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RESPONSE OF GRAPEVINE CV. 'BRANCELLAO' AND 'SOUSÓN' TO SUPPLEMENTARY IRRIGATION: WATER RELATIONS, VINE GROWTH, YIELD AND BERRY AND WINE COMPOSITION

RESPOSTA DAS VARIEDADES 'BRANCELLAO' E 'SOUSÓN' À REGA SUPLEMENTAR: RELAÇÕES HÍDRICAS, CRESCIMENTO VEGETATIVO, PRODUÇÃO E COMPOSIÇÃO DA UVA E DO VINHO

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SUMMARY

Growers are concerned about the negative impacts on vine yield and berry quality caused by global warming. Irrigation systems are increasingly being installed in vineyards in order to counteract those problems. Therefore, an efficient irrigation management is required. In this context, a field experiment was carried out over three years (2012-2014) on red *Vitis vinifera* (L.) cv. 'Brancellao' and 'Sousón' in order to assess the effects of supplementary irrigation on vine performance and must and wine composition. Rain-fed vines were compared with a treatment irrigated to 40% of potential evapotranspiration (ET0) from bloom to two/three weeks before harvest. Both cultivars showed less negative stem water potentials under irrigation than under rain-fed conditions; however, stomatal conductance was similar between treatments. Yield was unaffected by irrigation, whereas pruning weight tended to increase in both cultivars. Must and wine composition, including amino acids and volatile compound concentrations, were mostly unaffected by irrigation. Water productivity was higher under rain-fed conditions and, as a consequence, gross incomes were not increased by irrigation. Therefore, irrigation does not seem an economically viable agricultural practice under the conditions of this trial.

RESUMO

Os viticultores estão preocupados com os impactos negativos no rendimento da videira e na qualidade da uva causados pelo aquecimento global. Estão a ser instalados sistemas de irrigação nas vinhas para ultrapassar estes problemas. Portanto, é necessária uma gestão eficiente da irrigação. Neste contexto, um ensaio de campo foi realizado durante três anos (2012-2014) em castas tintas 'Brancellao' e 'Sousón' (*Vitis vinifera* L.) a fim de avaliar os efeitos da irrigação suplementar no desempenho da videira e na composição da uva e do vinho. Videiras em sequeiro foram comparadas com um tratamento irrigado a 40% da evapotranspiração potencial (ET0) desde a floração até duas/três semanas antes da vindima. Ambas as variedades mostraram potenciais hídricos do ramo menos negativos sob irrigação que em sequeiro; no entanto, a condutância estomática foi similar entre os tratamentos. O rendimento não foi afetado pela irrigação, mas o peso de poda tendeu a aumentar em ambas as variedades. A composição do mosto e do vinho, incluindo concentrações de aminoácidos e de compostos voláteis, foram afetadas pela irrigação. A produtividade da água foi maior em condições de sequeiro e, como consequência, o rendimento bruto não sofreu aumento com a irrigação. Portanto, a irrigação não parece ser uma prática agrícola economicamente viável nas condições deste estudo.

Key words: irrigation, must composition, Ribeiro, water potential, volatile compounds.
Palavras-chave: irrigação, composição do mosto, Ribeiro, potencial hídrico, compostos voláteis.

INTRODUCTION

Global warming predictions estimate reductions in rainfall and increasing evapotranspiration for the near future (IPCC, 2014). Modifications in the spatial and temporal distribution of rainfall (Mirás-Avalos *et al.*, 2009) as well as temperature increases (Cruz *et al.*, 2009) have already been reported in Galicia (NW Spain). These changes might cause that grapevine (*Vitis vinifera* L.) cultivars adapted to temperate and cool climates reach their maximum temperature threshold and lose their specific organoleptic

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qualities. The negative impacts of water deficit on vine growth, yield and the need to secure and stabilize production (Gouveia *et al.*, 2012) have raised vinegrowers concern for using irrigation in their vineyards, even in humid regions such as northwest Iberian Peninsula. In this sense, deficit irrigation strategies have been successfully adopted as management tools to ensure an adequate balance between vegetative growth and yield while preserving water resources and enhancing berry composition (Dry *et al.*, 2001; dos Santos *et al.*, 2003; Intrigliolo and Castel, 2009). Therefore, irrigation (amount and time) must be applied in accordance with the "terroir" and type of wine to be produced (Ojeda, 2008) in order to maintain an appropriate soil water content, according to grapevine phenological stage, and avoid potential negative impacts on vine vigour, berry composition and wine quality (Jackson and Lombard, 1993; Deloire *et al.*, 2004). Furthermore, the need for customizing irrigation according to cultivar and region of production has been highlighted (Basile *et al.*, 2012).

Even though a great effort on research has been devoted to determine the effects of irrigation and vine water status on yield and berry composition for several grapevine cultivars (e.g. Jackson and Lombard, 1993; Reynolds *et al.*, 2007; van Leeuwen *et al.*, 2009; Intrigliolo *et al.*, 2012), the results were different due to the great variety of climate conditions and vineyard managements. These studies lack from a thorough assessment of the chemical composition of the musts and wines produced, so they do not reflect the possible influence that irrigation may exert on the volatile composition of the wines. Moreover, no studies about irrigation have been conducted on red grapevine cultivars growing in the northwest of the Iberian Peninsula, except for 'Touriga Nacional' (Gouveia *et al.*, 2012), and the research effort has concentrated on white cultivars (Fandiño *et al.*, 2012; Trigo-Córdoba *et al.*, 2015; Cancela *et al.*, 2016).

Although in Galicia white grapevine (*Vitis vinifera* L.) cultivars are mainly grown, red varieties are also spreading. Among them, 'Sousón' and 'Brancellao' represent a significant area of cultivation in Galicia (NW Spain) and north of Portugal. These two autochthonous cultivars are used for plurivarietal wines. 'Sousón' is a traditional red grapevine cultivar that is spreading over the Ribeiro Designation of Origin (DO), nowadays it is ranked first in production. This cultivar is characterized by its high polyphenolic potential and is used for providing colour to plurivarietal wines (Consello Regulador Ribeiro, 2016); however its growing cycle is long. 'Brancellao' is the third autochthonous red cultivar in the Ribeiro DO. Its growing cycle is medium and it is used as a complement for plurivarietal wines since it has low polyphenolic content but a good aromatic expression (Consello Regulador Ribeiro, 2016). The potential of 'Brancellao' as a possible alternative to the 'Mencía' cultivar for the elaboration of quality red wines, due to its maturation characteristics and phenolic composition has been already pointed out (Cortés and Díaz, 2011).

Therefore, the aim of the current study was to assess the effects of supplementary irrigation on the agronomic performance of two red grapevine cultivars, 'Brancellao' and 'Sousón', grown in Galicia. We also determined the influence of supplementary irrigation on must and wine composition of both cultivars, including amino acids and volatile compounds. The current study tried to determine whether it is necessary or not to apply supplementary irrigation to two red grapevine cultivars in Galicia in order to counteract the negative effects of climate change.

MATERIAL AND METHODS

Description of the study site

The experiment was carried out, over three consecutive years (2012-2014), in two adjacent 0.2-ha vineyards (*Vitis vinifera* L.) planted in 1998 with red cultivars 'Brancellao' and 'Sousón' grafted on 196-17C rootstock. The vineyards were located at the experimental farm of the Estación de Viticultura e Enoloxía de Galicia (EVEGA) in Leiro (Ourense, NW Spain), within the Ribeiro DO (42º 21.62' N, 8º 7.02' W, elevation 115 m). Vines were trained to a vertical trellis on a single cordon system (10-12 buds per vine). Spacing was 2.4 m between rows and 1.25 m between plants (3333 vines/ha). Rows were East-West oriented. Cultural practices were those commonly applied in the area and weed growth was controlled mechanically.

Soil was sandy textured (64% sand, 16.4% silt and 19.6% clay), slightly acidic (pH (H_2O) 6.3) and of medium fertility (2.7% organic matter). Its average depth and available water capacity were 1.2 m and 100 mm/m, respectively.

Climate is Atlantic with average annual rainfall of 900 mm, of which about 70% falls during the dormant period. It has been described as temperate, humid with cool nights (Fraga *et al.*, 2014).

Experimental design

For each cultivar, two treatments were established: rain-fed and drip irrigation to 40% potential evapotranspiration (ET_0). Weather data for calculating ET0, using the Penman-Monteith equation (Allen *et al.*, 1998), were recorded at a weather station located 150 m from the experimental site. Precipitation was subtracted from ET0 each week. The calculated amount of water was applied the following week. Irrigation was triggered in late June (just after bloom) and water was applied till mid-August, between two and three weeks before harvest.

Water was applied early in the morning, for minimizing evaporation losses, through two pressure-compensated emitters (4 L/h) per vine, located 25 cm on either side of the trunk. Frequency of water applications varied from 3 to 5 days per week, depending on the amount of water required. Irrigation water was of good quality, with pH of 6.5, electrical conductivity of 163.4 μS/cm and 0.4 mg/L of suspended solids. Total irrigation depth per season was 50, 79.3 and 50 mm, in 2012, 2013 and 2014, respectively.

The experiment was laid out in randomised blocks, with three replicates. Each of the replicates consisted of three rows with 12 vines per row. The eight vines in the centre of the middle row were used for measurements and the rest acted as buffer vines. In summary, for each year and cultivar, two treatments and three replicates per treatment were considered.

Field measurements

Midday stem water potential (Ψs) was measured at noon (12:00 – 13:00 h) on non-transpiring leaves that had been bagged with both plastic sheet and aluminium foil for at least 1 hour before measurements (Choné *et al.*, 2001). Readings were taken fortnightly from mid-May until mid-September using a pressure chamber (Pump-Up, PMS Instruments, Albany, OR, USA). One leaf from two vines per replicate was used per date (6 leaves per treatment and cultivar). This modality of leaf water potential has been proven the most useful for assessing vine water status in Galician cultivars (Mirás-Avalos *et al.*, 2014).

On the same days as Ψs, stomatal conductance was measured around solar midday using a leaf porometer (Model SC-1, Decagon Devices, WA, USA) on one fully-exposed and healthy leaf per vine and two vines per replicate (6 leaves per treatment and cultivar).

Chlorophyll concentration index (CCI) was estimated non-destructively with a portable chlorophyll meter (CCM-200, Opti-Sciences, Tyngsboro, MA, USA), which calculates a unitless CCI value from the ratio of optical absorbance at 655 nm to that at 940 nm. This value is correlated with total foliar extractable chlorophyll (Steele *et al.*, 2008). These measurements were performed from veraison to harvest on three leaves per plant and three vines per replicate (9 vines per treatment). Major veins and areas of obvious visual damage or disease were avoided.

Yield was determined at harvest on each of the internal rows (8 vines/row) of each replicate. The number of clusters per vine was recorded. Average cluster weight was calculated by dividing yield per vine by the number of clusters. Berry fresh weight was determined on random samples of 200 berries per replicate.

Pruning weight (PW) and external leaf area (SA) were determined in six vines per replicate. External leaf area was estimated after veraison, when shoot growth had ceased, following the method proposed by Sánchez-de-Miguel *et al.* (2010), in which the width and height of the canopy are collected at five different spots along the vine using a measurement tape. Data on SA could not be obtained in 2013 because vines were trimmed prior to performing of these measurements.

Water productivity (WP) was assessed as yield (in kg per ha) divided to the amount of water received during the growing season (rainfall + irrigation).

Gross incomes were assessed using the prices issued by Xunta de Galicia (Diario Oficial de Galicia 16th July 2008) for the Ribeiro DO. Grape is paid according to its probable alcoholic grade. Hence, one kg of ‘Brancellao’ or ‘Sousón’ grapes with 13% vol. probable alcoholic grade would be paid at 1.39 €; whereas 1 kg with 11% vol. would be paid at 1.06€. In our case, both rain-fed and irrigated vines received the same management practices except for the additional costs of irrigation. Therefore, gross incomes might provide an indication of the sustainability of irrigation in the medium term.

Winemaking

Grapes from each cultivar and treatment were manually harvested and transported to the experimental winery in field boxes. Winemaking was performed at EVEGA separately on samples of about 40 kg from each field replication.

Grapes from each cultivar and treatment were separately destemmed and transferred to 35-L stainless steel containers. During grape processing SO_2 (50 mg/L) was added to the mass in order to avoid oxidation and for microbiological control. Fermentations were carried out at room temperature (22-24 ºC). Excellence XR (Lamothe-Abiet, Bordeaux, France) yeast was added according to manufacturer’s instructions. Density and temperature of fermentations were daily monitored. Wine lots were punched down daily until the end of alcoholic fermentation (8 days). Then, they were pressed, racked into new tanks and kept at room temperature for a couple of days. A tartaric stabilization was carried out at 4 ºC for one month, approximately. Finally, wines were filtered, sulphited to 35 ppm of free sulphur dioxide, bottled and stored.

Analytical determinations in the wines were performed just after alcoholic fermentation.

Must and wine basic attributes

The analyzed attributes of musts comprised total soluble solids content (SSC), pH, titratable acidity (TA) and malic and tartaric acid concentrations. They were determined using the official methods for must analysis (OIV, 2009)

Wine alcohol content, pH, and TA were determined by Infrared Spectrometry with Fourier Transformation (IRFT) using a WineScanTM FT120 (FOSS®, Barcelona, Spain) calibrated according to official protocols (OIV, 2009). Malic and tartaric acids were determined by enzymatic methods using a LISA200 Analyzer System.

Wine colour characteristics, including total polyphenol index (TPI), anthocyanins and tannins concentrations were assessed with an ultraviolet-visible Themo Helios Zeta spectrophotometer using the methods described by Glories (1984) and Zamora (2003). TPI was directly measured after diluting 1% the wines. Total anthocyanins were quantified according to the decolouration experienced by adding metabisulphite and were expressed as mg/L; total tannins were determined after wine treatment with concentrated hydrochloric acid.

Analysis of amino acids and aroma compounds

Amino acids concentrations in must and wine samples were assessed following the method proposed by Gómez-Alonso *et al.* (2007) and Garde-Cerdán *et al.* (2009), with slight modifications. Briefly, must and wine samples were subject to a 30-minute derivatization reaction using DEEMM (diethylethoxymethylenemalonate) as derivatizing agent. Then, they were heated at 70 ºC for 2 hours in order to degrade the excess of DEEMM and reagent by-products. Finally, amino acids were eluted and their concentrations detected at 280 and 269 nm using the photodiode array detector of a High Performance Liquid Chromatography (HPLC) equipment (1100 series, Agilent Technologies, Palo Alto, CA, USA). Determinations were carried out on one sample per replicate (hence, three samples per treatment and year for each cultivar). Each of these determinations was performed three times in order to account for the reproducibility of the methods. Further details for these determinations, including a complete list of chemical reagents and elution conditions, can be found in Bouzas-Cid *et al.* (2015).

Higher alcohols and other volatile compounds were determined by gas chromatography (GC). Each wine sample from each cultivar, treatment replication and year was analyzed in triplicate. An Agilent (model 7890A) chromatograph equipped with a flame ionization detector was used. A capillary column CP-Wax57CB (Chrompack) (50 m x 0.32 mm x 0.20 μm) was employed. The operating conditions were the following: injector and detector temperatures, 230 ºC; column temperature, programmed from 40 ºC to 200 ºC at 4 ºC/minute and final isotherm for 15 minutes; carrier gas (Hydrogen) flow 3.3 mL/minute; auxiliar gas (Nitrogen) flow, 30 mL/minute. A 2 μL sample of the extract was injected in split mode (purge rate 5.2 mL/minute, split 125 mL/minute, attenuation of 1).

Statistical analysis

A two-way ANOVA was carried out on the agronomic and chemical data (R software v. 3.2.3; R Core Team, 2015) accounting for irrigation treatment, year and their interaction as factors. Differences were considered significant when $p < 0.05$, according to the Tukey's Honest Significant Difference (HSD) test. Cultivar was accounted for in the first analysis of the data set (reflected on Table I) in order to ascertain if both cultivars behaved differently; then, data from each cultivar were independently analyzed..

RESULTS AND DISCUSSION

Climate conditions and vine water relations

The time course of daily ET0 and rainfall over the growing seasons (April to late-September) studied is shown in Figure 1. Rainfall was concentrated on the beginning of the growing season in 2012, whereas it was evenly distributed in 2014. The growing season of 2013 showed less rainfall events than the other two years, and they were mainly concentrated at the beginning of the season. Maximum ET0 values were similar among seasons; however, these records were higher at the beginning of the season in 2013 and 2014 than in 2012. A summary of the total values for the climate variables recorded at this site can be found in Trigo-Córdoba *et al.* (2015); briefly, the driest year was 2012 with 841 mm of annual rainfall, slightly lower than the 900 mm average for the area. However, the 2012 growing season (April to harvest) was the wettest one with 313 mm rainfall. In contrast, 2013 and 2014 were wet years with, approximately, 1300 mm rainfall; nevertheless, the growing seasons of these years were drier than that of 2012 (163 and

185 mm for 2013 and 2014, respectively). Rainfall over the irrigation period ranged from 22 mm in 2013 to 84 mm in 2014. Annual and growing season average temperatures increased from 2012 (13.1 and 17.2 ºC, respectively) to 2014 (14.2 and 18 ºC, respectively). Consequently, a higher ET0 was observed in 2013 and 2014 when compared with 2012.

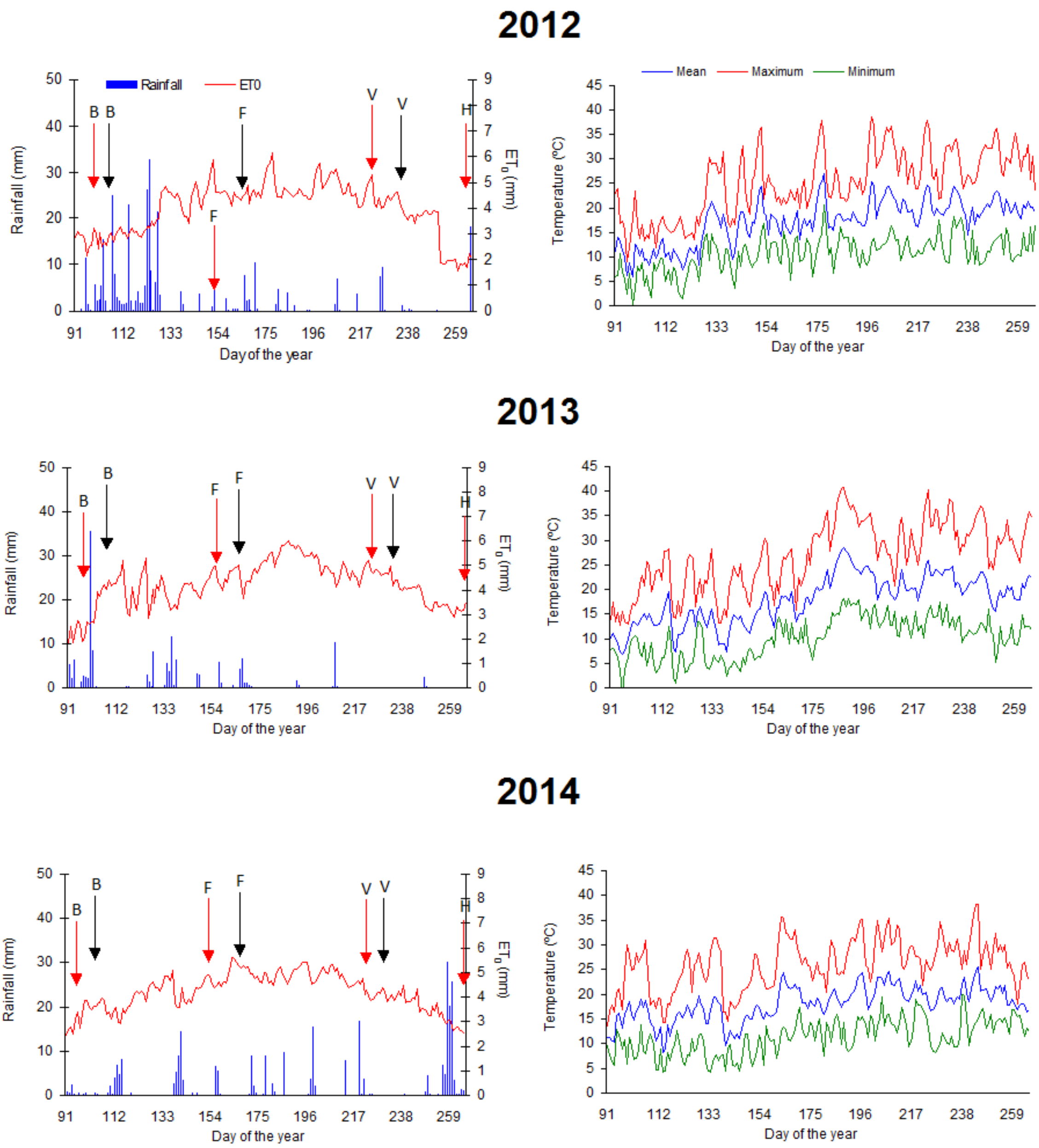

Figure 1. Daily rainfall (bars) and reference evapotranspiration (lines) at the experimental site during the seasons of 2012–2014. Maximum, minimum and mean daily temperatures for each season are also shown. Downward arrows (red for ‘Brancellao’ and black for ‘Sousón’) indicate the occurrence of bud-break (B), flowering (F), veraison (V) and harvest (H).

Precipitação (barras) e evapotranspiração de referência (linhas) diárias na finca experimental durante os anos 2012 a 2014. Se amostram as temperaturas máximas, mínimas e médias diárias para cada ano. As frechas (vermelhas para ‘Brancellao’ e pretas para ‘Sousón’) indicam as fases de brotação (B), floração (F), pintor (V) e colheita (H).

Irrigation allowed vines of both cultivars to maintain a higher water status than those rain-fed, as proven by the midday stem water potential values (Figure 2), which tended to decline progressively over the course of the growing season due to higher temperatures and solar radiation values that caused an increasing evaporative demand (as shown in Figure 1). This decreasing pattern reflects the decrease in soil water availability along the season. The lowest values of midday stem water potential were observed in 2013 and 2014 (Figure 2) for both cultivars. In the case of 'Brancellao', minimal values of -0.88 MPa were measured in 2013 and 2014. In the case of 'Sousón', minimal stem water potentials reached -1.08 MPa in 2013. Both cultivars showed differences between rain-fed and irrigation conditions regarding water status. These differences were significant from day 200 (19th July), approximately (Figure 2) and were detected on more occasions for 'Brancellao' than for 'Sousón', indicating that these cultivars present a different behaviour in regard to water status, as previously observed for two white cultivars (Trigo-Córdoba *et al.*, 2015). Therefore, this kind of studies must be conducted for each cultivar in order to schedule irrigation appropriately, as suggested by Basile *et al.* (2012). It is no doubt that vine response to water stress depends on the phenological stage (Jackson and Lombard, 1993); therefore, irrigation can be used as a tool to control vine water status as a function of phenological stage, water stress degree and the aims of the vineyard (Ojeda, 2008).

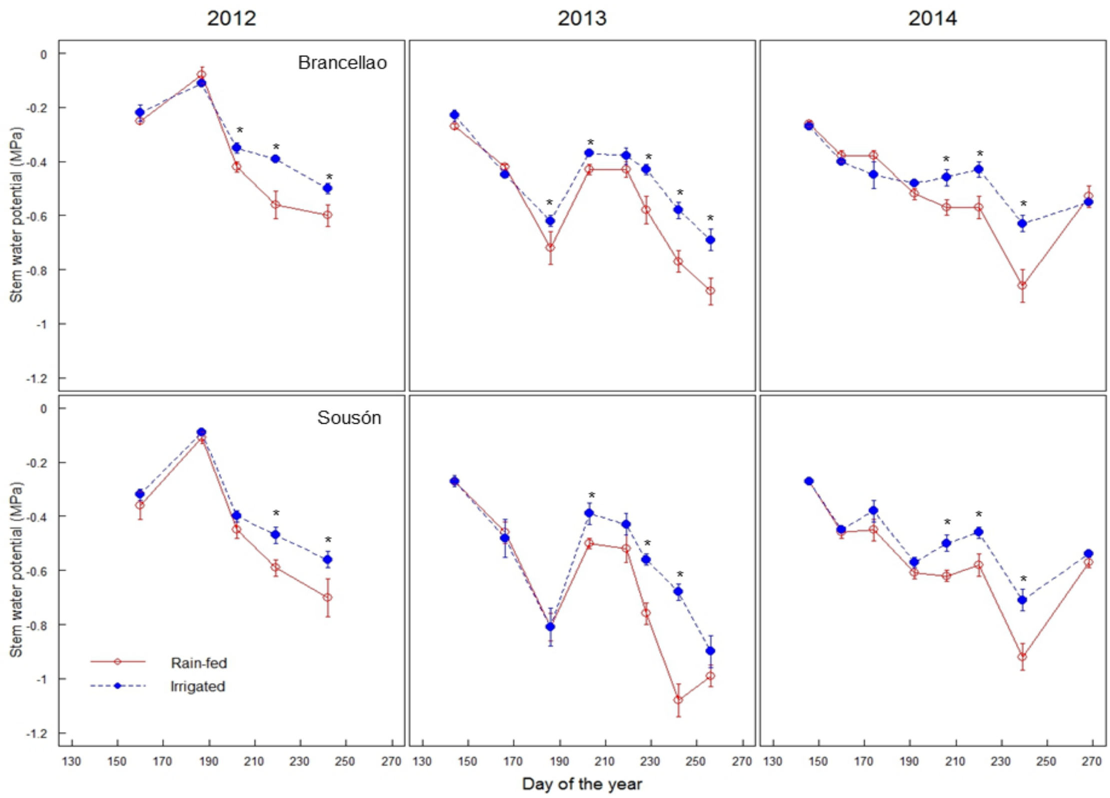

Figure 2. Seasonal variation of midday stem water potentials for 'Brancellao' and 'Sousón' cultivars under rain-fed and irrigation conditions. Values are treatment means±standard error. Asterisks indicate significant differences between treatments at $p < 0.05$.

Evolução temporal do potencial hídrico foliar para as variedades 'Brancellao' e 'Sousón' sobre condições de sequeiro e rega. Os valores são médias±desvios padrão. Os asteriscos mostram diferenças significativas entre tratamentos (p < 0.05).

Both cultivars showed high values of stomatal conductance (Figure 3), greater than those reported by other authors (Williams and Araujo, 2002; Intrigliolo and Castel, 2009), probably caused by a sufficient soil water availability and a high relative humidity in the atmosphere during our study. The fact that the measurement interval of the equipment used is rather narrow, measurements greater than 0.7 mol $H_2O/m^2/s$

should be taken with caution. However, comparisons between treatments are allowed because all the measurements were performed with the same equipment and conditions. Rain-fed vines had lower stomatal conductance values than irrigated vines on certain dates, especially for 'Sousón'; although no significant differences between treatments were observed in most of the measurement dates due to the high variability in these readings. In addition, the values observed in both rain-fed and irrigated vines did not reflect stomatal closure by the end of the season, suggesting that plant functioning was not altered by the treatment. Most of the published reports indicate an increase in stomatal conductance under irrigation conditions (e.g. de Souza *et al.*, 2003; Intrigliolo and Castel, 2009); however, these studies were carried out under Mediterranean or semi-arid conditions, where water constrains were higher than those observed in our study; thus, the high values found in our study are somewhat expected. In addition, Williams and Trout (2005) and Teszlák *et al.* (2013) observed peak values of stomatal conductance greater than 0.8 mol $H_2O/m^2/s$ in well-irrigated 'Thompson Seedless' and 'Riesling' vines, similar to our findings and those from a previous study on two white grapevine cultivars (Trigo-Córdoba *et al.*, 2015). Furthermore, midday stem water potential and stomatal conductance values measured in the current work are over the optima (-1.25 to -1.4 MPa and 0.12 to 0.15 mol $H_2O/m^2/s$, respectively) suggested by Romero *et al.* (2010) for 'Monastrell' cultivar under the semi-arid conditions of south east Spain, which might be the reason for the absence of differences among treatments in the present study.

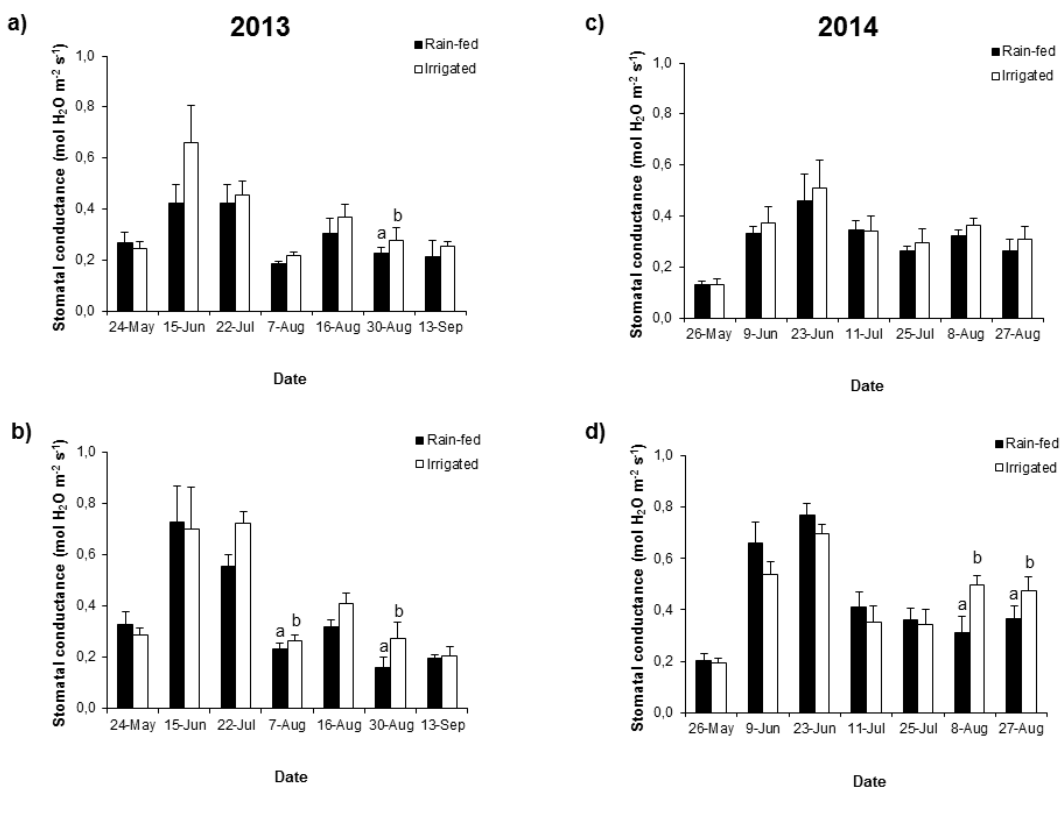

Figure 3. Effect of irrigation on the stomatal conductance during the 2013 and 2014 growing seasons for B'rancellao' (a and c) and 'Sousón' (b and d) cultivars. Bars indicate the standard error of the mean. Different letters on the columns indicate significant differences ($p < 0.05$) for each date.

Efeito da rega na condutância estomática durante os anos 2013 e 2014 para as variedades 'Brancellao' (a e c) e 'Sousón' (b e d). As barras indicam o erro padrão. Letras diferentes indicam diferenças significativas entre tratamentos (p < 0.05) para cada data.

In ‘Brancellao’, CCI values were lower under rain-fed than under irrigation conditions only in 2013 (Figure 4 a and c). However, ‘Sousón’ also showed significant differences for this variable on one date in 2014 (Figure 4 b and d). The absence of differences between treatments in 2014 might have been caused by the even distribution of rainfall over the growing season. Due to the fact that CCI is directly correlated to extractable chlorophyll content (Richardson *et al.*, 2002), our results suggest that leaf chlorophyll in the two cultivars studied was very sensitive to slight changes in vine water status.

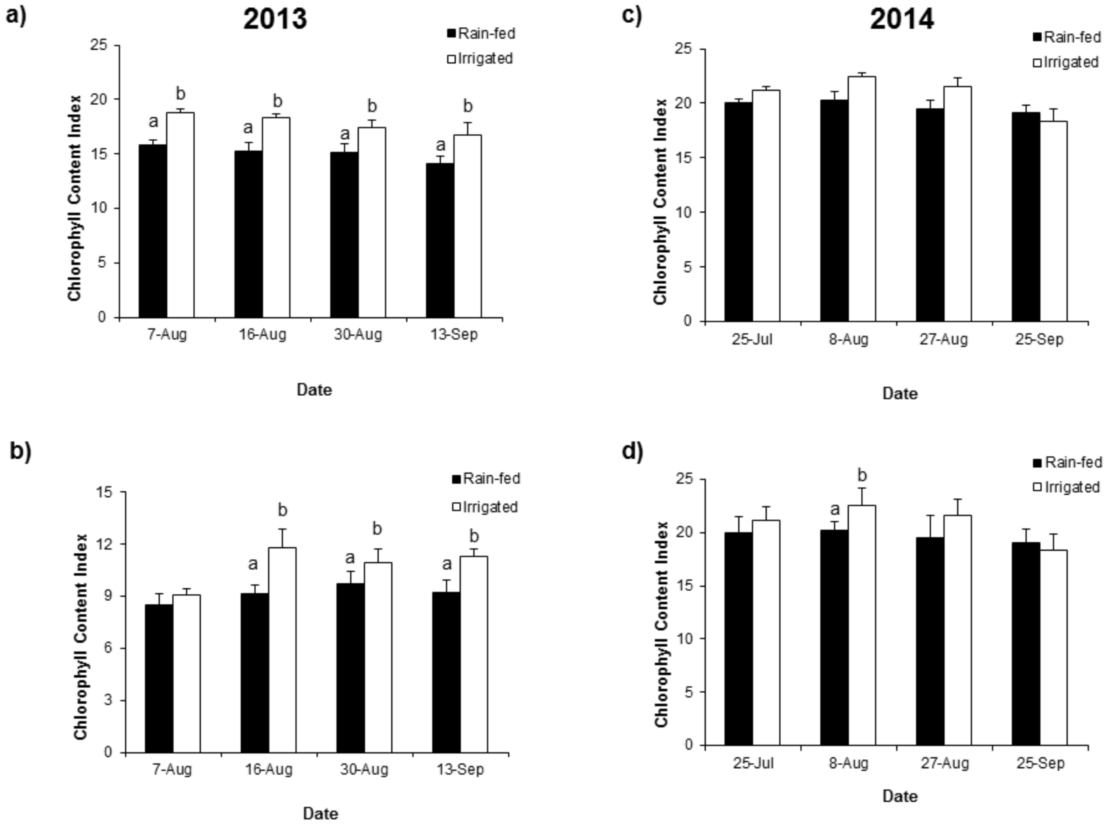

Figure 4. Effect of irrigation on the relative chlorophyll content index during the 2013 and 2014 growing seasons, from veraison to harvest, for ‘Brancellao’ (a and c) and ‘Sousón’ (b and d) cultivars. Bars indicate the standard error of the mean. Different letters on the columns indicate significant differences ($p < 0.05$) for each date.

Efeito da rega no conteúdo relativo de clorofila durante os anos 2013 e 2014, do pintor à vindima, para as variedades ‘Brancellao’ (a e c) e ‘Sousón’ (b e d). As barras indicam o erro padrão. Letras diferentes indicam diferenças significativas entre tratamentos (p < 0.05) para cada data.

Vegetative growth and yield components

Most of the vegetative growth and yield variables measured in the current study were affected by cultivar and season (Table I). In contrast, treatment only exerted a significant effect on pruning and berry weights. The interaction between cultivar and treatment was significant for pruning, cluster and berry weights.

In the case of ‘Brancellao’, most of vegetative growth and yield components were unaffected by irrigation (Table II). Greater PW values were observed in irrigated vines in 2012 and 2013, but not in 2014, probably due to the higher amount of annual rainfall during this year. Similarly, cluster weight was higher in irrigated vines for the 2012 growing season. Finally, berry weight was significantly greater in irrigated vines in 2013 and 2014.

Table I

Effects of cultivar (C), irrigation treatment (T), season (S) and their interactions on the variables considered in the current study (data are p-values from an ANOVA)

Efeitos da variedade (C), tratamento de rega (T), ano (S) e as suas interações sob as variáveis consideradas no presente estudo (os dados são p-valores do ANOVA)

	Variable	Cultivar	Treatment	Season	C x T	C x S	T x S	C x T x S
Vegetative growth	**External leaf area**	0.168	0.325	**< 0.001**	0.050	0.482	0.521	0.467
	Pruning weight	**< 0.001**	**0.023**	**0.019**	**0.040**	0.924	0.506	0.859
Yield components	**Yield**	**0.005**	0.114	0.882	**0.002**	0.816	0.556	0.263
	Clusters per vine	0.093	0.100	**< 0.001**	0.235	0.102	0.707	0.832
	Cluster weight	**< 0.001**	0.459	**< 0.001**	**< 0.001**	0.133	0.609	0.125
	Berry weight	**< 0.001**	**0.023**	**0.019**	**0.040**	0.924	0.506	0.859
Economic indicators	**Water productivity**	0.148	**< 0.001**	**< 0.001**	0.120	0.772	0.559	0.925
	Gross incomes	0.904	**0.048**	0.805	**0.018**	0.611	0.989	0.826
Must attributes	**Total soluble solids**	**< 0.001**	**0.044**	0.365	0.912	0.310	0.437	0.437
	Titratable acidity	**< 0.001**	**0.003**	1.000	0.792	0.296	0.907	1.000
	pH	**< 0.001**	**0.029**	0.151	0.503	**< 0.001**	0.715	0.616
	Tartaric acid	0.225	0.057	**0.010**	0.373	**0.019**	0.912	0.952
	Malic acid	**< 0.001**	**0.019**	**< 0.001**	0.550	0.140	0.586	0.763
Wine attributes	**Alcohol content**	**< 0.001**	**0.038**	0.068	0.566	0.381	0.295	0.681
	Titratable acidity	**< 0.001**	**0.033**	0.285	0.202	**< 0.001**	1.000	1.000
	pH	**< 0.001**	**0.008**	**0.002**	0.844	**0.005**	0.435	0.810
	Tartaric acid	**< 0.001**	**0.009**	0.401	0.955	0.094	0.809	0.755
	Malic acid	**< 0.001**	0.238	0.664	0.074	**0.003**	0.969	0.843
	Total polyphenol index	**< 0.001**	0.435	**< 0.001**	0.173	0.581	0.387	0.854
	Anthocyanins	**< 0.001**	0.461	**0.016**	0.322	**0.012**	0.874	0.626
	Tannins	**< 0.001**	0.833	0.817	0.562	0.817	0.562	0.935

Significant values are highlighted in bold.

Table II

External leaf area, pruning weight, yield components, water productivity and expected gross incomes of rain-fed and irrigated vines during each season for the two cultivars considered in this study.

Superfície foliar externa, peso de poda, componentes da produção, produtividade da água e renda bruta esperada para as vinhas em sequeiro e regadas durante cada ano para as duas variedades estudadas.

Parameter	Year	Brancellao		Sousón	
		Rain-fed	Irrigated	Rain-fed	Irrigated
External leaf area (m^2/m^2)	2012	**1.18 b**	**1.11 a**	1.10	1.16
	2014	1.58	1.51	1.50	1.49
	Average	*1.38*	*1.31*	*1.30*	*1.33*
Pruning weight (kg/vine)	2012	**1.63 a**	**1.93 b**	1.24	1.15
	2013	**1.18 a**	**1.71 b**	1.00	1.04
	2014	1.85	2.27	1.40	1.52
	Average	*1.55*	*1.97*	*1.21*	*1.24*
Yield (kg/vine)	2012	3.37	4.01	**5.10 b**	**3.76 a**
	2013	4.09	3.84	**4.55 b**	**3.82 a**
	2014	3.46	3.91	4.76	4.07
	Average	*3.64*	*3.92*	*4.76*	*3.88*
Clusters per vine	2012	32.07	32.25	**38.57 b**	**31.90 a**
	2013	38.17	34.96	41.46	43.21
	2014	39.58	40.50	**40.33 b**	**34.96 a**
	Average	*36.61*	*35.90*	*40.12*	*36.69*
Cluster weight (g)	2012	**103.76 a**	**119.56 b**	**133.04 b**	**115.62 a**
	2013	101.94	105.91	**110.43 b**	**87.59 a**
	2014	84.98	93.50	115.56	114.43
	Average	*96.89*	*106.32*	*119.68*	*105.88*
Berry weight (g)	2012	1.77	1.79	1.83	1.80
	2013	**1.35 a**	**1.44 b**	**1.65 a**	**1.72 b**
	2014	**1.86 a**	**1.94 b**	2.29	2.35
	Average	*1.66*	*1.72*	*1.92*	*1.96*
Water productivity (kg/m^3)	2012	2.44	2.14	**5.43 b**	**3.45 a**
	2013	7.79	6.00	**9.29 b**	**5.26 a**
	2014	7.31	6.89	**8.32 b**	**5.70 a**
	Average	*5.85*	*5.01*	*7.68*	*4.80*
Gross incomes (€/ha)	2012	11231.1	13675.3	**18860.7 b**	**13892.1 a**
	2013	13640.3	12807.1	**18784.1 b**	**14645.1 a**
	2014	16041.3	16659.7	**19738.5 b**	**15177.6 a**
	Average	*13637.6*	*14380.7*	*19127.8*	*14571.6*

Different letters in the row, for each cultivar, indicate significant differences between treatments at $p < 0.05$.

In the case of ‘Sousón’, neither SA nor PW were affected by irrigation (Table II). In contrast, all of the yield components accounted for in this study were negatively influenced by irrigation at least on one season. Hence, yield was lower in irrigated than in rain-fed vines in 2012 and 2013 due to lower number of clusters per vine and lower cluster weights, which could have been caused by a greater incidence of bunch rot detected on irrigated vines. On the contrary, berry weight was greater in irrigated vines in 2013.

Both cultivars showed higher PW in irrigated vines, although these differences were not always significant, especially for ‘Sousón’. Overall, these results suggest that vine vegetative growth was stimulated by the water applied, as observed by Intrigliolo and Castel (2010) for ‘Tempranillo’.

The lack of effects of irrigation on vine yield may be attributed to high water supply by rainfall, which was sufficient for fulfilling vine water requirements. These results are in disagreement with previous reports for other cultivars and climate conditions (Reynolds *et al.*, 2007; Intrigliolo and Castel, 2010; Gouveia *et al.*, 2012). However, Junquera *et al.* (2012) and Lanari *et al.* (2014) did not detect any difference in yield until the third year of experiments, suggesting that vineyard irrigation studies might be carried out for longer time periods. Moreover, under the conditions of the current study, rainfall events and the volume and characteristics of the soil explored by the root system may have buffered the yield response under irrigation shortage ensuring full canopy transpiration until July. Consequently, there was a very slow development of water stress, which only was mild to moderate (van Leeuwen *et al.*, 2009), depending on the cultivar, over the three years of study. Hence, rain-fed vines reached lower plant water status than the irrigated ones only late in the season (mid-July, August). Moreover, it is important to take into account the different length of the growing cycle for the two cultivars studied; ‘Sousón’ has a more lengthy maturation period, thus it is not harvested till October or even November, when rainfall is already occurring in the region, whereas ‘Brancellao’ is harvested by the end of September. This has probably caused the lack of differences in yield and its components between treatments.

Water productivity was similar between treatments for ‘Brancellao’. However, WP was greater for ‘Sousón’ rain-fed vines than for those irrigated (Table II). These observations can be explained by the fact that WP index was developed for Mediterranean climates, where summer rainfall amounts are much lesser than those registered in our study, as previously suggested by Cancela *et al.* (2016).

Gross incomes were similar between treatments in the case of ‘Brancellao’; however, they were higher for rain-fed vines than for those irrigated in the case of ‘Sousón’ (Table II). From the economic viewpoint, irrigation was not a viable practice for these red cultivars under the conditions of this study, especially for ‘Sousón’, due to the fact that gross incomes were not increased and higher costs must be faced when using irrigation.

Must and wine basic attributes

Cultivar and irrigation treatment influenced all the must attributes considered in the current study, except for tartaric acid concentration (Table I). Season affected the concentrations of tartaric and malic acids; whereas the interaction between cultivar and season was significant for pH and tartaric acid concentration.

In the case of ‘Brancellao’, significant differences between treatments were only observed for pH and TA (Table III). Rain-fed vines produced musts with lower values of TA in 2012 and 2014 than irrigated vines. Moreover, in 2012, musts from rain-fed vines showed a higher pH than those from irrigated vines. In the case of ‘Sousón’, must attributes remained unaffected by the irrigation treatment. However, in 2014, musts from rain-fed vines presented a higher SSC value than those from irrigated vines (Table III).

Table III

Must quality attributes of rain-fed and irrigation treatments during each season

Atributos da qualidade do mosto para os tratamentos de sequeiro e rega durante cada ano

Attribute	**Year**	**Brancellao**		**Sousón**	
		Rain-fed	Irrigated	Rain-fed	Irrigated
Total soluble solids (ºBrix)	2012	22.7	21.7	20.1	20.1
	2013	23.7	23.4	21.5	20.7
	2014	22.7	21.6	**21.7 b**	**20.2 a**
	Average	*23.0*	*22.2*	*21.1*	*20.3*
Titratable acidity (g/L tartaric acid)	2012	**5.9 a**	**6.7 b**	8.4	9.0
	2013	6.3	7.1	8.4	9.3
	2014	**5.6 a**	**6.4 b**	8.7	9.3
	Average	*5.9*	*6.7*	*8.5*	*9.2*
pH	2012	**3.23 b**	**3.15 a**	3.08	3.00
	2013	3.23	3.13	3.04	3.00
	2014	3.39	3.30	3.00	2.98
	Average	*3.28*	*3.19*	*3.00*	*2.99*
Tartaric acid (g/L)	2012	6.6	7.3	7.1	7.2
	2013	7.3	8.0	6.5	6.9
	2014	4.8	5.7	6.9	7.2
	Average	*6.2*	*7.0*	*6.8*	*7.1*
Malic acid (g/L)	2012	1.6	1.9	2.8	3.0
	2013	2.1	2.3	3.7	4.4
	2014	2.3	2.6	3.9	4.3
	Average	*2.0*	*2.3*	*3.5*	*3.9*

Different letters in the row, for each cultivar, indicate significant differences between treatments at $p < 0.05$.

These observations seem to indicate that berry composition of both cultivars was not affected by irrigation, and the values found for SSC and TA under irrigation were very similar to those of the rain-fed treatment, suggesting an excess of source compared to sink demand. External leaf area values are within the range considered optimal to ensure maturation of up to 12 t/ha (Schneider, 1989). Our yield ranged from 11 to 16 t/ha, depending on the cultivar and year, and thus differences in must composition is not expected. However, these results are in disagreement with reports for other cultivars grown in other regions under Mediterranean or semi-arid conditions, where sink demands are not covered due to severe water restrictions (Intrigliolo and Castel, 2010; Romero *et al.*, 2010), proving that irrigation might not provide any benefit under the current Galician climate conditions, as previously reported for two white grapevine cultivars (Trigo-Córdoba *et al.*, 2015).

Wine attributes depended on the cultivar (Table I). However, irrigation treatment only influenced alcohol content, TA, pH and the concentration of tartaric acid; whereas season only affected pH, TPI and anthocyanins (Table I). The interaction between cultivar and season was significant for TA, pH, malic acid and anthocyanins.

'Brancellao' wine basic attributes were only affected by irrigation in the last year (Table IV). Wines from the rain-fed treatment showed higher values of pH and TPI, whereas wines from the irrigated treatment had a higher concentration of tartaric acid.

'Sousón' wine composition was very similar between treatments for the three years of study (Table IV). Significant differences were only detected for alcohol content and TPI.

Similarly to the observations in must composition, wines were mainly unaffected by irrigation. Nevertheless, those slight variations might be detected at the sensory level, as previously reported for white cultivars (Trigo-Córdoba *et al.*, 2014). In fact, wines from the irrigated vines tended to have lower alcohol concentrations and higher total acidities than those from the rain-fed treatment. This trend was observed for both cultivars and has been previously reported for Spanish red grapevine varieties (e.g. Intrigliolo and Castel, 2010).

Amino acids and volatile compounds

Concentrations of individual amino acids in the musts from both cultivars varied from year to year (Tables V and VI). In the case of 'Brancellao', the concentrations of seven amino acids remained unaffected by the year: arginine, alanine, tyrosine, isoleucine, phenylalanine, ornithine and lysine, as well as the ammonium ion (Table V). In the case of 'Sousón' musts, only three amino acids showed similar concentrations over the three studied years: glutamic acid, tyrosine and ornithine (Table VI). This strong effect of vintage on the amino acids concentrations in musts has been previously observed in Galician white cultivars (Bouzas-Cid *et al.*, 2015); and it can be explained by the different degree of berry maturation due to the particular weather conditions of a given year, as reported also for other cultivars (Ortega-Heras *et al.*, 2014).

Table IV

Wine quality attributes of rain-fed and irrigation treatments during each season.

Atributos da qualidade do vinho para os tratamentos de sequeiro e rega durante cada ano.

Attribute	Year	Brancellao		Sousón	
		Rain-fed	Irrigated	Rain-fed	Irrigated
Wine alcohol (% Vol)	2012	13.2	12.6	11.3	11.3
	2013	13.0	12.6	11.1	10.9
	2014	13.7	12.7	**12.5 b**	**11.7 a**
	Average	*13.3*	*12.6*	*11.6*	*11.3*
Titratable acidity (g/L tartaric acid)	2012	7.2	7.6	8.0	8.5
	2013	6.3	6.3	8.1	9.3
	2014	6.5	6.8	9.3	9.8
	Average	*6.7*	*6.9*	*8.5*	*9.2*
pH	2012	3.46	3.37	3.33	3.25
	2013	3.52	3.46	3.23	3.05
	2014	**3.50 b**	**3.30 a**	3.02	2.89
	Average	*3.49*	*3.38*	*3.19*	*3.06*
Tartaric acid (g/L)	2012	2.8	3.3	4.1	4.8
	2013	3.0	3.4	4.4	4.7
	2014	**2.5 a**	**3.3 b**	4.7	4.3
	Average	*2.8*	*3.3*	*4.4*	*4.6*
Malic acid (g/L)	2012	2.5	2.4	2.2	2.3
	2013	1.5	1.4	1.6	2.9
	2014	1.7	1.6	2.8	2.9
	Average	*1.9*	*1.8*	*2.2*	*2.7*
TPI	2012	40.5	42.1	**62.6 a**	**68.1 b**
	2013	32.	30.6	**44.0 a**	**48.4 b**
	2014	**31.0 b**	**27.7 a**	49.7	52.0
	Average	*34.6*	*33.5*	*52.1*	*56.2*
Anthocyanins (mg/L)	2012	195.3	205.2	1064.0	1128.7
	2013	158.1	139.7	558.9	612.2
	2014	222.8	192.2	704.7	849.0
	Average	*192.1*	*179.0*	*775.9*	*863.3*
Tannins (g/L)	2012	1.3	1.5	2.1	2.3

Different letters in the row, for each cultivar, indicate significant differences between treatments at $p < 0.05$.

In contrast, irrigation only affected the concentrations of certain amino acids in some of the studied years (Table V and VI). For instance, in the case of 'Brancellao', irrigation increased the concentrations of alanine, γ-aminobutyric acid and ammonium ion in 2013, and tryptophan in 2014 (Table V). Similarly, concentrations of amino acids in 'Sousón' musts were very similar between treatments in 2012 and 2013 (only tyrosine concentration was increased under irrigation conditions in 2013). However, in 2014, 'Sousón' musts from the irrigated treatment showed higher concentrations in asparagine, threonine, valine, cysteine, isoleucine, tryptophan and leucine that led to a greater concentration of total amino acids in musts from the irrigation treatment (Table VI).

Table V

Amino acids and ammonium concentrations (mg/L) in the musts from 'Brancellao' red grapevine cultivar in the three vintages studied

Concentração de aminoácidos e ião amónio (mg/L) nos mostos da variedade tinta 'Brancellao' nas três vindimas estudadas

Compound	2012		2013		2014		Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	
Aspartic acid	37.01	37.45	24.60	22.51	12.55	15.22	***
Glutamic acid	19.26	23.82	68.21	61.65	52.35	54.45	**
Asparagine	2.56	2.56	1.60	2.60	3.56	4.97	*
Serine	27.89	22.30	21.91	23.33	14.91	15.58	**
Glutamine	73.32	54.50	62.50	68.62	33.81	43.98	*
Histidine	14.75	11.68	7.85	9.05	6.36	8.12	*
Glycine	2.02	1.39	1.67	1.86	3.29	3.17	***
Threonine	28.13	23.05	24.79	28.45	14.98	17.58	*
Arginine	116.07	84.97	66.05	87.84	61.62	90.85	ns
Alanine	50.07	37.58	**34.89 a**	**40.83 b**	34.54	40.25	ns
γ-Aminobutyric acid (GABA)	81.60	67.49	**39.73 a**	**45.36 b**	37.64	42.74	***
Proline	8.54	8.48	7.52	7.82	1.12	1.75	**
Tyrosine	5.19	3.53	0.85	0.99	2.39	2.58	ns
Ammonium ion	92.99	99.83	**143.49 a**	**186.53 b**	78.04	105.76	ns
Valine	13.94	11.36	10.76	11.62	8.42	8.70	*
Methionine	1.30	1.02	0.89	0.73	0.61	0.59	***
Cysteine	2.22	2.12	3.09	2.88	0.84	0.91	*
Isoleucine	6.61	5.77	4.82	5.46	7.06	6.66	ns
Tryptophan	8.82	7.17	7.31	5.39	**0.95 a**	**1.24 b**	***
Leucine	9.69	8.10	5.98	6.85	4.12	4.48	**
Phenylalanine	7.24	5.82	4.77	5.18	7.21	6.89	ns
Ornithine	1.35	0.79	1.04	1.20	0.90	1.31	ns
Lysine	2.49	1.88	1.81	2.02	2.67	3.04	ns
Sum of amino acids	*520.08*	*423.02*	*402.64*	*442.20*	*311.90*	*375.06*	*

Different letters in the row for each year indicate significant differences between treatments at $p < 0.05$. The significance of the year as factor is expressed as ns = non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table VI

Amino acids and ammonium concentrations (mg/L) in the musts from 'Sousón' red grapevine cultivar in the three vintages studied

Concentração de aminoácidos e ião amónio (mg/L) nos mostos da variedade tinta 'Sousón' nas três vindimas estudadas

Compound	2012		2013		2014		Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	
Aspartic acid	9.09	9.62	9.93	7.10	3.20	4.07	**
Glutamic acid	9.44	10.59	12.61	14.58	12.14	13.30	ns
Asparagine	2.24	2.26	0.86	1.10	**1.25 a**	**1.65 b**	*
Serine	16.99	17.50	10.94	9.14	7.59	9.01	***
Glutamine	23.45	25.93	16.51	14.80	14.62	16.17	*
Histidine	9.30	10.18	4.48	3.53	3.15	3.96	***
Glycine	3.17	3.79	2.01	1.79	1.95	2.20	**
Threonine	16.45	18.01	11.23	9.08	**7.69 a**	**10.39 b**	**
Arginine	75.45	80.18	37.33	30.07	27.87	46.92	*
Alanine	28.41	29.37	10.23	9.12	10.19	13.27	**
γ-Aminobutyric acid (GABA)	68.27	78.80	30.57	26.54	21.68	25.68	***
Proline	9.96	8.71	6.56	4.72	0.58	0.65	***
Tyrosine	3.83	4.36	**0.45 a**	**0.57 b**	2.06	1.93	ns
Ammonium ion	92.77	92.82	96.82	112.74	38.06	58.67	*
Valine	10.92	11.24	6.82	5.49	**5.58 a**	**6.49 b**	***
Methionine	2.99	2.73	0.56	0.59	0.63	0.70	**
Cysteine	2.12	2.50	2.50	2.22	**0.91 a**	**1.02b**	***
Isoleucine	6.34	6.66	4.75	3.97	**3.40 a**	**4.23 b**	***
Tryptophan	24.71	21.94	13.86	10.42	10.33	11.61	***
Leucine	9.91	10.36	6.46	5.46	**4.15 a**	**5.21 b**	***
Phenylalanine	3.85	3.37	3.09	2.78	4.94	5.92	*
Ornithine	0.75	0.72	0.85	0.73	0.65	0.82	ns
Lysine	1.79	2.04	1.60	1.68	3.53	4.04	**
Sum of amino acids	*339.46*	*360.86*	194.20	*165.48*	***148.10 a***	***189.24 b***	**

Different letters in the row for each year indicate significant differences between treatments at $p < 0.05$. The significance of the year as factor is expressed as ns = non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the case of 'Brancellao', all the amino acids were present in the range of concentrations reported in the literature (Bell and Henschke, 2005), except for proline and methionine, which appeared in lower concentrations. In the case of 'Sousón', amino acids concentrations were low when compared to those previously reported, especially for glutamic acid, glutamine and proline. Both cultivars were arginine accumulators since their ratio proline/arginine was lesser than 1, as observed for other cultivars traditionally grown in this region (Bouzas-Cid *et al.*, 2015), and other widely-grown cultivars such as ‘Syrah’ (Garde-Cerdán *et al.*, 2009). In fact, proline and arginine metabolism are linked and the final concentration of one of them is influenced by the concentration of the other, with arginine as a precursor for at least some of the proline accumulated in the grapes (Kliewer, 1968). The most abundant amino acids found in ‘Brancellao’ and ‘Sousón’ musts were arginine, GABA, alanine, glutamine and glutamic acid although their concentrations changed over the study period. These amino acids constituted 64-71% of the total free amino acids in musts from ‘Brancellao’, and 56-61% in musts from ‘Sousón’.

Irrigation did not affect the total concentration of amino acids in the wines (Figure 5), except for ‘Brancellao’ wines in 2013. Amino acids concentrations in ‘Sousón’ wines increased from year to year. Slight differences between treatments were detected for certain amino acids concentrations in some years for both cultivars (data not shown).

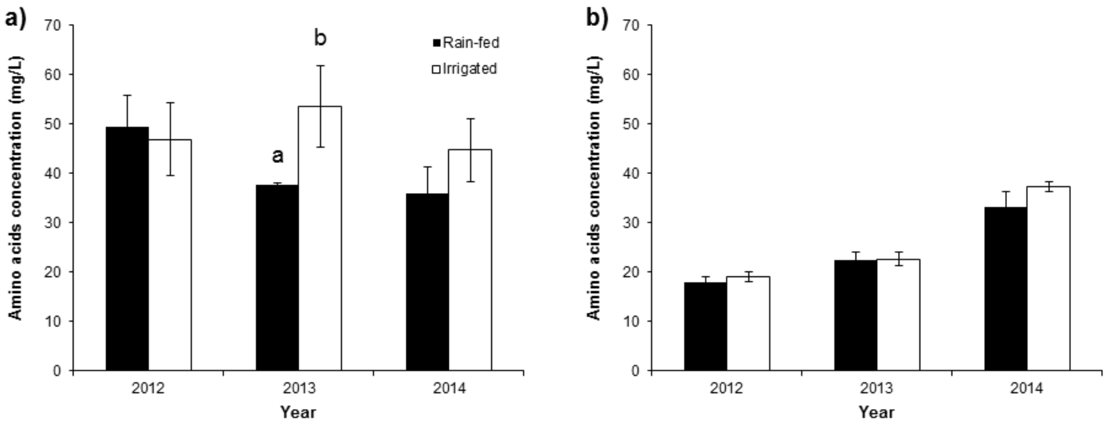

Figure 5. Effect of irrigation on the concentration of amino acids in wines from ‘Brancellao’ (a) and ‘Sousón’ (b) cultivars. Bars indicate the standard error of the mean. Different letters on the columns indicate significant differences ($p < 0.05$).

Efeito da rega na concentração de aminoácidos dos vinhos das variedades ‘Brancellao’ (a) e ‘Sousón’ (b). As barras indicam o erro padrão da média. Letras diferentes indicam diferenças significativas entre tratamentos (p < 0.05).

In general, the concentrations of higher alcohols and other volatile compounds were affected by the year, as previously reported by Vilanova *et al.* (2012). In ‘Brancellao’, all compounds were affected except for methanol, ethyl acetate, 1-propanol, acetol and 2,3-butanediol levo (Table VII). In contrast, concentrations of these compounds in ‘Sousón’ wines remained unaltered from year to year except for those of methanol, ethyl acetate, acetaldehyde, benzylic alcohol and ethyl lactate (Table VIII).

Irrigation only affected wines from ‘Brancellao’ in 2013. The concentrations of benzylic alcohol, 2-phenylethanol, 2,3-butanediol levo and meso were significantly greater in the wines from the rain-fed treatment (Table VII). This suggests that irrigation might exert an effect on wine aroma compounds. In fact, acetaldehyde concentration was lower than the threshold for this compound, which causes wine oxidation (Swiegers *et al.*, 2005). Moreover, irrigation reduced the concentration of 2-phenylethanol, an aromatic compound that provides floral notes, which could lead to a reduction in the wine aroma complexity.

In the case of ‘Sousón’, irrigation influenced the concentrations of several of these compounds in the three years of the study. In 2012, acetol concentrations were lower in wines from irrigation than in those from the rain-fed treatment. In 2013, ethyl acetate and acetaldehyde showed, respectively, a greater and a lower concentration in wines from the rain-fed treatment. Finally, in 2014, wines from the irrigation treatment presented lower concentrations of ethyl lactate, 2,3-butanediol levo and meso and γβ-lactone than the wines from the rain-fed treatment (Table VIII).

Recently, Talaverano *et al*. (2016) found higher alcohols, C6 compounds and phenol volatiles concentrations in 'Tempranillo' wines from their rain-fed treatment than in those from the deficit irrigated treatments, whereas the highest concentrations of ethyl esters were detected in wines from the irrigated treatments. These findings are in accordance with the ones reported in the current study.

Table VII

Irrigation effects on volatile compounds concentrations (mg/L) in wines from 'Brancellao'. The significance of the year is also shown

Efeitos da rega na concentração de compostos voláteis (mg/L) nos vinhos da variedade tinta 'Brancellao'. A significância do ano também é indicada

Compound	2012		2013		2014		Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	
Methanol	127	131	170	154	116	110	ns
Ethyl acetate	47	43	34	31	40	36	ns
Acetaldehyde	12	20	19	17	33	28	**
Higher alcohols							
1-Propanol	19	17	17	18	15	16	ns
2-Methyl-1-propanol	68	70	69	72	60	55	**
2-Methyl-1-butanol	253	257	294	286	196	176	*
3-Methyl-1-butanol	75	76	81	77	64	58	**
R2(3Me/2Me)	0.30	0.30	0.28	0.27	0.33	0.33	ns
Total higher alcohols	414	420	461	453	335	305	**
Other alcohols							
Benzylic alcohol	2.98	3.15	**3.55 b**	**2.65 a**	2.22	2.16	*
2-Phenylethanol	41	38	**55 b**	**42 a**	32	28	*
Other compounds							
Ethyl lactate	48	36	13	14	8	12	*
Acetoine	15.6	12.3	5.6	6.0	3.4	3.1	*
Acetol	31.4	33.7	57.4	53.2	30.4	21.1	ns
2,3-Butanediol levo	830	677	**672 b**	**508 a**	832	701	ns
2,3-Butanediol meso	412	341	**327 b**	**299 a**	172	146	**
γβ-Lactone	32	29	37	35	22	19	*

Different letters in the row for each year indicate significant differences between treatments at $p < 0.05$. The significance of the year as factor is expressed as ns = non-significant; * $p < 0.05$; ** $p < 0.01$. LOD = Limit of Detection

Table VIII

Irrigation effects on volatile compounds concentrations (mg/L) in wines from 'Sousón'. The significance of the year is also shown.

Efeitos da rega na concentração de compostos voláteis (mg/L) nos vinhos das variedade tinta 'Sousón'. A significancia do ano também é indicada.

Compound	2012		2013		2014		Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	
Methanol	199	194	229	244	126	126	**
Ethyl acetate	41	43	**37 b**	**31 a**	**36 b**	**31 a**	**
Acetaldehyde	19	12	**21 a**	**27 b**	33	26	*
Higher alcohols							
1-Propanol	15	13	18	15	12	13	ns
2-Methyl-1-propanol	57	56	68	80	52	56	ns
2-Methyl-1-butanol	192	192	237	251	158	156	ns
3-Methyl-1-butanol	55	53	61	65	51	50	ns
R2(3Me/2Me)	0.29	0.28	0.26	0.26	0.32	0.32	ns
Total higher alcohols	319	315	383	410	271	275	ns
Other alcohols							
Benzylic alcohol	4.1	4.1	3.9	4.2	2.8	258	***
2-Phenylethanol	33	30	46	46	30	27	ns
Other compounds							
Ethyl lactate	87	102	16	19	6	6	***
Acetoine	5.7	16.0	4.2	4.8	3.8	3.7	ns
Acetol	**49.3 b**	**29.6 a**	52.3	63.1	37.8	30.9	ns
2,3-Butanediol levo	763	708	606	537	**809 b**	**640 a**	ns
2,3-Butanediol meso	175	1154	321	303	**178 b**	**137 a**	ns
γβ-Lactone	16	16	20	23	**17 b**	**15 a**	ns

Different letters in the row for each year indicate significant differences between treatments at $p < 0.05$. The significance of the year as factor is expressed as ns = non-significant; * $p < 0.05$; ** $p < 0.01$. LOD = Limit of Detection.

CONCLUSIONS

Independently of the cultivar, irrigated vines showed less negative stem water potential values than vines under rain-fed conditions. However, stomatal conductance was not altered, indicating that water supplied by rainfall was sufficient for adequate plant functioning in both cultivars. Pruning weight was higher in irrigated vines, suggesting a greater vegetative growth under this treatment. In contrast, irrigation did not increase yield in these cultivars. Must and wine composition, including amino acids and volatile compounds concentrations, were negatively affected by irrigation. Moreover, water productivity was greater in rain-fed vines in the case of 'Sousón', whereas no significant differences were detected for 'Brancellao'. Consequently, gross incomes were not increased by the irrigation practice. The results from this study indicated that irrigation in vineyards from a humid region such as Galicia might still not be necessary, although research in other areas within the region and with other irrigation protocols (timeframes and amounts) may provide other results.

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REFERENCES

Allen R.G., Pereira L.S., Raes D., Smith M., 1998. *Crop evapotranspiration: guidelines for computing crop water requirements*. Irrigation and Drainage Paper 56. United Nations FAO, Rome, 300 p.

Basile B., Girona J., Behboudian M.H., Mata M., Rosello J., Ferré M., Marsal J., 2012. Responses of "Chardonnay" to deficit irrigation applied at different phenological stages: vine growth, must composition, and wine quality. *Irrig. Sci.*, **30**, 397-406.

Bell S.J., Henschke P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.*, **11**, 242-295.

Bouzas-Cid Y., Falqué E., Orriols I., Trigo-Córdoba E., Díaz-Losada E., Fornos-Rivas D., Mirás-Avalos J.M., 2015. Amino acids profile of two Galician white grapevine cultivars (Godello and Treixadura). *Ciência Téc. Vitiv.*, **30**, 84-93.

Cancela J.J., Trigo-Córdoba, E., Martínez E.M., Rey B.J., Bouzas-Cid Y., Fandiño M., Mirás-Avalos J.M., 2016. Effects of climate variability on irrigation scheduling in white varieties of *Vitis vinifera* (L.) of NW Spain. *Agric. Water Manage.*, **170**, 99-109.

Choné X., van Leeuwen C., Dubourdieu D., Gaudillère J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.*, **87**, 477-483.

Consello Regulador Ribeiro. 2016. http://ribeiro.es/nuestros-vinos/variedades-de-uva/ (last accessed June 6th 2016).

Cortés S., Díaz E., 2011. Characterization of autochthonous monovarietal wines from red Brancellao L. cultivars. *J. Food Compost. Anal.*, **24**, 154-159.

Cruz R., Lago A., Rial M.E., Díaz-Fierros F., Salsón S., 2009. Evolución recente do clima de Galicia. Tendencias observadas en variables meteorolóxicas. *In: Evidencias e Impactos do Cambio Climático en Galicia*. 19-58. Santiago de Compostela: Consellería de Medio Ambiente e Desenvolvemento Sostible.

de Souza C.R., Maroco J.P., dos Santos T.P., Rodríguez M.L., Lopes C.M., Pereira J.S., Chaves M.M., 2003. Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines (*Vitis vinifera* cv. Moscatel). *Funct. Plant Biol.*, **30**, 653-662.

Deloire A., Carbonneau A., Wang Z.P., Ojeda H., 2004. Vine and water a short review. *J. Int. Sci. Vigne Vin*, **38**, 1-13.

dos Santos T.P., Lopes C.M., Rodrigues M.L., Souza C.R., Maroco J.P., Pereira J.S., Silva J.R., Chaves M.M., 2003. Partial rootzone drying: effectsongrowth and fruit quality of field-grown grapevines *Vitis vinifera* L.. *Funct. Plant Biol.*, **30**, 663-671.

Dry P.R., Loveys B.R., McCarthy M.G., Stoll M., 2001. Strategic irrigation management in Australian vineyards. *J. Int. Sci. Vigne Vin*, **35**, 129-139.

Fandiño M., Cancela J.J., Rey B.J., Martínez E.M., Rosa R.G., Pereira L.S., 2012. Using the dual-Kc approach to model evapotranspiration of Albariño vineyards (*Vitis vinifera* L. cv. Albariño) with consideration of active ground cover. *Agric. Water Manag.*, **112**, 75-87.

Fraga H., Malheiro A.C., Moutinho-Pereira J., Cardoso R.M., Soares P.M.M., Cancela J.J., Pinto J.G., Santos J.A., 2014. Integrated analysis of climate, soil, topography and vegetative growth in Iberian viticultural regions. *PLOS One*, **9**, e108078.

Garde-Cerdán T., Lorenzo C., Lara J.F., Pardo F., Ancín-Azpilicueta C., Salinas M.R., 2009. Study of the evolution of nitrogen compounds during grape ripening. Application to differentiate grape varieties and cultivated systems. *J. Agric. Food Chem.*, **57**, 2410-2419.

Glories Y., 1984. La couleur des vins rouges, 2eme partie. *Connaiss. Vigne Vin*, **18**, 253-271.

Gómez-Alonso S., Hermosín-Gutiérrez I., García-Romero E., 2007. Simultaneous HPLC analysis of biogenic amines, amino acids and ammonium ion as aminoenone derivatives in wine and beer samples. *J. Agric. Food Chem.*, **55**, 608-613.

Gouveia J., Lopes C.M., Pedroso V., Martins S., Rodrigues P., Alves I., 2012. Effect of irrigation on soil water depletion, vegetative growth, yield and berry composition of the grapevine variety Touriga Nacional. *Ciência Téc. Vitiv.*, **27**, 115-122.

Intrigliolo D.S., Castel J.R., 2009. Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: water relations, growth, yield and fruit and wine quality. *Agric. Water Manage.*, **96**, 282-292.

Intrigliolo D.S., Castel J.R., 2010. Response of grapevine cultivar 'Tempranillo' to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. Irrig. Sci., **28**, 113-125.

Intrigliolo D.S., Pérez D., Risco D., Yeves A., Castel J.R., 2012. Yield components and grape composition responses to seasonal water deficits in Tempranillo grapevines. *Irrig. Sci.*, **30**, 339-349.

IPCC, 2014. *Climate Change 2014: Synthesis Report*. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC; Geneva, Switzerland, 151 pp.

Jackson D.I., Lombard P.B., 1993. Environmental and management practices affecting grape composition and wine quality: A review. *Amer. J. Enol. Vitic.*, **44**, 409-430.

Junquera P., Jiménez L., Linares R., Baeza P., Lissarrague J.R., 2012. Long-term effects of different irrigation strategies on yield components, vine vigour, and grape composition in cv. Cabernet sauvignon (*Vitis vinifera* L.). *Irrig. Sci.*, **30**, 351-361.

Kliewer W.M., 1968. Changes in the concentration of free amino acids in grape berries during maturation. *Am. J. Enol. Vitic.*, **19**, 166-174.

Lanari V., Palliotti A., Sabbatini P., Stanley Howell G., Silvestroni O., 2014. Optimizing deficit irrigation strategies to manage vine performance and fruit composition of field-grown 'Sangiovese' (*Vitis vinifera* L.) grapevines. *Sci. Hortic.*, **179**, 239-247.

Mirás-Avalos J.M., Mestas-Valero R.M., Sande-Fouz P., Paz-González A., 2009. Consistency analysis of pluviometric information in Galicia (NW Spain). *Atmos. Res.*, **94**, 629-640.

Mirás-Avalos J.M., Trigo-Córdoba E., Bouzas-Cid Y., 2014. Does predawn water potential discern between irrigation treatments in Galician white grapevine cultivars? *J. Int. Sci. Vigne Vin*, **48**, 123-127.

Ojeda H., 2008. L'irrigation de précision en fonction des particularités et les objectifs du vignoble. *Cahier Technique Revue Française d'œnologie*, **229**, 1-8.

OIV, 2009. *Compendium of International Methods of Wine and Must Analysis vols. 1 and 2*. Paris, France: Office International de la Vigne et du Vin.

Ortega-Heras M., Pérez-Magariño S., Del-Villar-Garrachón V., González-Huerta C., Moro-González L.C., Guadarrama-Rodríguez A., Villanueva-Sánchez S., Gallo-González R., Martín de la Helguera S., 2014. Study of the effect of vintage, maturity degree and irrigation on the amino acid and biogenic amine content of a white wine from the Verdejo variety. *J. Sci. Food Agric.*, **94**, 2073-2082.

R Core Team, 2015. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reynolds A.G., Lowrey W.D., Tomek L., Hakimi J., de Savigny C., 2007. Influence of irrigation on vine performance, fruit composition, and wine quality of Chardonnay in a cool, humid climate. *Am. J. Enol. Vitic.*, **58**, 217-228.

Richardson A.D., Duigan S.P., Berlyn G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol.*, **153**, 185-194.

Romero P., Fernéndez-Fernández J.I., Martínez-Cutillas A., 2010. Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. *Am. J. Enol. Vitic.*, **61**, 300-312.

Sánchez-de-Miguel P., Baeza P., Junquera P., Lissarrague J.R., 2010. Vegetative development: total leaf area and surface area indexes. *In: Methodologies and Results in Grapevine Research.* Delrot, S., Medrano, H., Bavaresco, L., Grando, S. (Eds.), 31-44, Springer.

Schneider C.H., 1989. Introduction à l'écologie viticole. Application aux systèmes de conduite. *Bulletin de l'OIV*, **701-702**, 498-515.

Steele M.R., Gitelson A.A., Rundquist D.C., 2008. A comparison of two techniques for nondestructive measurement of chlorophyll content in grapevine leaves. *Agron. J.*, **100**, 779-782.

Swiegers J.H., Bartowsky P.A., Henschke P.A., Pretorius I.S., 2005. Yeast and bacterial modulation of wine aroma and flavor. *Aus. J. Grape Wine Res.*, **11**, 139-173.

Talaverano I., Valdés E., Moreno D., Gamero E., Mancha L., Vilanova M., 2016. The combined effect of water status and crop level on Tempranillo wine volatiles. *J. Sci. Food Agric.*, doi: 10.1002/jsfa.7898

Teszlák P., Kocsis M., Gaál K., Nikfardjam M.P., 2013. Regulatory effects of exogenous gibberelic acid (GA3) on water relations and CO2 assimilation among grapevine (*Vitis vinifera* L.) cultivars. *Sci. Hortic.*, **159**, 41-51.

Trigo-Córdoba E., Bouzas-Cid Y., Orriols-Fernández I., Mirás-Avalos J.M., 2014. Irrigation effects on the sensory perception of wines from three white grapevine cultivars traditional from Galicia (Albariño, Godello and Treixadura). *Ciência Téc. Vitiv.*, **29**, 71-80.

Trigo-Córdoba E., Bouzas-Cid Y., Orriols-Fernández I., Mirás-Avalos J.M., 2015. Effects of deficit irrigation on the performance of grapevine (*Vitis vinifera* L.) cv. 'Godello' and 'Treixadura' in Ribeiro, NW Spain. *Agric. Water Manage.*, **161**, 20-30.

van Leeuwen C., Tregoat O., Choné X., Bois B., Pernet D., Gaudillère J.-P., 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *J. Int. Sci. Vigne Vin*, **43**, 121-134.

Vilanova M., Campo E., Escudero A., Graña M., Cacho J., 2012. Volatile composition and sensory properties of *Vitis vinifera* red cultivars from North West Spain: Correlation between sensory and instrumental analysis. *Anal. Chim. Acta*, **720**, 104-111.

Williams L.E., Araujo F.J., 2002. Correlations among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera*. *J. Amer. Soc. Hort. Sci.*, **127**, 448-454.

Williams L.E., Trout T.J., 2005. Relationships among vine- and soil-based measures of water status in a Thompson seedless vineyard in response to high-frequency drip irrigation. *Am. J. Enol. Vitic.*, **56**, 357-366.

Zamora F., 2003. *Elaboración y crianza del vino tinto: aspectos científicos y prácticos*. Ed. Mundi-Prensa, Madrid, 225 p.