



# Effects of deficit irrigation with saline water on yield and grape composition of *Vitis vinifera* L. cv. Monastrell

A. Martínez-Moreno<sup>1,2</sup> · E. P. Pérez-Álvarez<sup>1</sup> · D. S. Intrigliolo<sup>3</sup> · J. M. Mirás-Avalos<sup>4</sup> · R. López-Urrea<sup>5</sup> · R. Gil-Muñoz<sup>6</sup> · V. Lizama<sup>7</sup> · M. J. García-Esparza<sup>7</sup> · M. I. Álvarez<sup>7</sup> · I. Buesa<sup>1,8</sup>

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## Abstract

Warm and semi-arid climates are characterized by rainfall scarcity, resulting in the frequent use of low-quality water for irrigation. This work was undertaken to study the effects of water stress and saline irrigation on yield and grape composition of Monastrell grapevines grafted onto 1103P rootstock. The experiment was carried out during three consecutive seasons in a commercial vineyard located in Jumilla (SE Spain) with a loamy-sandy soil. Rainfed vines were compared with five watering regimes including a Control, irrigated with standard water, and four treatments that combined two different schedules for irrigation initiation (pre- and post-veraison) with saline water obtained by adding two types of salts (sulphates and chlorides). Vines from treatments with more severe water stress (i.e., rainfed) showed lower yields and vegetative growth. Moreover, the Rainfed treatment clearly modified grape composition when compared with the Control treatment by increasing berry phenolic content. The application of saline water slightly affected vine performance and grape composition regardless of the type of salts added to the irrigation water. Indeed, the watering regime had a greater effect on yield, vegetative growth and grape composition than the use of different saline waters. Our results suggest that, in the mid-term (3 years), and with a vineyard soil with good drainage, the use of saline waters is not detrimental to vine performance, but does not improve grape composition. Further research is required to assess the long-term effects of saline water application, particularly in view of the important accumulation of chlorides and sodium in leaf tissues observed in vines watered with salty water at the last season of this experiment.

**Keywords** Water stress · Wine · Salinity · Semi-arid climate · Vegetative growth · Grape composition

✉ A. Martínez-Moreno  
martinezamoreno@gmail.com

<sup>1</sup> Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Campus Universitario de Espinardo, 30100 Murcia, Spain

<sup>2</sup> Department of Food Science and Technology, Faculty of Veterinary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain

<sup>3</sup> Departamento de Ecología, Centro de Investigación sobre Desertificación (CSIC-UV-GV), Carretera CV-315, km 10.7, 46113 Moncada, Valencia, Spain

<sup>4</sup> Departamento de Sistemas Agrícolas, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Forestales y Medio Ambiente (Unidad de Suelos y Riegos, asociada a EEAD-CSIC), Avda. Montañana 930, 50059 Zaragoza, Spain

<sup>5</sup> Instituto Técnico Agronómico Provincial (ITAP), Parque Empresarial Campollano, 2ª Avda. Nº 61, 02007 Albacete, Spain

<sup>6</sup> Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), 30150 La Alberca, Murcia, Spain

<sup>7</sup> Instituto de Ingeniería de Alimentos para el Desarrollo (IIAD), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

<sup>8</sup> Present Address: Departamento de Biología, Universidad de las Islas Baleares (UIB), Ctra. Valldemossa km 7.5, 07122 Palma, Balearic Islands, Spain

## Introduction

Water stress and salinity are the most common abiotic constraints in many wine grape production areas (Mirás-Avalos and Intrigliolo 2017). Mediterranean climates are characterized by warm and dry summers. In these areas (such as the study area), where water is a limited resource, the driest months (with a high evaporative demand in the atmosphere) coincide with the highest water requirements of plants, being quite common the use of low quality water for irrigation of vineyards (Medrano et al. 2015). Moreover, the evaporative demand of the atmosphere is expected to increase as a consequence of increased global air temperature (Vicente-Serrano et al. 2014), as well as long periods of drought and heat waves (IPCC 2019). These events will compromise the availability of good quality water for irrigation, making it difficult to improve grape productivity and fruit quality.

Although grapevines are species with a relatively high tolerance to water stress (Cramer et al. 2007), restricted water availability has a direct effect on vegetative growth, yield components, canopy microclimate and berry metabolism (Pellegrino et al. 2005; Ezzhaouani et al. 2007), resulting in a significant reduction in grape yield and quality (Ramos et al. 2020; Zufferey et al. 2017). A great effort has been devoted (mainly on red varieties under semi-arid conditions) to assess the influence of grapevine water status on berry composition, mainly in primary (total soluble solids and titratable acidity) and secondary metabolites, such as anthocyanins and tannins (Van Leeuwen et al. 2009; Mirás-Avalos and Intrigliolo 2017). In general, a moderate water stress reduces berry weight, but increases total anthocyanin and phenolic concentrations in red grapes (Romero et al. 2010). However, the impact of water deficits on grape composition depends on the severity of stress, its duration, and the phenological period in which vines experience this water stress (Ramos et al. 2020). In addition, under water scarcity conditions, there is a high risk of soil salinization due to high concentrations of dissolved salts in the irrigation water and the low water leaching fractions (Keller 2015). Under these circumstances, the quality of irrigation water plays an important role and affects both yield and grape quality (Mirás-Avalos and Intrigliolo 2017).

High salt concentrations in the soil caused by irrigation with saline water could limit vine growth and development and can also lead to modifications of vine physiology caused by water stress and ion toxicity (Zhu 2007). In this sense, grapevines are classified as moderately sensitive to salinity, with a threshold value of electrical conductivity (EC) in the saturated soil extract ranging from 1800 to 4000 ( $\mu\text{S}/\text{cm}$ ), and a decrease in yield between 2.3 and 15.0% per unit increase of EC (Walker et al. 2002). The grapevine's first response to salinity is a reduction in vegetative growth and a reduction

in leaf area (Walker et al. 1981). This reduction in vegetative growth is determined by a severe water deficit in the vines, caused by a decrease in soil water potential, which leads to a greater difficulty for vines to absorb water and nutrients (Walker et al. 1981; Cramer et al. 2007). Walker et al. (2002) observed a significant reduction in pruning wood weights, after irrigation with saline water. Salinity can also affect bunch number (Prior et al. 1992), berry size, and sugar concentration (Hawker and Walker 1978), leading to a significant reduction in grape yield (Degaris et al. 2016; Maas and Hoffman 1977; Walker et al. 2002). On the other hand, the effects of salinity on grape composition seem to depend on the combination of cultivar and rootstock and on the salt concentration in the irrigation water, as well as on its time of application and exposure over the growing season (Mirás-Avalos and Intrigliolo 2017). While salinity itself affects the grapevine's capacity to uptake water from the soil by increasing the osmotic potential, the accumulation of some specific ions such as chloride in leaf tissues can directly affect plant conductance and photosynthesis (Downton, 1977; Walker et al., 1997). However, to our knowledge, studies that focus on how the type of salt and timing of application affect yield and grape composition for a given variety and rootstock are lacking.

Although previous studies assessed drought tolerance and salt effects on yield and grape composition by focusing on different varieties and rootstocks (Downey et al. 2006), there is less information on the combined effects of water and salt stresses and their impact on grape and wine composition (Suarez et al. 2019). It is hypothesized that increasing irrigation water electrical conductivity using sodium chloride to simulate the effect of sea salt intrusion into water aquifers can have a more detrimental effect on the overall vine performance than when water salinity is mainly due to the high concentration of sulphates, which are often found in the bedrocks of certain aquifers. In this sense, the studies by Martínez-Moreno et al. (2021) and (2022) determined the effects of drought stress and saline water application on the resultant wine composition. In the present research, the agronomic effects of different saline water application on vine water status, yield and its components and fruit composition are studied in order to determine the mid-term effects of drought and salinity on vineyard performance. To this end, a Rainfed regime was compared with saline water applications that varied according to the phenological period of its applications and the type of salts added for increasing the irrigation water electrical conductivity.

## Materials and methods

### Field conditions and plant material

The study was carried out in a commercial vineyard located in the municipality of Fuente-Álamo (38°43' N, 1°28' W, elevation 820 m a.s.l.), Albacete, Spain, during three consecutive seasons (2016–2018). The plant material was cv. Monastrell (syn. Mourvedre) (*Vitis vinifera* L.) grafted onto 1103P rootstock. This cultivar is the most cultivated in Southeast Spain and is characterized by its high resistance to drought, in addition to its requirement of a high number of hours of sun to achieve an adequate fruit ripening (Romero et al. 2018). From an oenological point of view, Monastrell cultivation generally results in musts of high alcohol degrees with a medium–low acidity, and a medium–high aroma intensity (Riquelme and Martínez-Cutillas 2018).

In the experimental vineyard located within the Denomination of Origin Jumilla, vines were planted in 2007 at a spacing of 1.5 m between vines and 3.0 m between rows (2222 vines/ha). Vines were trained to a vertical trellis on a bilateral cordon system with north–south orientation. Sixteen buds per vine were left after winter pruning. The vineyard management and cultural practices (soil cultivation, shoot hedging and lateral shoot thinning, among others) were those commonly applied in the area and were carried out by the winegrower. The vineyard soil was loamy-sandy (56 sand, 27% silt and 17% clay) and 90 cm in depth. Soil pH and bulk density were 8.86 and 1.17 g/cm<sup>3</sup>, respectively. Climate in the study area is Mediterranean semiarid, with hot and dry summers. The long-term average for annual rainfall is 290 mm and the total annual reference evapotranspiration (ET<sub>0</sub>) is approximately 1,279 mm.

### Experimental design

The experimental design was laid out in completely randomized blocks with four replicates. Each block contained all

the treatments (six), and each experimental unit consisted of four rows with 12 vines each (total of 48 vines). The number of vines per replicate used varied among the different determinations, up to a maximum of 20 vines (e.g., yield), being the rest buffer vines. The experiment involved six treatments (five irrigation treatments and a rainfed) (Table 1) that were applied for 3 years (2016–2018). The treatments were: (1) Rainfed, vines were not irrigated; (2) Control, irrigation with the same water available by the winegrowers of the area (standard water) (average electrical conductivity (EC) of 1,900 µS/m); (3) Sulphate treatment “Sul”, vines were irrigated with emulated saline water (Na<sub>2</sub>SO<sub>4</sub>+ MgSO<sub>4</sub>); (4) Chloride treatment “Chl”, vines were irrigated with emulated saline water (NaCl). In these treatments (Control, Sul and Chl), irrigation was triggered when grapevine stem water potential (Ψ<sub>s</sub>), measured at midday, reached – 0.8 MPa and vines were steadily irrigated until after harvest. The other two treatments in which vines were not irrigated until veraison were considered: (5) Sulphate after veraison treatment “SulV”, vines were irrigated with the same water

**Table 2** Chemical composition of the water used for irrigation in the different treatments

	Standard water	Standard water + chlorides	Standard water + sulphates
EC (dS/m)	1.89	4.95	4.98
SAR	1.06	7.83	3.95
B	12	13	10
Ca <sup>+2</sup>	6.64	6.15	6.30
K <sup>+</sup>	0.26	0.41	0.41
Mg <sup>+2</sup>	3.92	5.03	12.51
Na <sup>+</sup>	3.43	26.20	17.26
SO <sub>4</sub> <sup>-2</sup>	10.22	14.28	35.76
Cl <sup>-</sup>	4.33	27.79	5.16

EC electrical conductivity, SAR sodium adsorption ratio. The values presented are the average values of the 3 years of study. All values except boron (µmol/l), are expressed in mmol/L

**Table 1** Summary of the experimental treatments carried out (Rainfed, Control, Sul: Control + Sulphates; Chl: Control + Chloride; SulV: Control + Sulphates post-veraison; ChIV: Control + Chloride

Treatment	Irrigation period	Added salts	Electrical conductivity (dS/m)	Irrigation (mm)
Rainfed	No irrigation	No salts	–	0
Control	Pre- and post-veraison	No salts	1.9	108
Sul	Pre- and post-veraison	Sulphates (Na <sub>2</sub> SO <sub>4</sub> + MgSO <sub>4</sub> )	5.0	112
Chl	Pre- and post-veraison	Chlorides (NaCl)	5.0	109
SulV	Post-veraison	Sulphates (Na <sub>2</sub> SO <sub>4</sub> + MgSO <sub>4</sub> )	5.0	102
ChV	Post-veraison	Chlorides (NaCl)	5.0	95

post-veraison). More detailed data about water application are published in Martínez-Moreno et al. (2021).

as “Sul” treatment and (6) Chloride after veraison treatment “ChIV”, vines were irrigated with the same water as “ChI” treatment. In the SulV and ChIV treatments, irrigation began after full veraison (after berries coloration) and ended after harvest.

The EC of the water in all the saline treatments was the same: 5000  $\mu\text{S}/\text{m}$ . This EC was obtained by adding different concentrations of salts to the “Control water” (Table 2). For the ChI and ChIV treatments, 28 mmol/L of NaCl were added. For the Sul and SulV treatments, 6 mmol/L of  $\text{Na}_2\text{SO}_4$  and 8 mmol/L of  $\text{MgSO}_4$  were added. The amount of water applied to all the treatments was measured with water meters (one per replicate) and the cumulated yearly values (averaged for the 3 years of study) are shown in (Table 1).

Deficit irrigation scheduling was carried out weekly using the soil water balance method proposed in FAO56 (Allen et al. 1998; Pereira et al. 2020). Crop evapotranspiration ( $\text{ET}_c$ ) was computed by multiplying the grass reference evapotranspiration ( $\text{ET}_o$ ) by a dimensionless crop coefficient ( $K_c$ ) (i.e.,  $\text{ET}_c = \text{ET}_o \times K_c$ ).  $\text{ET}_o$  values were calculated daily with the FAO56 Penman–Monteith equation (Allen et al. 1998), using the climate data provided by an agrometeorological station located in the municipality of Ontur, belonging to the Spanish Agro-climatic Information System for Irrigation, and handled by the irrigation advisory service of Castilla-La Mancha (Spain) (SIAR) (Ortega et al. 2005). This station is located 10 km away from the experimental plot. Crop coefficient values were derived from those reported by López-Urrea et al. (2012) and adjusted to supply a deficit regime that mimics the water allocation regimes established by the water basin authority, which limits the water application to a maximum of 1000  $\text{m}^3/\text{ha}/\text{year}$ . The water was applied through a drip irrigation system with one emitter (3.8 L/h) per linear meter of pipe. The treatments in which irrigation started prior to veraison (C, Sul and ChI) received two irrigation events per week of, approximately, 4.5 mm each, with a total of 20–25 irrigation events per year. The treatments that began to be watered after veraison (SulEnv and ChIEnv) generally received three irrigation events per week of, approximately, 7.6 mm each, amounting to 12–15 irrigation events per year.

## Field measurements

Grapevine water status was assessed through fortnightly measurements of midday  $\Psi_s$ , using a pressure chamber (Model 600, PMS Instrument, Albany, OR, USA). These measurements began, usually, in June and were performed at solar noon on bag-covered leaves from three representative vines per replicate. The leaves used for these measurements were enclosed in hermetic plastic bags and covered with aluminium foil for at least 1 h prior to measurement (Choné

et al. 2001). The water stress integral ( $S_\Psi$ ) was calculated using the equation proposed by Myers (1988).

To calculate the soil electrical conductivity, a 1:5 soil: water extract was measured. Two samples per replicate were taken at two different depths (30 and 60 cm), obtaining 16 soil samples per treatment for each time of measurement. These samples were collected under the vine rows, in the irrigated treatments, sampled within the wet bulb. Soil samplings were performed at four times from November 2017 to November 2018: (1) after leaf fall (2) before the beginning of irrigation, (3) at veraison, and (4) after harvest. In the laboratory, 20 g of sampled soil were mixed with 100 mL of distilled water, then the samples were shaken for 120 minutes and left to decant for 30 minutes. Lastly, the electrical conductivity was measured with a multi-parameter instrument (Bench PC2700, Eutech Instruments, Nijkerk, Netherlands).

Grape yield, number of clusters per vine and cluster weight were determined at harvest on each experimental vine. The initial harvest criterion was to reach a TSS of approximately 23°Brix and harvest dates were the same for all treatments during the 3 years of study (10/10/2016–26/09/2017–10/10/2018). However, particularly in 2018, grapes could not reach this desired TSS level and had to be harvest at lower TSS before rots and other diseases could have compromised yield. Five clusters were selected from each replicate for the determination of the number of berries per cluster, rachis weight, and berry weight. The number of shoots per vine was determined in all experimental vines (80 vines per treatment) after veraison.

Five shoots from six vines per replicate were marked and used for measuring the lengths of the main and secondary shoots (24 vines per treatment). To determine the leaf area, allometric equations were obtained relating total shoot length and leaf surface, separating main and lateral shoots. These equations were used to calculate the total leaf area per vine (24 vines per treatment). Pruning weight was recorded in each experimental vine in winter and the Ravaz index calculated as the ratio of fruit yield to the pruning weight. Finally, the irrigation water productivity (WP) was calculated as the relationship between the kilograms of fruit produced by the vines and the irrigation water applied to each treatment (Fernández et al. 2020).

To determine the vine nutritional status, the concentrations of macronutrients and micronutrients in the leaf tissues (petioles) were analysed. Leaf samples were taken at veraison. In 2018 (the only year for which the results are shown), 10–15 complete leaves were collected per sample (without apparent symptoms of nutritional deficiencies, viruses or fungi) in each experimental unit. Therefore, a total of four independent samples per treatment were analysed. The leaves opposite to the second bunch of fruiting branches and medium vigour were picked, according to the

methodology described by Romero et al. (2010). Once all the leaf samples were collected, they were placed in a portable refrigerator and transported to the laboratory, where the petioles and blades were separated. Petioles were washed three times with running water and then twice with distilled water. The plant material was then placed in an oven at 60 °C for at least 48 hours. Once dried, the samples were crushed using a mill (batch mill, A 10 basic, IKA, Staufen, Germany) and sieved (1 mm mesh).

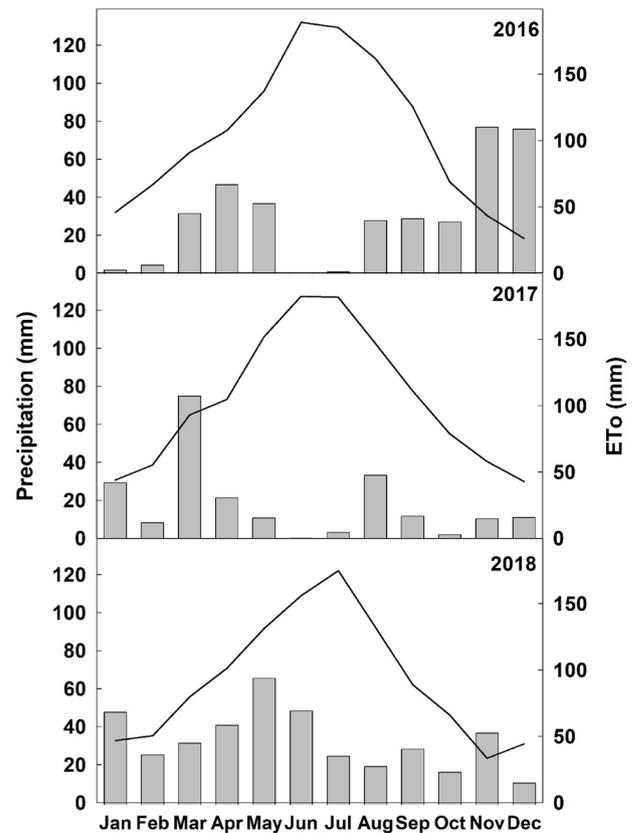
The leaf material was analysed in the Ionomics service facilities, where the mineral elements were determined by means of an inductively coupled plasma analyser linked to an optical emission spectrophotometer (iCAP 7200, ICP-OES, Thermo-Fisher Scientific, MA, USA).

### Grape composition

Grape analysis was carried out on 16 independent samples (500 berries each) per treatment (4 per field replicate), three different times throughout the ripening period. Total soluble solids (TSS) were determined using a digital refractometer (Atago RX-5000, Tokyo, Japan). Juice pH and total acidity (TA) were determined by titration with 0.1 N NaOH using an automatic titrator (Crisón mod. Basic 20, Barcelona, Spain). To assess the extractable phenolic compounds, 50 g of a solution composed of 5 g of tartaric acid, 2.5 mL of 32% NaOH solution and water up to 1 litre (pH 3.2) were added to 50 g of grapes. Then, this mix was crushed and homogenized in a Thermomix F6 (Vorwerk Spain M.S.L., S.C., Madrid, Spain) for 2 min at a constant and smooth speed (3). Then, the samples were macerated for 4 h. After this time, the samples were filtered in a funnel with glass wool, and the filtrates were centrifuged for 15 minutes at 4000 rpm. The phenolic composition of the extracts was determined with UV and visible spectrophotometry, using a UV–visible JASCO V-630 spectrophotometer (JASCO Analytical Instruments, Tokyo, Japan). Colour intensity (CI) and total phenol index (TPI) were estimated using the method described by Glories (1984). Anthocyanins were determined using the method of Puissant-León described by Blouin (1992). The concentration of tannins was estimated according to Sarneckis et al. (2006).

### Statistical analysis

The significances of the treatment, year, and their interaction on vine performance and grape composition parameters were assessed using a two-way analysis of variance (ANOVA). The means were separated by Duncan's multiple range test ( $p < 0.05$ ) when the ANOVA test was significant (IBM SPSS Statistics 26, Armonk, New York, USA). Linear regressions between the  $S_{\psi}$  and agronomic and grape composition variables, using data from each replication, were



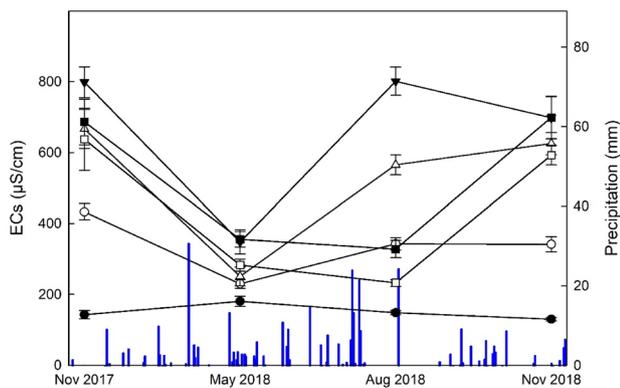
**Fig. 1** Monthly accumulated precipitation (mm) and reference evapotranspiration ( $ET_0$ ) (mm) during the 3 years of study (2016, 2017 and 2018). Data were collected from the Ontur meteorological station. (<http://crea.uclm.es/siar/datmeteo/consulta.php=6>)

calculated using SigmaPlot software (version 11.0) (Systat Software, San Jose, CA, USA). In addition, the residuals from the regression between TSS and the leaf area-to-yield ratio were used to assess differences between treatments.

## Results

### Meteorological conditions

The rainfall regime in the study area differed among the three experimental seasons (Fig. 1). The total precipitation recorded in 2016 and 2018 (360 and 405 mm, respectively) was higher than the average for the 20 years prior (291 mm), while 2017 was a dry year (224 mm). In contrast, the total annual  $ET_0$  was fairly similar in the three experimental years (1,230, 1,250 and 1,280 mm in 2016, 2017 and 2018, respectively) (Fig. 1), although in 2018, during the summer period,  $ET_0$  values were lower in June August and September than in 2016 and 2017. The thermal growing degree days (GDD) from April to October with a base temperature of 10 °C



**Fig. 2** Evolution of the electrical conductivity of the 1:5 soil extract (EC) ( $\mu\text{S}/\text{cm}$ ) in the different treatments: Rainfed (●), Control (○), Sul: Control + Sulphates (▼); Chl: Control + Chloride (△); SulV: Control + Sulphates post-veraison (■); ChIV: Control + Chloride post-veraison (□), during a complete year ( $n = 16$ ), from November 2017 to November 2018. Error bars represent the standard error of the means. Blue bars represent the daily precipitation (mm)

(Amerine and Winkler 1944), were 1706, 1921 and 1737 °C in 2016, 2017 and 2018, respectively.

### Salt accumulation in soil and leaves

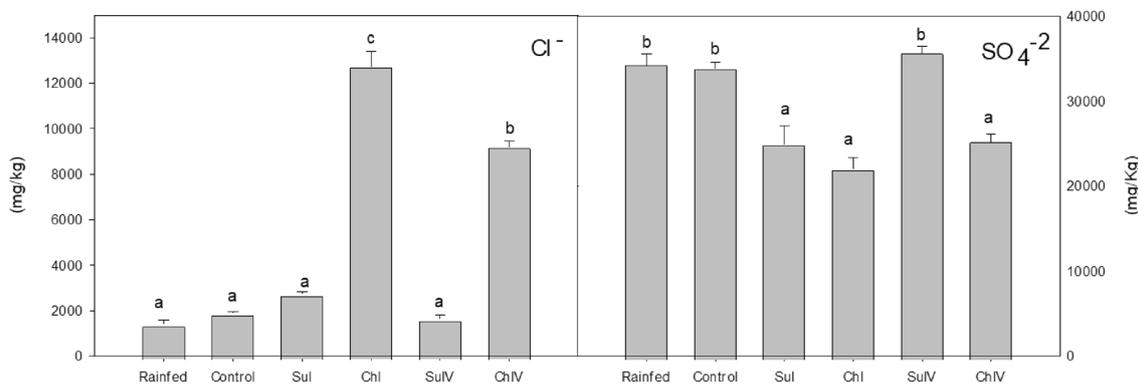
The saline irrigation treatments resulted in considerable differences in the soil electrical conductivity ( $\text{EC}_{1.5}$ ) during the period analysed (Fig. 2). The Rainfed treatment maintained the lowest  $\text{EC}_{1.5}$  values, which remained stable at around 150  $\mu\text{S}/\text{cm}$ . The Control treatment presented higher  $\text{EC}_{1.5}$  values than the Rainfed treatment, but did not show high variations in  $\text{EC}_{1.5}$  over time (Fig. 2). Treatments irrigated with saline water (Sul, Chl, SulV and ChIV) showed high  $\text{EC}_{1.5}$  values in November 2017, with an average of 697  $\mu\text{S}/\text{cm}$

cm. In the sampling carried out in May 2018,  $\text{EC}_{1.5}$  values significantly decreased to 300  $\mu\text{S}/\text{cm}$ . It must be noted that irrigation ended after harvest and was restored in June 2018 (Control, Sul and Chl). Before veraison, Sul (800  $\mu\text{S}/\text{cm}$ ) and Chl (565  $\mu\text{S}/\text{cm}$ ) significantly increased their  $\text{EC}_{1.5}$  values as compared to SulV (327  $\mu\text{S}/\text{cm}$ ) and ChIV (232  $\mu\text{S}/\text{cm}$ ), while those treatments with irrigation starting after veraison (SulV and ChIV) maintained an  $\text{EC}_{1.5}$  similar to those observed in May. Finally, in the last sampling carried out after the last harvest, all the treatments irrigated with saline water (Sul, Chl, SulV, and ChIV) showed similar  $\text{EC}_{1.5}$  values, which were higher than those from the Control and Rainfed treatments (Fig. 2).

In the last experimental season, the concentration of chlorides and sulphates accumulated in plant leaves were assessed (Fig. 3). Both treatments irrigated with chloride saline water (Chl and ChIV) significantly increased (up to 5 times) the chloride accumulated in the leaf petioles. In relation with the concentration of sulphates in the petioles, although certain differences were observed among treatments (Fig. 3), no clear effect of saline irrigation was observed. In both saline waters treatments, there was a tendency ( $p$  value = 0.08) to increase sodium levels in leaf petioles (Supp. Table 1).

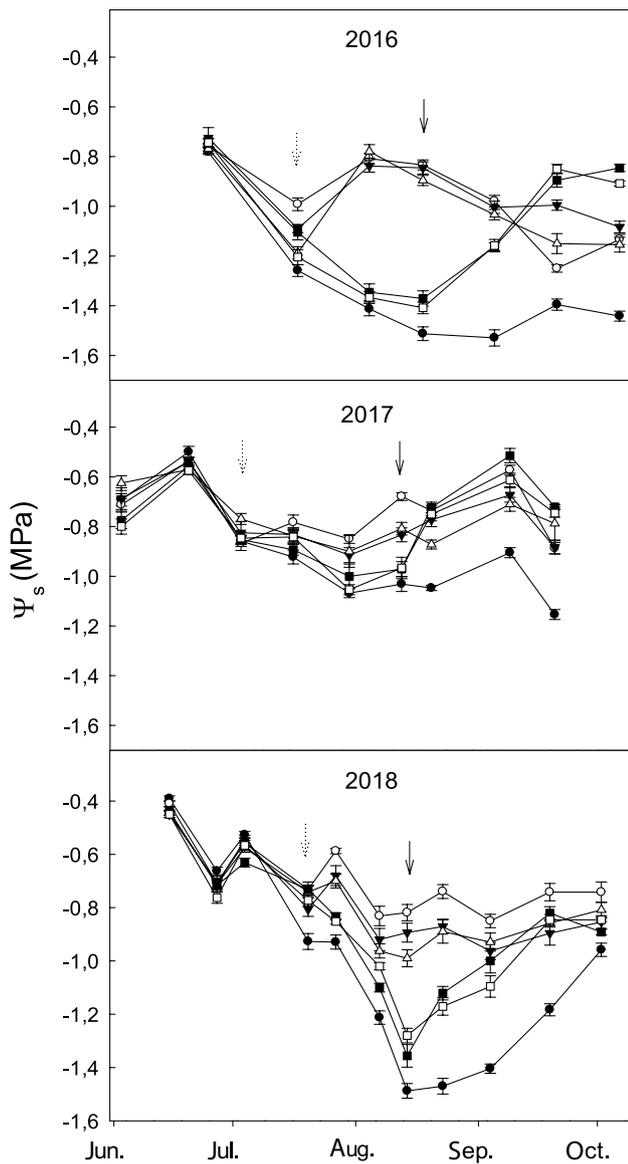
### Water status

A gradual decrease in  $\Psi_s$  was observed in all the treatments as the season progressed (Fig. 4), although this trend changed at the moment in which irrigation began. The most negative  $\Psi_s$  was recorded in the Rainfed treatment during the 3 years of study, with values of  $-1.53$  MPa in 2016,  $-1.15$  MPa in 2017 and  $-1.49$  MPa in 2018. In 2016, after the start of the post-veraison irrigation, the  $\Psi_s$  of the ChIV and SulV treatments increased significantly to values



**Fig. 3** Accumulation (mg/kg) of chlorides  $\text{Cl}^-$  and sulphates ( $\text{SO}_4^{-2}$ ) in the petioles of the leaves ( $n = 12$ ) from vines from the different treatments. The error bars represent the standard error of the means

and different letters indicate significant differences among treatments ( $p < 0.05$ ) according to Duncan's multiple range test



**Fig. 4** Evolution of the stem water potential ( $\Psi_s$ ) measured at midday ( $n = 12$ ) in the different treatments Rainfed (●), Control (○), Sul: Control + Sulphates (▼); Chl: Control + Chloride (△); SulV: Control + Sulphates post-veraison (■); ChlV: Control + Chloride post-veraison (□), applied during the 3 years of study. Error bars represent the standard error of the means

even higher than those found in the treatments which were irrigated over the entire season (Control, Sul and Chl). Throughout 2017, the treatments did not reach  $\Psi_s$  values as low as in 2016 and 2018. Since the beginning of July 2016, significant differences were observed between the treatments irrigated throughout the season (Control, Sul and Chl) and those irrigated after veraison (SulV and ChlV). In 2016 after veraison, the water status of all the irrigated treatments was similar between them and significantly higher than that of the Rainfed treatment. Non-consistent differences were observed between treatments with the same irrigation start date and different type of salt (Sul, Chl, or SulV–ChlV). During August (2018), significant differences were observed between the treatments grouped by the same irrigation start time, with the Control treatment showing higher  $\Psi_s$  values. These differences between irrigation treatments disappeared when irrigation started after veraison (SulV–ChlV).

Considering the cumulative water stress experienced, the Rainfed treatment reached the highest  $S_\psi$  values as compared to the rest of the treatments during the three seasons (Table 3). In 2016 and 2018 seasons, the treatments irrigated after veraison (SulV and ChlV) tended to show a  $S_\psi$  higher than the treatments irrigated throughout the whole growing season (Control, Sul and Chl), although these differences were not significant. After comparing the treatments with the same irrigation starting time (Control, Sul and Chl), it was observed that the saline treatments (Sul and Chl) reached a higher (but not significant)  $S_\psi$  than the Control in every season studied.

### Vegetative growth and yield

The year exerted a significant influence on all the vegetative growth and yield parameters measured (Table 4). Similarly, the watering regimes had a significant influence on these parameters, except for the number of berries per cluster. The interaction between year and watering regime was significant for shoot length, yield, number of clusters per vine, and cluster and berry weights (Table 4).

The number of shoots per vine was only different between treatments in the first year of the trial (Table 5). Shoot length in 2016 was higher in the Rainfed, Control, Sul and Chl treatment, while in 2017 and 2018, the greatest main shoot

**Table 3** Water stress integral (MPa \* day) for each treatment (Rainfed, Control, Sul: Control + Sulphates; Chl: Control + Chloride; SulV: Control + Sulphates post-veraison; ChlV: Control + Chloride post-veraison) and year

	Rainfed	Control	Sul	Chl	SulV	ChlV	ANOVA
2016	120 d	74 a	77 ab	85 abc	91 bc	94 c	***
2017	76 d	51 a	56 abc	53 ab	53 ab	57 cd	**
2018	76 c	40 a	50 ab	49 ab	61 bc	58 bc	**

Within each row, values followed by a different letters are significantly different at  $P < 0.05$ . \*\* and \*\*\* mean statistical significance at  $P < 0.01$  and  $P < 0.001$ , respectively

**Table 4** Significance of the effects of year, treatment and their interaction on the vegetative growth, yield and berry composition parameters studied

Parameter	Year	Treatment	Year x Treatment	Parameter	Year	Treatment	Year x Treatment
No. of shoots/ vine	***	***	ns	TSS (°Brix)	***	ns	ns
Shoot length (cm)	***	**	***	pH	***	**	ns
Secondary Shoot length (cm)	***	***	ns	Total acidity (g/L)	ns	**	***
Leaf area (m <sup>2</sup> )	***	*	ns	CI	***	***	*
Yield (t/ha)	***	***	***	TPI	***	***	***
No. of clusters/ vine	***	***	**	Tannin (mg/L)	***	*	**
Cluster weight (g)	***	***	*	Anthocyanin (mg/L)	***	***	**
Rachis weight (g)	***	**	ns				
No. of berries/ cluster	***	ns	ns				
Berry weight (g)	***	***	**				
LA to yield ratio	***	***	ns				

LA leaf area, TSS total soluble solids, CI colour intensity, TPI Total polyphenol index. \*, \*\* and \*\*\* indicate significant differences at  $p < 0.05$ , 0.01 and 0.001, respectively, ns: indicates non-significant effects ( $p > 0.05$ )

**Table 5** Vegetative growth parameters, pruning weight and Ravaz index of Monastrell vines under the different treatments (Rainfed, Control, Sul: Control + Sulphates; Chl: Control + Chloride; SulV: Control + Sulphates post-veraison; ChlV: Control + Chloride post-veraison) during the 3 years of study (2016-2018)

Parameter	Year	Rainfed	Control	Sul	Chl	SulV	ChlV	ANOVA
No. of shoots/vine	2016	14 a	15 ab	13 a	13 a	15 ab	16 b	*
	2017	13	14	13	13	14	13	ns
	2018	18	19	19	18	20	19	ns
Shoot length (cm)	2016	97.5 c	91.3 abc	90.5 abc	94.2 bc	87.8 ab	85.0 a	**
	2017	99.5 a	120.8 c	114.3 bc	115.5 bc	118.4 c	102.9 ab	**
	2018	87.3 a	102.1 b	81.5 a	81.7 a	85.3 a	83.2 a	**
Secondary shoot length (cm)	2016	10.9 a	16.7 b	13.8 ab	14.6 b	15.3 b	16.1 b	*
	2017	13.9 a	20.1 b	14.4 a	19.2 b	17.4 ab	16.1 ab	*
	2018	7.2 a	10.3 b	6.9 a	8.2 ab	5.7 a	5.5 a	*
Total leaf area (m <sup>2</sup> /vine)	2016	5.70	5.63	5.30	6.24	5.32	5.73	ns
	2017	4.85 a	6.61 c	5.08 b	6.01 b	6.25 b	4.81 a	**
	2018	4.70 a	5.82 b	4.58 a	4.49 a	4.69 a	4.43 a	**
Pruning weight (kg/ vine)	2016	—	—	—	—	—	—	
	2017	0.75 a	1.13 c	1.02 b	1.13 c	1.08 bc	0.82 a	***
	2018	0.88 a	1.25 c	1.08 b	0.96b	0.92 b	0.95 b	*
LA to yield ratio	2016	1.60 b	1.07 a	1.34 ab	1.18 ab	1.56 ab	1.39 ab	**
	2017	1.26 c	1.01 bc	0.82 ab	0.72 a	0.90 ab	1.04 bc	**
	2018	0.74 b	0.60 a	0.53 a	0.52 a	0.59 a	0.54 a	***
Ravaz index	2016	—	—	—	—	—	—	
	2017	3.24	3.53	3.68	3.31	3.31	3.89	ns
	2018	7.25 a	7.70 ab	7.91 ab	9.07 b	8.63 ab	8.64 ab	*

LA leaf area. \*, \*\* and \*\*\* indicate significant differences among treatments at  $p < 0.05$ , 0.01 and 0.001, respectively, ns indicates non-significant effects ( $p > 0.05$ ). Different letters indicate significant differences among treatments ( $p < 0.05$ ) within a given year according to the Duncan's multiple range test. In 2016, pruning weight data are not shown because the wine grower performed a mechanical pruning prior to the measurements

length was observed in the Control treatment, which was significantly different from that measured in the Rainfed treatment in both years. In 2018, significant differences were found between the Control and the Sul and Chl treatments.

On the other hand, the Control treatment had the greatest secondary shoot length every year, although this difference was only consistently significant when compared to the Rainfed treatment. A slight reduction in secondary shoot

length was also observed in the treatments irrigated with saline water (Sul and Chl) as compared to the Control, being this reduction statistically significant with the Sul treatment. In this sense, a reduction was also observed in the growth of the secondary shoots in the treatments in which irrigation started after veraison (SulV and ChlV), as compared with the Control treatment, although it was statistically different in 2018 (Table 5). In fact, the reduction in the secondary shoot length increased over the years. The Control treatment showed the highest leaf area in 2017 and 2018, when compared with the rest of the treatments. In 2017, the Rainfed and ChlV treatments had the lowest leaf area ( $<5 \text{ m}^2$ ). In 2018, all the treatments had a significantly reduced leaf area as compared to the Control, without significant differences between them. Overall, the saline treatments slightly reduced the leaf area compared to Control, with the exception of the first year of the study (Table 5). Pruning weight and Ravaz index for the year 2016 were not recorded due to an early pruning carried out by the winegrower. In 2017 and 2018, the Rainfed treatment showed the lowest pruning weight. Although significant differences were observed in 2018, a clear effect of the treatments was not observed (Table 5). In addition, the evaluation of leaf area (LA) to yield ratio was performed by estimation, using the total leaf area ( $\text{m}^2/\text{vine}$ ) and yield ( $\text{kg}/\text{vine}$ ). In the first 2 years, the treatments

resulted in a leaf area to yield ratio that ranged from 0.72 to  $1.60 \text{ m}^2/\text{kg}$ . In the last year, the ratio decreased in all the treatments ( $0.52$  to  $0.74 \text{ m}^2/\text{kg}$ ), as observed in (Table 5). The LA/yield ratio was higher in the Rainfed treatment during the 3 years of study, although these differences were only significant in 2018. Finally, a clear effect was not observed among the rest of the treatments, with a high variability observed in the treatments depending on the year of study.

In terms of grapevine yield, the Rainfed treatment showed a significantly lower yield in comparison with the Control in the three experimental seasons (Table 6). Moreover, in 2017 and 2018 the yield of the Rainfed treatment was significantly lower than that of the rest of the treatments. In addition, the Control obtained the highest yields in every year of study, but this parameter was significantly different as compared to all the other treatments only in 2016 and 2018 (Table 6). In 2017, the yields from the Sul and Chl treatments were similar to the Control. Moreover, no significant differences were detected between treatments that had been watered starting at the same moment in time, but differed in the type of salt applied (Chl–Sul and ChlV–SulV) (Table 6).

Regarding yield components, the Rainfed treatment showed the lowest values for the number of clusters per vine, cluster weight, and berry weight in the 3 years of study,

**Table 6** Yield components of Monastrell vines subjected to different irrigation treatments (Rainfed, Control, Sul: Control + Sulphates; Chl: Control + Chloride; SulV: Control + Sulphates post-veraison; ChlV: Control + Chloride post-veraison) during the 3 years of study (2016–2018)

Parameter	Year	Rainfed	Control	Sul	Chl	SulV	ChlV	ANOVA
Yield (t/ha)	2016	7.6 a	12.0 c	8.7 ab	9.8 b	8.5 ab	9.6 b	***
	2017	5.3 a	8.8 c	8.4 c	8.4 c	7.9 b	7.1 b	***
	2018	14.1 a	21.3 c	18.9 b	19.3 b	17.6 b	18.2 b	***
No. of clusters/ vine	2016	17 a	23 c	19 ab	20 b	18 ab	18 ab	***
	2017	14 a	19 c	20 c	20 c	18 bc	16 b	***
	2018	22 a	27 b	25 ab	24 a	24 a	24 a	***
Cluster weight (g)	2016	213 a	217ab	218 ab	239 bc	232 bc	242 c	*
	2017	173 a	200 c	195 bc	194 bc	190 abc	181 ab	*
	2018	282 a	356 c	351 bc	368 c	325 b	326 b	***
Rachis weight (g)	2016	4.47 a	5.99 b	5.46 ab	6.01 b	4.96 ab	4.64 a	*
	2017	8.25 a	11.30 bc	11.99 c	9.27 ab	8.49 a	9.89 abc	*
	2018	7.34 a	10.64 b	9.81 ab	9.84 ab	9.71 ab	8.68 a	**
No. of berries/cluster	2016	168	140	143	175	165	150	ns
	2017	114	142	142	124	114	118	ns
	2018	223	233	237	275	265	231	ns
Berry weight (g)	2016	1.13 a	1.57 c	1.37 bc	1.37 bc	1.34 ab	1.31 ab	**
	2017	1.37 a	1.82 abc	1.62 ab	2.24 c	1.81 abc	2.04 bc	*
	2018	1.10 a	1.69 c	1.68 c	1.44 b	1.40 b	1.38 b	***
WP ( $\text{kg}/\text{m}^3$ )	2016	6.81 b	5.12 a	3.53 a	4.04 a	3.98 a	4.60 a	**
	2017	7.13 b	4.94 a	4.66 a	4.76 a	4.33 a	4.03 a	**
	2018	6.80	6.77	6.05	6.17	5.61	5.98	ns

WP water productivity. \*, \*\* and \*\*\* indicate significant differences among treatments at  $p < 0.05$ , 0.01 and 0.001, respectively, ns indicates non-significant effects ( $p > 0.05$ ). Different letters indicate significant differences among treatments within a given year ( $p < 0.05$ ) according to the Duncan's multiple range test

although these differences were not statistically significant (Table 6). In general, the treatments in which irrigation started early in the season, showed a higher number of clusters per vine, and higher cluster, rachis and berry weights. Regarding the number of berries per cluster, no significant differences were observed among treatments in any of the years studied (Table 6). In general, the treatments irrigated with saline waters since pre-veraison (Sul and Chl), did not show a consistent effect of the treatments on the yield components analysed. Finally, water productivity (WP) was significantly higher in the Rainfed treatment when compared to the rest of the treatments in 2016 and 2017 (Table 6). However, the other treatments did not differ between them in regard to WP values, independently of water regime and salinity.

### Grape composition

Annual average TSS values were similar in 2016 and 2017 (24 and 23°Brix). However, the average TSS concentrations were lower in 2018, due to an incomplete grape ripening. In every year studied, there was an increasing trend for grape total soluble solids concentration during the maturation period, although this trend was not equal for all the treatments (Supp. Figure 1). In 2016 and 2017, no clear effects

of the irrigation treatments on grape TSS were observed (Table 7). However, in 2018, the treatments that began to be irrigated after veraison (SulV and ChIV) showed a reduction in berry sugar concentration during the last days of grape maturation, while the remaining treatments continued with a positive trend in TSS level (Table 7). The evolution of pH was very similar among treatments, increasing as the grapes matured (Supp. Figure 1). In the last year, the Rainfed treatment reached higher pH values at harvest as compared to the rest of the treatments (Table 7). Contrary to pH, the evolution of total acidity during the 3 years of study decreased over the ripening period in all treatments, this decline being more pronounced in 2018. The Rainfed treatment showed the lowest values of total acidity at harvest in 2 of the 3 years of study, but only was statistically significant in the last year (Table 7).

In general, the concentrations of all the phenolic substances (anthocyanins, tannins, CI and TPI) increased throughout maturation in every treatment (Supp. Figure 2). Although the concentration of TPI increased during maturation in all the treatments, the treatments with the highest water stress (Rainfed, SulV and ChIV) tended to reach the highest TPI values at harvest. On the other hand, a clear effect of salinity was not observed on TPI (Table 7). Berries from Rainfed treatments showed the

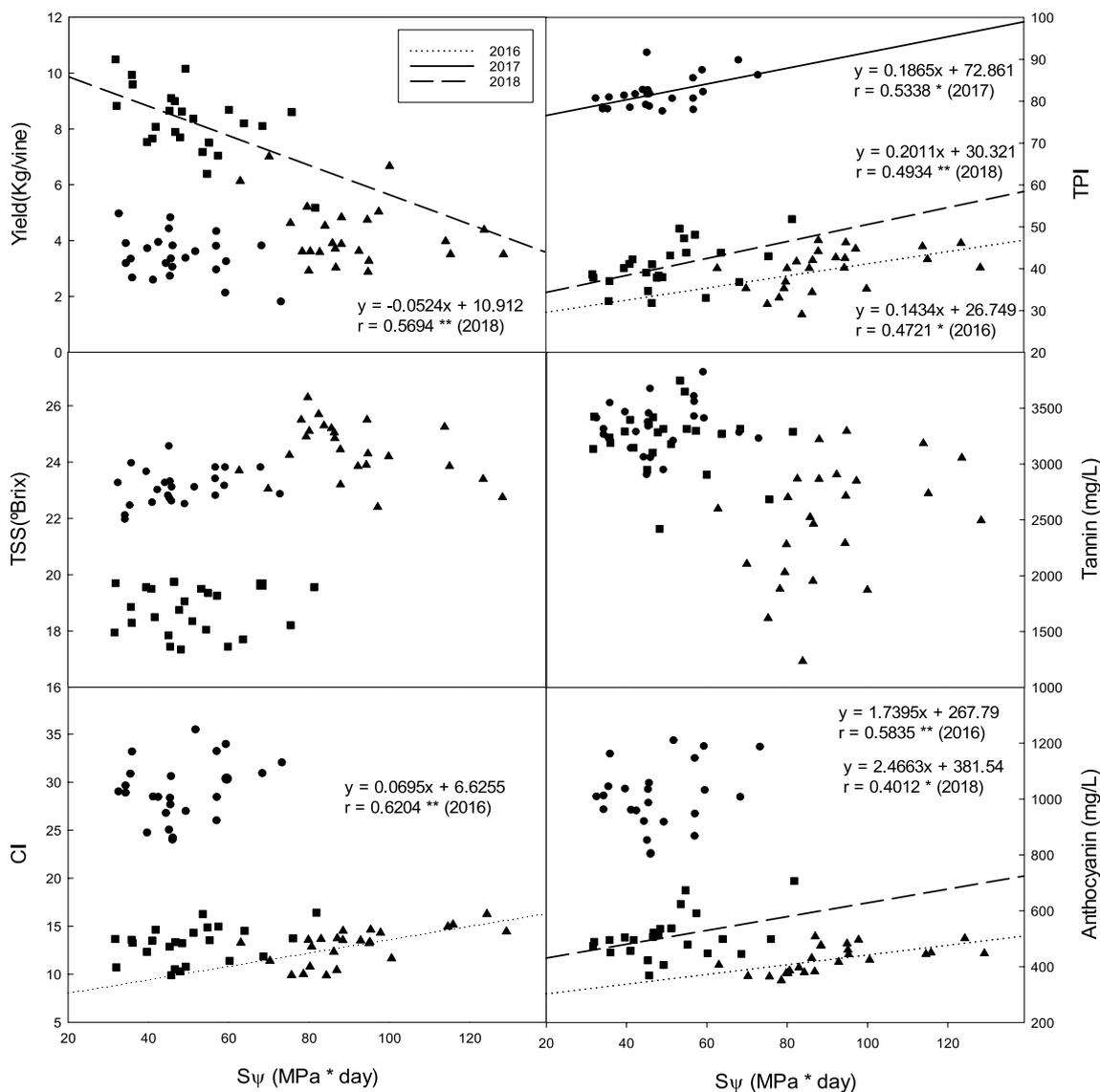
**Table 7** Fruit quality parameters in the grape from Monastrell at harvest for each treatment (Rainfed, Control, Sul: Control + Sulphates; Chl: Control + Chloride; SulV: Control + Sulphates post-veraison; ChIV: Control + Chloride post-veraison) during the 3 years of study (2016–2018)

Parameter	Year	Rainfed	Control	Sul	Chl	SulV	ChIV	ANOVA
TSS (°Brix)	2016	23.8 a	24.3 ab	25.0 b	25.2 b	24.3 ab	23.7 a	**
	2017	23.5 c	23.3 bc	23.1 bc	22.4 a	23.1 bc	22.8 ab	**
	2018	19.0 ab	18.1 a	18.6 ab	19.2 b	18.8 ab	18.21 a	*
pH	2016	3.49 bc	3.46 ab	3.53 bc	3.55 c	3.52 bc	3.41 a	**
	2017	3.42	3.39	3.42	3.39	3.40	3.38	ns
	2018	3.39 c	3.28 b	3.28 b	3.31 c	3.27 ab	3.21 a	***
Total acidity (g/L)	2016	4.6 a	4.9 ab	5.1 b	4.9 ab	5.1 b	5.5 c	***
	2017	5.1 b	5.7 c	5.5 c	4.9 b	4.4 a	4.5 a	***
	2018	4.0 a	4.8 b	4.8 b	4.7 b	4.8 b	5.6 c	***
TPI	2016	43.5 c	38.1 b	36.2 ab	33.9 a	43.7 c	43.5 c	***
	2017	51.1 c	52.5 c	48.4 bc	49.6 bc	47.6 a	48.1 b	***
	2018	49.2 c	35.2 a	36.4 a	37.6 a	42.5 b	41.5 b	***
CI	2016	15.2 d	12.9 b	11.2 a	10.6 a	13.8 c	13.8 c	***
	2017	12.7 d	10.4 c	10.0 bc	9.8 b	8.9 a	10.0 bc	***
	2018	15.6 d	12.9 b	11.3 ab	10.9 a	13.8 c	13.8 c	***
Anthocyanin (mg/L)	2016	460 b	386 a	382 a	390 a	460 b	478 b	***
	2017	418 d	367 c	370 c	322 b	288 a	312 ab	***
	2018	648 c	467 a	450 a	463 a	482 ab	523 b	***
Tannin (mg/L)	2016	2868 d	2401 ab	2181 a	1835 a	2664 c	2768 c	***
	2017	2778 c	2573 c	2325 b	2650 c	1961 a	2091 a	***
	2018	3495 b	3116 a	3175 a	3334 b	3134 a	3070 a	***

TSS total soluble solids, CI colour intensity, TPI total polyphenol index. \*, \*\* and \*\*\* indicate significant differences among treatments at  $p < 0.05$ , 0.01 and 0.001, respectively, ns: indicates non-significant effects ( $p > 0.05$ ). Different letters indicate significant differences among treatments within a given year ( $p < 0.05$ ) according to the Duncan's multiple range test

highest concentration of anthocyanins during all years of study (Table 7). Moreover, berries from the Rainfed, SulV and ChlV treatments showed higher concentrations of anthocyanins than the treatments irrigated throughout the season (Control, Sul and Chl) in 2 out of 3 years (2016 and 2018). Moreover, the Rainfed treatment had a significantly higher concentration of anthocyanins in comparison with the rest of the treatments in the last 2 years. No clear effects of salinity were observed on anthocyanin concentration. In 2 of the 3 years, the anthocyanin concentration was similar in all the treatments (Control, Sul, and Chl). The CI values were in agreement with the anthocyanin concentrations, with the Rainfed treatment showing a

significantly higher colour intensity at harvest than the rest of the treatments in every year studied (Table 7). The treatments irrigated for a longer period had a lower CI than the Rainfed treatment and the treatments in which irrigation started after veraison. In the 3 years of study, a slightly negative trend in CI was observed for the treatments irrigated with saline water (Sul and Chl) as compared to the Control. The accumulation of tannins was positive in 2016 and 2018, and the highest tannin concentration during the entire ripening period, in these 2 years, was observed under the Rainfed treatment (Table 7). However, in 2017, the concentration of tannins remained constant or even decreased. In that year, SulV and ChlV had a significantly



**Fig. 5** Relationships between the water stress integral and different traits over the three years of study: 2016 (▲), 2017 (●) and 2018 (■). Both regression equations and the Pearson correlation coefficients (r)

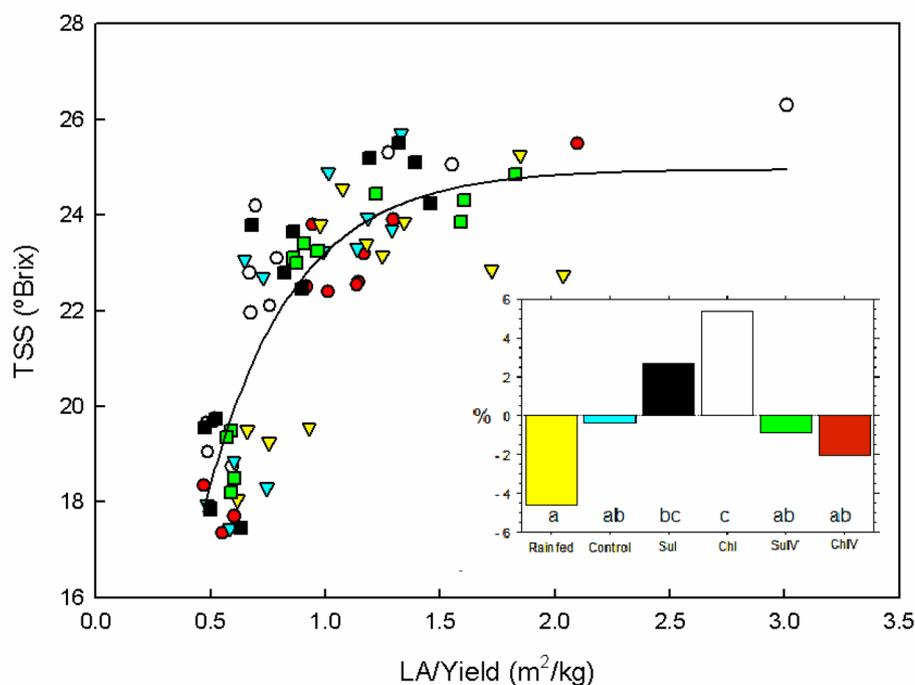
are shown. S $\psi$ : Water stress integral, CI: colour intensity, TPI: Total Polyphenolic Index. \*, \*\* and \*\*\*: indicate significant relationships for  $p < 0.05$ , 0.01 and 0.001, respectively

lower accumulation of tannins than the rest of the treatments during the final days of ripening (Supp. Figure 2).

### Relationships among indicators

The relationships between  $S_{\psi}$  and yield, TPI, TSS, CI, anthocyanins and tannins were calculated to obtain a comprehensive view of how water stress affected vine performance and grape composition (Fig. 5). No significant relationships were found between  $S_{\psi}$  and TSS and tannin concentration in any year of study. In contrast, significant linear relationships between  $S_{\psi}$  and the other indicators were observed (Fig. 5). However, TPI was the only parameter that showed significant linear relationships with  $S_{\psi}$  in all 3 years of the study (Fig. 5). Finally, the relationship between TSS and the leaf-area ratio to yield across seasons showed that the increase in TSS slowed down when this ratio reached values between 1.2 and 1.4  $m^2/kg$  (Fig. 6). Moreover, by analysing the residuals of each treatment in this regression, statistical differences in the accumulation of sugars in the grapes among treatments could be established regardless of their source-to-sink balance, calculated as the leaf area to yield ratio. This analysis showed that the Rainfed treatment reduced the relative TSS accumulation by 4%, significantly differing from treatments irrigated from the start with saline water, Sul or Chl, which increased it by 3% and 5%, respectively (Fig. 6).

**Fig. 6** Relationships between the LA-to-yield ratio and TSS ( $y = 24.95 * (1 - e^{-2.66 * x})$ ;  $r^2 = 0.71$ ;  $p < 0.0001$ ). Rainfed ( $\blacktriangledown$ ), Control ( $\blacktriangledown$ ), Sul: Control + Sulphates ( $\blacksquare$ ); Chl: Control + Chloride ( $\circ$ ); SulV: Control + Sulphates post-veraison ( $\blacksquare$ ); ChlV: Control + Chloride post-veraison ( $\bullet$ ). Data are averages of each replicate and season. Bar plots show regression residuals for each treatment with different letters indicating significant differences between them ( $p < 0.05$ )



## Discussion

### Responses to irrigation with saline water

Even if grapevines are considered moderately sensitive to salinity (Walker et al. 2002), the application of saline water did not drastically affect the vine performance. This was likely due to the fact that the  $EC_{1.5}$  values measured throughout the trial never exceeded 1,200  $\mu S/cm$  (threshold value of EC from which the yield is reduced by 9.6% for each unit increase of EC (Nauriyal and Gupta 1967)). This low effect of saline water on vine performance could be due to the characteristics of the soil texture, and the typical Mediterranean rainfall regime that occurs mainly during the dormant part of the season, with all the irrigated treatments showing a significantly reduced  $EC_{1.5}$  during winter (Fig. 2).

The dynamics of  $\Psi_s$  throughout the growing season was greatly determined by irrigation timing rather than water salinity (Fig. 4). Nevertheless, on most dates, Control vines showed a slightly (0.1–0.2 MPa) better water status than the treatments with the same irrigation rate but with saline water (Sul and Chl). This suggests that the high salt concentration of the irrigation water slightly increased vine water stress, likely due to an osmotic effect. The type of salt applied did not consistently affect  $\Psi_s$  under any of the watering regimes (Table 3) perhaps because the osmotic effect due to salinity was similar, considering that both types of saline water had the same electrical conductivity.

The experiment was carried out using the 1103P rootstock, which is known for its high tolerance to salinity (Walker et al. 2002; Zhang et al. 2002; Keller 2015). This rootstock is considered to confer the ability to reduce salt accumulation in plant tissues (Walker et al. 2010), buffering the possible detrimental effects of phytotoxic ion buildup on vine tissues and increasing the overall performance. In fact, the application of salty water alleviated the severe water stress experienced by Rainfed vines (Fig. 4) and improved vine performance. Nevertheless, in 2 of the 3 years of the study, the Control treatment yielded more than the treatments with the same irrigation strategy, but with saline water (Sul and ChI), although the loss of yield in 2016 may be more determined by the differences in the number of bunches than by the effect of the treatments; therefore, an overall 17% yield loss due to the saline effect was observed. In addition, in the last year of study, a significant accumulation of chlorides in the petiole tissues was detected when NaCl was added in the saline water (Fig. 3), suggesting that the continued application of salty water could compromise vine performance in the long term. In a study on irrigation with different salinity levels (EC = 1,200, 2,700, and 4,200  $\mu\text{S}/\text{cm}$ ) in Cabernet Sauvignon grafted onto 1103 Paulsen, Dag et al. (2015) found concentrations in leaf petioles about twice as high as in our Chloride treatments. Over 2 years, these authors did not detect significant differences in terms of yield, although a slight tendency towards yield reduction was observed with the increase in salinity. Nevertheless, these authors found mortality of 2.5 and 17.5% for vines irrigated with 2700, and 4200  $\mu\text{S}/\text{cm}$  water and suggested that this could be due to sodium reaching critical levels in woody tissues. In a 6 year trial carried out in Superior Seedless grapes irrigated with reclaimed water with a high sodium concentration, Netzer et al. (2014) observed significant differences between treatments in the concentration of  $\text{Na}^+$  in the soil, leaves and xylem. However, they did not report significant differences among treatments for grape yield despite the fact that the Na concentrations reached were four times higher than in our petioles (Supp. Table 1) and explained this as the result of the vines being grafted onto 1103P rootstock.

In terms of grape composition, no consistent differences were observed among treatments with the same irrigation regime but different types of salts (Sul-Clo and SulV-ChIV). Nevertheless, the relationship between the LA-to-yield ratio and the TSS did show a significant treatment effect (Fig. 6). This relationship shows that the accumulation of TSS in rainfed grapes was lower than expected for its source-to-sink balance. This could be explained by the fact that the water stress experienced could lead to a slight reduction in the photosynthetic capacity of the Rainfed vines (Romero et al. 2010). In contrast, irrigation with

saline water before veraison in the Sul and ChI treatments seemed to induce an increase in grape TSS accumulation (Supp. Figure S1). This may be due to the osmotic effect caused by irrigation with saline water from the start of grape ripening, which may have led to a better osmotic adjustment capacity (Keller et al. 2015).

In general, salinity did not affect berry weight at harvest; therefore, it might not have modified the skin-to-pulp ratio, what might explain the absence of a clear effect of salinity on the concentration of phenolic substances. In contrast to these results, Scacco et al. (2010), in a trial with different concentrations of soil salinity (ECs of 700, 1200 and 2100  $\mu\text{S}/\text{cm}$ ), observed that an increase in soil salinity improved the colour intensity of Nero d'Avola grapes. In the present study, the observed reduction in leaf vegetative growth due to salinity could not have a clear impact on cluster micro-climate, as the irrigation application started by mid-summer when most of the canopy growth within the cluster zone had already developed (Keller 2015).

### Effects of the watering regime

The  $\Psi_s$  results obtained in the three seasons of the study were indicative of severe water stress for the years 2016 and 2018 and moderate water stress for the 2017 season (Van Leeuwen et al. 2009). It is worth noting that 2017 was the driest year with the highest  $\text{ET}_o$ , yet it was the year with the lowest water stress. This is most likely because of the important rainfalls that occurred at the end of 2016 (163 mm in November and December). During the period, vines were dormant and therefore that precipitation accumulated in the soil and was available for the next season. In addition, the fact that 2017 was a year within general lower yield levels probably resulted in vines with higher capacity to mitigate the soil water deficit. In two studies on regulated deficit irrigation carried out on the Monastrell variety, Romero et al. (2016, 2018) observed similar values of  $\Psi_s$  to those obtained in the current research, in which the evolution of the  $\Psi_s$  was strongly associated to irrigation scheduling. In this sense, treatments in which more irrigation water was applied, produced less negative  $\Psi_s$  values (Fig. 4). As expected, Rainfed vines reached the highest  $S_\psi$  values as compared to those from the rest of the treatments in the 3 years studied (Table 3). In 2016 and 2018, the treatments watered after veraison (SulV and ChIV) showed higher values than the treatments watered before veraison (Control, Sul and ChI), probably because these treatments received higher volumes of irrigation.

Overall, the higher the  $S_\psi$ , the lower is the shoot growth. In this sense, treatments that suffered from a higher water stress also had lower pruning weights (Table 5). It should be noted that the difference between the mean values of the

leaf area-to-yield ratio were largely due to the difference in yield between the seasons. In fact, the values obtained in 2017 were on average 180% higher than in 2018 (1.6 vs. 0.6 m<sup>2</sup>/kg). The values of this last year, according to the optimal ones presented by Kliewer and Dokoozlian (2005), suggest that, in this season, the vines from all the treatments were over-cropped and unable to reach commercial ripeness, as was the case in the study (Table 7). The relationship between the LA-to-yield ratio and TSS observed in (Fig. 6) allows us to identify the optimum source-to-sink balance threshold for Monastrell cv. in relation to commercial ripeness, setting it between 1 and 1.4 m<sup>2</sup>/kg. Similarly, Intrigliolo and Castel (2011) found that ratios lower than 1.5 m<sup>2</sup>/kg negatively affected grape composition in the Tempranillo cultivar. In addition, the experimental treatments significantly affected the relationship between the LA-to-yield ratio and the TSS (Fig. 6). This regression analysis shows that the accumulation of TSS in Rainfed grapes was lower than expected from its source-to-sink balance.

Treatments that started to be irrigated before veraison showed higher values of shoot length, leaf area, and pruning weight than the treatments which began to be irrigated after veraison, although these differences were not significant (Table 5). This implies that water availability at the beginning of the growing season could have had a positive effect on vegetative growth as compared to the water availability at the end of the growing season regardless of salinity, as also reported by Munitz et al. (2018). Additionally, the Rainfed treatment reduced the growth of the secondary shoots by 33% as compared to the Control. Also, the salinized treatments that used the same irrigation scheduling as the Control reduced their secondary shoot length by almost 10% as compared to the Control, likely due to the osmotic effect on the soil water potential. Therefore, the growth of the secondary shoots was strongly influenced by the vine water status (Peréz-Álvarez et al. 2021). In the case of our vineyard, the winegrower trimmed the apex of vines, so the longitudinal growth of the shoots was inhibited and the growth of the lateral shoots was enhanced. In addition, the Rainfed treatment and the treatments irrigated after veraison (SulV and ChIV) reduced the growth of the lateral shoots as compared to the Control, with a greater reduction observed in the Rainfed treatment, as expected. Remarkably, the reductions observed in the Rainfed and treatments irrigated after veraison as compared to the Control, in the main and secondary shoot lengths and leaf areas, increased over the experimental seasons (Table 5). This suggests that there may be a carryover effect that is more marked in the treatments irrigated with saline water.

In the 3 years of the study, the Control treatment significantly improved yields, but reduced WP the first 2 years of study as compared to the Rainfed treatment (Table 6).

Similar to our study, Intrigliolo et al. (2016), for Cabernet Sauvignon, found significant WP improvements in Rainfed vines as compared to deficit irrigation treatment (75% ET<sub>c</sub>). It should be noted that in the last year of our study, the significant improvement of the WP in the Rainfed treatment was not observed. These yield and WP results were in agreement with those obtained by Romero et al. (2010, 2013, and 2016) in the Monastrell variety. Furthermore, similar results have been described in other varieties such as Tempranillo (Santesteban et al. 2011), Cabernet Sauvignon (Junquera et al. 2012), and Pinot Noir (Zufferey et al. 2017). In our study, the treatments watered after veraison (SulV and ChIV) slightly reduced yield (7%) as compared to those treatments that were irrigated before (Sul and Clo), because of both a decrease in cluster weight and a reduction of berry growth. These results suggest that greater water volumes applied after veraison do not restore the negative effects on yield produced by water stress that occurs before veraison (Keller et al. 2015; Buesa et al. 2017).

Water deficits impaired vine growth and decreased yield; in turn, they can also improve grape and wine composition, unless water stress is too severe (Chaves et al. 2009; Gambetta et al. 2020). For instance, water deficit can reduce TSS accumulation during ripening (Sipiora and Gutierrez 1998; Romero et al. 2010). However, this effect was not observed in vines from the Rainfed and post-veraison irrigated treatments as compared to those from the Control treatment, probably because the source-to-sink balance buffered this effect. In Rainfed vines, the yield was lower and the LA-to-yield ratio was increased compared to that of the other treatments (Table 5). Nonetheless, the lack of association between TSS and S<sub>ψ</sub> was not expected (Romero et al. 2010, 2013) and may point to an osmotic adjustment in response to water salinity. Berries from the Rainfed treatment had a lower total acidity as compared to the Control in 2 of 3 years, which is in line with Santesteban et al.'s (2011) results. This effect may be due to a decrease in tartaric and/or malic acid concentration (data not shown) in response to reduced vegetative growth, and thus greater exposure of the clusters to sunlight increases their temperatures and results in a higher rate of malic acid catabolism (Santesteban et al. 2011; Buesa et al. 2017).

Grapes from the treatments that suffered a more severe water stress (Rainfed, SulV and ChIV) showed higher values of phenolic substances, colour intensity, and anthocyanin content than those from less water stressed treatments (Supp. Figure 2). In this sense, Romero et al. (2013) also observed an increase in total anthocyanins and colour intensity in Monastrell wines that came from plants that had suffered water stress. Moreover, similar results have been observed in different varieties such as: Pinot noir (Zufferey et al. 2017), Cabernet Sauvignon (Roby et al. 2004; Chalmers et al. 2010)

and Monastrell (Romero et al. 2010). Some authors have shown that the expression of some of the genes responsible for anthocyanin synthesis increased in response to water deficits (Castellarin et al. 2007; Deluc et al. 2009). According to our results, a severe water stress before veraison can result in a higher anthocyanin concentration in grapes.

The Rainfed treatment significantly increased the concentration of tannins in the grapes in 2 of 3 years, similarly to previous reports (Ojeda et al. 2002; Roby et al. 2004). However, the effect of water deficit was much greater on the concentration of anthocyanins than that of tannins. Moreover, no clear effects of water stress on tannin concentrations were observed in the treatments in which irrigation started after veraison (SulV-ChIV). Similarly, Kennedy et al. (2002), in Cabernet Sauvignon, and Matthews and Anderson (1989) in Cabernet Franc, obtained inconsistent results regarding tannin concentrations.

## Conclusions

In a semi-arid and warm Mediterranean climate, Monastrell vines grafted onto 1103P rootstock, tolerant to water and salinity stress, grown in a loamy-sandy soil, were more affected by the watering regime than by the water salinity. Nonetheless, the osmotic stress likely induced by soil salinity impaired vegetative growth and yield. Therefore, irrigation with saline water relieved vine water stress as compared to the Rainfed vines. However, grape composition was not clearly improved by irrigation regardless of water salinity, because it decreased phenolic substances and increased titratable acidity as compared to the Rainfed treatment. Therefore, saline waters with high electrical conductivity (5000  $\mu\text{S}/\text{m}$ ) can be employed as long as the vineyard soil has a high leaching capacity, avoiding the accumulation of salts in the short to medium term. Winegrowers who prioritize grape composition should focus on water regime rather than salinity. Nevertheless, the possible carryover effects on vine performance demands a long-term study for further analysis of the possible adverse effects of saline water irrigation on vine performance and grape composition.

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