and PRD (regulated deficit), and SDI and SPRD (sustained deficit). Different upper- and lower-case letters indicate significant differences between irrigation treatments according to Duncan's multiple range testing ($p \leq 0.05$) for pre- and post-veraison periods, respectively.

3.3. Gas Exchange Parameters

Mean values of net photosynthesis (P_n), stomatal conductance (g_s), and the intrinsic water use efficiency (P_n/g_s) at pre- and post-veraison periods are shown in Figure 5.

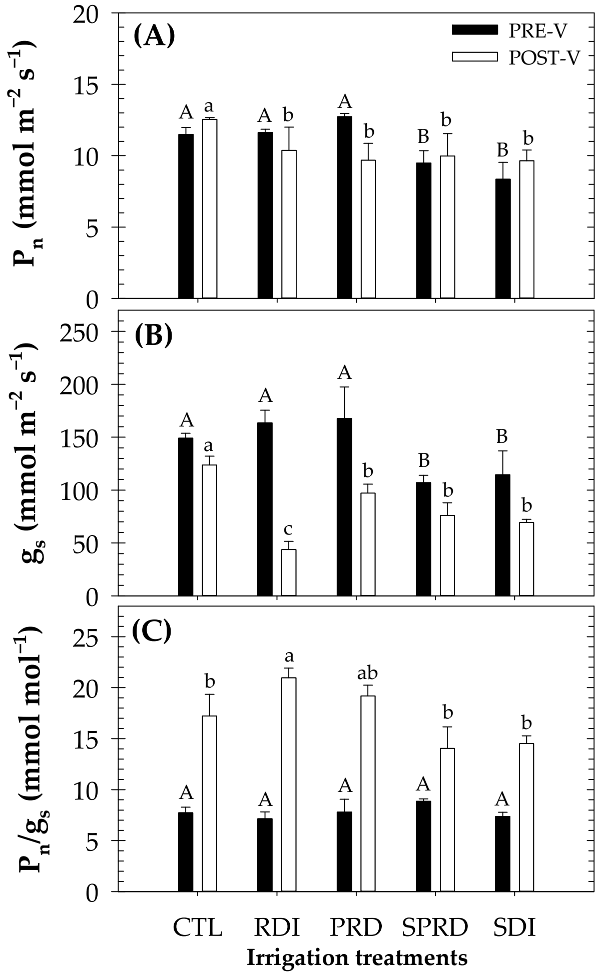

Figure 5. Mean values of (**A**) net photosynthesis (P_n); (**B**) stomatal conductance (g_s); (**C**) intrinsic water use efficiency (P_n/g_s) during pre-veraison (PRE-V, black bars), and post-veraison (POST-V, white bars) periods of 'Crimson Seedless' table grapes for each irrigation treatment: CTL (full-irrigated), RDI and PRD (regulated deficit, moderate deficit), and SDI and SPRD (sustained deficit, severe deficit). Each bar is the mean ± SE of 5 days of measurement of n = 6 leaves per treatment. Different upper- and lower-case letters indicate significant differences between irrigation treatments for each parameter according to Duncan's multiple range test ($p \leq 0.05$) for pre- and post-veraison periods, respectively.

During the pre-veraison period, the CTL treatment averaged P_n = 11.48 µmol m^{-2} s^{-1} and g_s = 149.16 mmol m^{-2} s^{-1}, similar to that obtained in the regulated irrigation practices of RDI and PRD (moderate deficit). However, the lowest values obtained in the sustained treatments (severe deficit) led to a significant reduction by 17% (in P_n) and 28% (g_s) for the SPRD treatment, and 27% (in P_n) and 23% (g_s) for the SDI treatment, respectively, as compared with the CTL treatment (Figure 5A,B). Moreover, at this phenological period, all irrigation treatments obtained the same levels of P_n/g_s in a range from 7.14–8.84 µmol mol^{-1} (Figure 5C).

During the post-veraison period, the CTL treatment averaged P_n = 12.53 µmol m^{-2} s^{-1} and g_s = 123.80 mmol m^{-2} s^{-1}. P_n values in the RDI and PRD treatments were significantly lower by 17% and 23% than the CTL treatment, respectively, whereas g_s values also decreased by 65% and 21% in the RDI and PRD treatments, respectively. These reductions, with respect to the CTL treatment, were approximately 20% and 33% in P_n and g_s for SPRD, and 23% and 44% in P_n and g_s for SDI, respectively (Figure 5A,B).

As expected, the P_n/g_s increased in the post-veraison deficit treatments (RDI and PRD) as compared with the CTL treatment, with the highest values found in the RDI treatments. However, this effect was not found in the sustained deficit treatments (Figure 5C).

3.4. Yield and Productive Efficiencies

No significant differences between the full-irrigated treatment (CTL) and the regulated (RDI and PRD) and sustained (SDI and SPRD) irrigation practices were observed in the yield parameters assessed, except in the mean weight of berries, which was significantly higher in the CTL and RDI treatments, respectively (Table 1). Furthermore, the values of the trunk cross-sectional area and the productivity efficiencies derived (PE and CLE) were similar between the irrigation treatments studied.

Interestingly, the earliness evaluated at the first harvestable pick was highest in all the deficit treatments as compared with the CTL treatment (Figure 6). Indeed, the values obtained at this time were higher in the SDI treatment, followed by RDI > SPRD > PRD > CTL. Lastly, in the last pick of the harvest, the SDI treatment obtained the lowest level of earliness as compared to the other irrigation treatments.

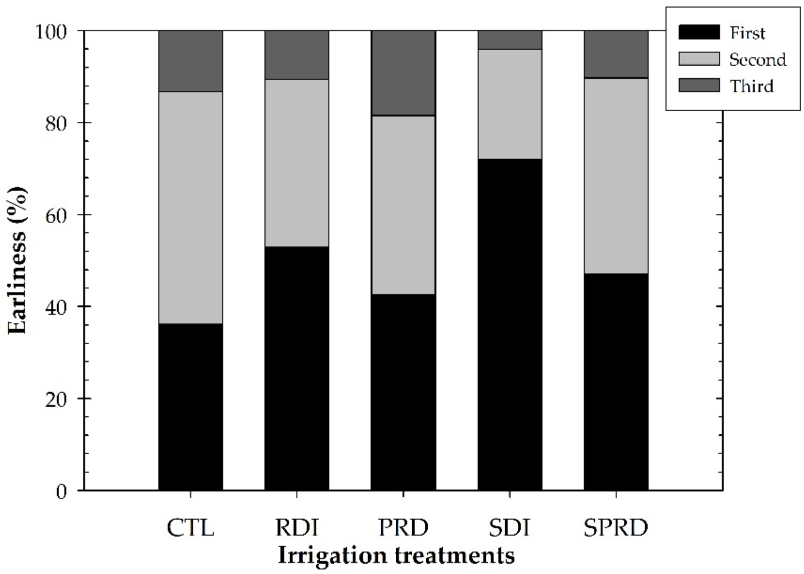

Figure 6. Earliness evaluated at each harvestable pick (first, second, and third) during the harvest period (from September to November) of 'Crimson Seedless' table grapes for CTL (full-irrigated) and deficit treatments: RDI and PRD (regulated deficit), and SDI and SPRD (sustained deficit). Values are means of n = 72 vines per treatment.

3.5. Berry Quality

Regarding the physical quality traits assessed, all the deficit treatments recorded a lower berry height as compared to the CTL treatment but similar berry equatorial diameters and firmness (Table 2). As for the objective color parameters, both SDI and SPRD treatments had lower significant values of hue h° and L*, and the highest CIRG, whereas chrome (C*) was not affect by the deficit irrigation. Altogether, the deficit irrigation practices assessed had an influence on the subjective berry color, as the panelists classified it at approximately between 80 and 85% in the Categories from III-IV. Moreover, only the sustained irrigation practices of SDI and SPPRD, which corresponded to a severe deficit, obtained berries classified in the Category V (1.67 and 2.33% respectively), which agrees with the subjective color performance values obtained in h°, L*, and CIRG. Meanwhile, the CTL treatment registered the worst levels of h° and L* along with a higher percentage (approximately 35%) of the berries classified in the Categories from I-II (indicating less reddish berries).

Table 2. Mean values for the chemical traits (TSS, total soluble solids; TA, titratable acidity; MI, maturity index; pH and $EC_{25\,°C}$, electrical conductivity) and physical traits (berry and height diameter and firmness), including skin objective color parameters (h°, hue angle; C*, chrome; L*, lightness), color index for red grapes (CIRG), and subjective color parameters of 'Crimson Seedless' table grapes, evaluated at harvest during the experiment, for CTL (full-irrigated) and deficit treatments: RDI and PRD (regulated deficit), and SDI and SPRD (sustained deficit).

Berry Quality	CTL	RDI	PRD	SDI	SPRD	ANOVA
Physical traits						
Berry diameter (mm)	17.79	17.12	16.66	16.86	16.69	n.s.
Berry height (mm)	28.71 a	25.37 b	24.72 b	24.17 b	25.16 b	*
Firmness (N)	13.53	11.2	11.83	9.98	11.01	n.s.
h°	57.72 a	57.66 ab	57.60 b	57.54 c	57.55 c	**
C*	13.53	12.67	12.7	13.9	12.36	n.s.
CIRG	2.53 b	2.61 ab	2.71 ab	2.77 a	2.77 a	*
L*	34.78 a	34.26 ab	32.60 bc	30.41 c	31.86 bc	**
I-II (%)	34.67	16.33	19.67	13	14	-
III-IV (%)	65.33	83.67	80.33	85.33	83.67	-
V (%)	0	0	0	1.67	2.33	-
Chemical traits						
TSS (°Brix)	19.46 b	20.06 b	20.20 b	21.33 a	20.13 b	*
TA (g L^{-1})	6.3	5.95	5.6	6.1	5.7	n.s.
MI	30.99	33.74	36.32	35.15	35.39	n.s.
pH	3.78	3.78	3.87	3.93	3.95	n.s.
$EC_{25\,°C}$	3.33 b	3.39 b	3.45 ab	3.75 a	3.25 b	*

Values are means of 60, 400, and 40 berries per treatment for physical and chemical measurements. Means within rows followed by a different letter were significantly different according to Duncan multiple range testing ($p \leq 0.05$). * and ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively; n.s.: not significant.

Regarding the chemical-quality traits assessed, the TSS (°Brix) was significantly higher in the SDI treatment than the other irrigation treatments. $EC_{25\,°C}$ increased in the SDI and PRD treatments. No significant differences were detected among irrigation treatments in the studied parameters of TA, MI, and pH.

3.6. Principal Components Analysis (PCA)

The PCA results explained 59.3% of the total variability of the observations in its first two components (Figure 7). The variables with the highest weight in PC1, from most to least important, were: $S\Psi_{stem}$, h°, berry weight and height, L*, and the CIRG. At the PC2 level, the variables with the highest weight, from highest to lowest importance, were: $EC_{25\,°C}$, C*, pH, and TSS. The control treatment clustered on PC1 with higher values of physical traits of berries: firmness, weight, height, equatorial diameter, L*, and higher tones (h°), i.e., more distant from the reddish color. On the opposite side, the SDI treatment clustered on PC1, where the intensity of water stress was higher, as confirmed by the highest $S\Psi_{stem}$ values observed at both pre- and post-veraison periods (Figure 4). This suggests that the SDI treatment had a stronger influence than the other studied deficit irrigation treatments on the increase of CIRG, maturity index, TSS, and pH of the berries (Figure 7).

The variables that correlated (r-Pearson) significantly with the water stress intensity were berry height (−0.754**), TSS (0.718**), berry weight (−0.678**), and L* (−0.678**). No significant correlations were detected between the variables evaluated and total production per vine. As expected, berry weight correlated with berry height (0.623*), but not with equatorial diameter. Berry weight was also positively correlated with firmness (0.694**) and the color components L* (0.644**), h° (0.764***), and CIRG (−0.806***). Regarding colorimetry, C* correlated with EC25 °C (0.664**) and h° with berry firmness (0.686**) and with CIRG (−0.784***). The rest of the variables were not significantly correlated (data not shown).

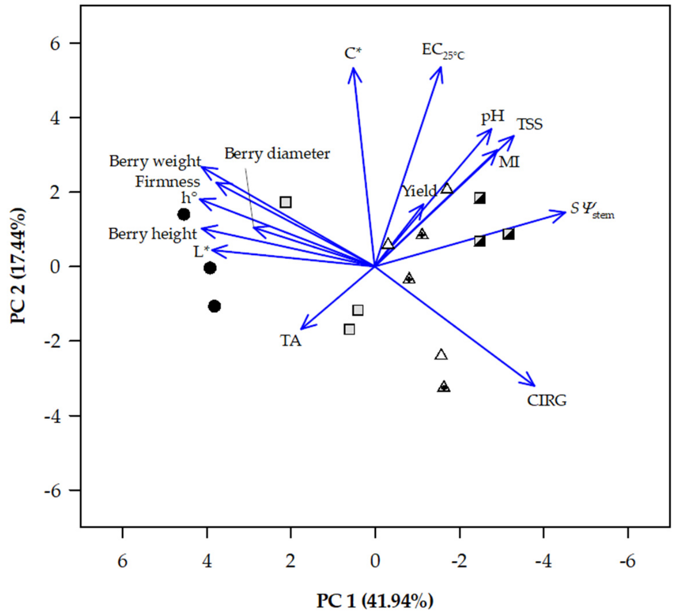

Figure 7. Biplot of the principal component analysis obtained with standardized data. Blue arrows represent the variables evaluated and symbols indicate the irrigation treatments in 'Crimson Seedless' table grapes: full-irrigated CTL (●); regulated deficit RDI (□) and PRD (△); sustained deficit SDI (◪) and SPRD (▲). CIRG: color index for red grapes, $S\Psi_{stem}$: water stress integral, MI: maturity index, TSS: total soluble solids, $EC_{25\,°C}$: electrical conductivity, C*: chroma, h°: hue angle or tone, L*: luminosity, and TA: titratable acidity. n = 15.

3.7. Water Productivity

The irrigation water use efficiency (IWUE) in the CTL treatment was 4.61 kg m^{-3} (Figure 8). As expected, IWUE improved in all the deficit treatments, with the increase observed being higher the greater the level of water deficit applied. In this sense, the sustained irrigation practices of SDI and SPRD attained the highest levels of IWUE as compared with the regulated irrigation practices of RDI and PRD. Comparing all the deficit irrigation treatments to the CTL treatment, the IWUE was significantly higher in SDI > SPRD > PRD > RDI, respectively.

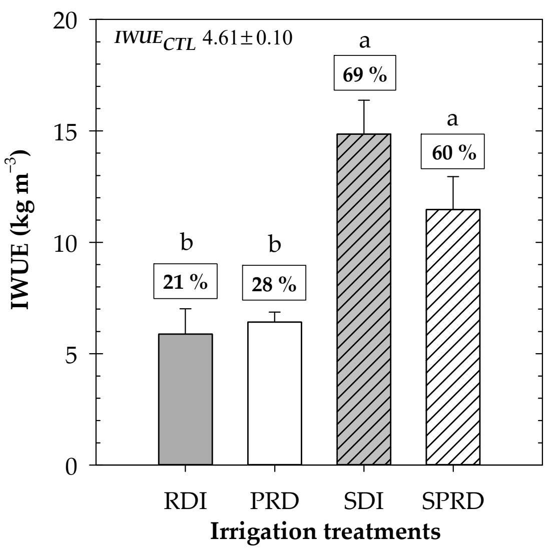

Figure 8. Irrigation water use efficiency (IWUE) of each irrigation treatment. The increase (%) in IWUE for each deficit irrigation treatment was: RDI and PRD (regulated deficit) and SPRD, and SDI (sustained deficit) with respect with the CTL treatment are shown in boxes. Different letters indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

4. Discussion

Field-grown 'Crimson Seedless' table grapes suffered from water deficit during two different periods: (i) post-veraison: known as the non-critical period of this cultivar [4–6,8] with the application of 'regulated' water deficit, and (ii) the entire berry development period, including pre- and post-veraison periods with the application of 'sustained' water deficit via different irrigation management practices. On the one hand, the irrigation management based on Regulated Deficit Irrigation (RDI) with one single-line of drippers, and on the other hand, the irrigation management design using two-lines of drippers, known as Partial Root-zone Drying (PRD), by alternating the irrigation applied on the dry side. To the best of our knowledge, the effects of RDI and PRD on yield and berry quality parameters of table grapes with both compared according to the application of regulated and sustained applications cannot be found in the literature.

Recent studies on 'Crimson Seedless' table grapes have demonstrated that both regulated practices of RDI and PRD led to remarkable water savings (~35%) without compromising the yield components and the main fruit quality parameters assessed as compared with CTL vines [4,5]. Temnani et al. [6] reported an average of 2054 m^3 ha^{-1} (a reduction of 30%) less water received in the RDI treatment with respect to the full-irrigated vines throughout the season. Our results are in agreement with these previous reports for 'Crimson Seedless', with reductions in the irrigation received with respect to the CTL treatment of 24% (in RDI) and 28% (in PRD), respectively (Figure 1). As expected, the water savings, in the sustained practices that were applied during the entire berry growth period (corresponding to a severe deficit), were higher as compared with the regulated practices that were applied after veraison (with a moderate water deficit). In this sense, the highest irrigation water reductions, with respect to the CTL treatment, were observed in the SDI treatment (65%) followed by the SPRD treatment (53%) (Figure 1). However, these reductions in the water applied did not result in negative effects on the yield or productive efficiencies (Table 1). The PCA analysis, shown in Figure 7, also indicated the decreased importance of the yield analysis (smaller length arrow). Only the berry weight was slightly lower in the DI treatments (except for the RDI treatment) as compared with the full-irrigated vines. In 'Italia' table grapes, Tarricone et al. [26] observed that the lowest irrigation amount (40% ET_c) yielded the lowest berry weight. In 'Sublima Seedless', a negative effect was reported on bunch weight, berry weight, and berry size during the early fruit growth stages when mild or severe water stress was applied at the beginning of berry set [27]. In 'Crimson Seedless', Conesa et al. [4,5] stated that severe drought stress during berry development (a reduction of 72%) also clearly affected the productivity of vines and, thus, berry growth. In our case, the lower berry weight observed in the DI treatments was compensated by an increase in the number of berries per cluster (Table 1). Weiler et al. [28] indicated that the total number of berries and number of marketable berries showed a cultivar-specific reaction to the water deficit. Peacock et al. [29] found a significant increase in the number of clusters with commercial color in 'Crimson Seedless' vines subjected to RDI during the last stages of maturation.

At pre-veraison, the CTL, RDI, and PRD treatments showed similar REW and the vine water status values (Figures 2B and 3B) within the range of non-stress conditions for vines [4–6,30]. However, the sustained SDI and SPRD treatments exhibited a mean reduction of 0.2 MPa with respect to the CTL treatment, coinciding with the lower REW, P_n, and g_s values during this period (Figures 2B, 3B, and 5). Conesa et al. [4] indicated that reductions in Ψ_{stem} of 0.2 MPa through water withholding promoted reductions in berry growth at pre-veraison. It is known that water deficit can decrease the vigor of the vines and causes competition for the carbohydrates necessary for growing [31], as well as increased cluster transpiration rates and subsequent berry dehydration [32,33]. However, despite the difference observed on the plant water status before veraison, the yield response was not affected in the sustained treatments (SDI and SPRD). This finding suggests that the higher stomatal control that promoted an increase in the P_n/g_s (Figure 5C) was sufficient to ensure water uptake by the roots and, thus, the supply of the water and carbohydrates needed for berry development. Romero et al. [34] reported that SDI vines increased sap flow rates and water and sugar accumulation as compared to RDI vines. Consequently, SDI berries had a greater average berry size, fresh and dry berry weights, dry matter, and water content than DI berries.

At post-veraison, both the RDI and PRD treatments resulted in moderate stress (with a mean difference of 0.18 MPa), which significantly reduced REW values and plant water relations (Figures 3A and 5). A similar response was obtained in the SDI and SPPRD treatments, but with a mean difference of 0.23 MPa with respect to the Control vines (Figure 3A). Conesa et al. [4] detected a maximum reduction of 0.3 MPa after veraison to avoid a negative impact on the yield components.

Generally, g_s decreases with an increase in water deficit due to being more sensitive than P_n (Figure 5). As a result, intrinsic water use efficiency (P_n/g_s) usually increases under moderate water stress conditions while ensuring the crop productivity [35,36]. Nevertheless, our results showed that despite the fact the P_n and g_s in the SDI and SPRD treatments significantly decreased, the total yield was not reduced compared with the CTL treatment. Indeed, the values of the yield parameters obtained in the vines from sustained irrigated practices were slightly higher than fully-irrigated vines. This fact suggests that the water deficit registered before veraison could promote an accumulation of carbohydrates reserves improved the berry development as well as encouraged the crop yield. We did not find significant differences in the yield components assessed among irrigation treatments (Table 1). Xue et al. [37] reported that in maize, when photosynthesis and biomass were reduced by water stress during grain filling, remobilization of pre-anthesis carbon reserves significantly contributed to the increased grain yield.

Berry quality was minimally affected by sustained and regulated deficit treatments. However, the size of the berries was slightly larger in the CTL than in the DI vines, as indicated by the lower weight and height values of the berries measured (Tables 1 and 2). Different authors have reported that water deficit often reduces the growth of the berries; however, this effect is dependent on the intensity and period of water stress [38]. Temnani et al. [6] also found that berry size was a very sensitive parameter to water deficit, as berry diameter decreased when differences of 0.15 MPa (in pre-veraison) and 0.32 MPa (in post-veraison), with respect to the Control vines, were obtained. Furthermore, Pinillos et al. [8] found a reduction in berry firmness due to the DI applications. The more intense, the greater the water restriction, but these were not significant, as we observed in our study (Table 2 and Figure 4). The authors suggested that this lower firmness was associated with a more advanced maturation stage of berries at harvest.

The implementation of DI strategies can improve the insufficient commercial reddish berry color that is characteristic of Crimson grapes [4,5,8,38]. Our findings showed that both sustained and regulated practices increased skin berry color, as indicated by the values of h°, L*, and CIRG obtained (Table 2). Indeed, the subjective color analysis performed by the panellists also confirmed an improvement in the berry color of DI berries (Table 2). Furthermore, the deficit treatments affected the earliness of the first harvestable pick of yield as compared with the CTL vines, with the SDI treatment underlined in 'Crimson Seedless'. Pinillos et al. [8] found a similar response in RDI vines (although without significant differences between irrigation treatments), as water deficit promoted an earlier harvest and higher CIRG values (indicated as a redder color). Faci et al. [3] did not find clear differences in the color of berries in response to post-veraison RDI. Finally, the SDI treatment promoted an increase in the TSS, as reported in other DI studies [29,39]. Interestingly, the PCA analysis was in line with these results, suggesting that the SDI treatment had a greater influence on the CIRG and TSS values than the other irrigation counterparts (Figure 7). Conesa et al. [4,5] stated that RDI and PRD treatments (that received the same amount of irrigation than CTL vines) enhanced berry coloration, providing grapes that were more acceptable to consumers than the CTL treatment. PRD induced a greater accumulation of skin anthocyanins and resveratrol, with the concomitant increase in the soluble phenolic content and antioxidant capacity evaluated at harvest.

Altogether, the DI treatments had an increased irrigation water use efficiency (IWUE), being highest in the sustained (SDI > SPRD) rather than the regulated (PRD > RDI) practices, while the Control treatment averaged 4.61 kg m^{-3} during the season (Figure 8). These results are in agreement with the accumulated water stress integral in each single treatment (Figure 4). For Crimson vines, a similar response in the IWUE values with respect to the CTL was found in Faci et al. [3] (19.6%), Conesa et al. [4,5] (32%), and Temnani et al. [6] (50%).

In short, both regulated RDI and PRD treatments (moderate deficit) not only allowed notable water savings without significant differences in yield, but also improved berry quality as compared with the CTL treatment. Meanwhile, both sustained SDI and SPRD treatments (severe deficit) reported considerable water reductions with respect to the CTL vines but without promoting yield penalties and enhancing berry quality (especially skin berry color) to a higher extent than regulated deficit treatments. In this sense, the SDI treatment stood out from the other irrigation counterparts, in addition to the fact that the PRD system was associated with a higher installation cost, in particular, at a commercial level. Nevertheless, it would be advisable to conduct a long-term study to evaluate the effects of the SDI treatment on Crimson grape productivity.

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