Effects of Irrigation and Cluster Thinning on Tempranillo Grape and Wine Composition

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Submitted for publication: October 2013
Accepted for publication: May 2014

Key words: Irrigation, must, wine, production, Tempranillo

With the aim of understanding the effects of water stress and cluster load on berry composition and wine quality, a four-year field test was conducted in a cv. Tempranillo vineyard in Extremadura (Spain). When the first berries appeared to be changing colour (onset of véraison), grapevines were subjected to two different irrigation regimes, one supplying 100% of crop evapotranspiration, and the other 25%. In addition, two cluster load levels were tested for each irrigation regime: seven to nine and four to five clusters/m^2 planting area. Both irrigation and thinning had an impact on most of the parameters analysed in the grapes and the wines, although the thinning effect was in general higher than the irrigation effect. Thus, deficit irrigation reduced malic acid and the titratable acidity of Tempranillo grape juice, while cluster thinning increased all parameters analysed, except potassium concentrations. Similarly, the wine composition was also affected differently by irrigation and by cluster thinning. Deficit irrigation reduced pH and increased titratable acidity, total phenol index and colour parameters, while cluster thinning increased alcohol content, anthocyanin and colour intensity, and reduced pH and colour. The effect of the cluster thinning technique was independent of the irrigation regime in most of the wine parameters analysed, except for total phenol index, where the effect of deficit irrigation (DI) was more pronounced in grapevines also subjected to the cluster thinning treatment.

INTRODUCTION

Although grapevines are cultivated in a wide variety of climates, grapevine development, and consequently must and wine composition, are dependent on edapho-climatological and crop conditions, and also are highly dependent on plant-water status (Valdés *et al*., 2009). Water stress can influence various physiological and metabolic plant processes, including growth, photosynthesis and respiration. These, in turn, can affect the production, composition and characteristics of the must (Wang *et al*., 2003) and, in consequence, the quality of the resulting wine (Intrigliolo & Castel, 2010). In arid climates, rainfall amount and regime are usually very irregular and often not synchronised at all with the grapevine's life cycle, so that grapevines are often subjected to severe water stress, which lowers both grape production and quality (Mullins *et al*., 1992). Irrigation is an effective way of regulating the availability of water for grapevines and, consequently, their yield.

If irrigation is to be applied in a vineyard, a number of factors need to be taken into consideration, the most significant of which involve the amount of water applied and the season in which it is applied. With respect to the amount of water, several studies have shown that wine quality falls if too much water is supplied. Vegetative growth increases, increasing cluster shading and resulting in higher total acidity and pH of the must, in addition to significant reductions in the final colour and phenolic content of red wines (Valdés *et al*., 2009; Basile *et al*., 2011). In contrast, it has been shown that controlled water deficit can lead to enhanced wine quality by limiting plant growth and so favouring cluster sunlight exposure, improving peel/pulp ratio and raising phenol production (Ojeda *et al*., 2002; Nadal & Lampreave, 2004). All of this can significantly affect the composition of the wines. Regarding the timing of deficit irrigation, pre-véraison stress resulted in berries with higher concentrations of anthocyanins and phenolic compounds (Ojeda *et al*., 2001), while in other studies, similar responses were obtained when stress was induced between véraison and harvesting (Ojeda *et al*., 2002; Valdés *et al*., 2009; Intrigliolo & Castel, 2010). In both situations,

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Acknowledgements: The authors are grateful to Mrs Raquel Manzano, for her technical assistance during the laboratory measurements. This research was supported by funds from INIA, FEDER and the Government of Extremadura, Project RTA2005-00038-C06-0 and RTA2008-00037-C04

the response will also depend on the duration and intensity of the stress endured by the plants. So, to ensure the success of any irrigation strategy, the decisions that need to be taken should be based on sensitive and reliable water indicators (Yuste *et al*., 2004).

The practice of cluster thinning influences the sink-source ratio, restricting part of the yield without lowering leaf surface area. Thinning allows the plant to concentrate its activity on the remaining clusters, making it an effective method of regulating production and modifying the composition of the berries (Keller *et al*., 2005; Gatti *et al*., 2012; Sun *et al*., 2012). Several authors have found that cluster thinning enhances berry ripening, affecting the contents of sugars, acids, polyphenols and aromas of the harvested grape and therefore positively affecting the quality of the wine (Ferrer *et al*., 2009; Sun *et al*., 2012). Its positive effects have been reported for the Tempranillo variety (Valdés *et al*., 2009; Intrigliolo & Castel, 2011). Studies on cluster thinning have also shown the importance of the time when it is performed. When thinning is performed at veraison, the effect on berry weight and cluster size is lower than when performed immediately after fruit set, since it is at veraison that potential berry size and the number of berries per cluster are determined, as well as some compounds, such as the organic acids (Valdés *et al*., 2009). In addition, the result of cluster thinning can depend to a large extent on the climatological conditions during the season, with contrasting and even opposite results being obtained depending on the varieties and yields (Guidoni *et al*., 2002, Prajitna *et al*., 2007).

Many research studies have been conducted previously on the influence of water stress and cluster thinning on berry composition, but few studies have been done in which the effect of cluster thinning is evaluated under different irrigation regimes on grapes and on the wines made from them (Intrigliolo & Castel, 2011; Santesteban *et al*., 2011). This type of study is particularly important in vineyards with resource limitations, as would be the case in deficit-irrigated grapevines with high crop loads. Such studies are also relevant in relation to red grapevine cultivars, since the repercussions of these practices on the phenolic and chromatic characteristics of the wines obtained need to be analysed. In this sense, the complexity of the fermentation process limits the value of the phenolic composition of the grape as a marker for certain phenolic compounds of the resulting wine (Bindon *et al*., 2011). It has been shown that minimal differences in tannin concentration in the berries, in response to water deficit, are magnified after vinification and give rise to significant increases in wine tannins (Kennedy *et al*., 2002). These increases in wine tannins in response to water deficit are also associated with increases in anthocyanins and flavonols. It is clear that the extraction and subsequent stabilisation of phenolic derivatives play an important role in the final composition of the wine, as much as or greater than the actual phenolic composition of the berry. The question of berry size and its effect on wine composition has produced inconclusive results, and the relationship between berry size and the phenolic composition of wine remains unclear (Holt *et al*., 2008). Consequently, there is a need for further study on the relationship between the berry and the composition of the wine in relation to the specific characteristics of the vineyard.

The diversity in the responses of different varieties means that studies need to be performed in relation to this practice in each case, without making generalisations that could lead to erroneous conclusions. The aim of the present work was to study the response of cv. Tempranillo to different irrigation regimes and cluster thinning treatments under the growing conditions found in Extremadura, in terms of the composition of the grapes and the wines made from them during four seasons.

MATERIALS AND METHODS

Site description and experiment design

The experiment was conducted over four consecutive crop seasons (2005 to 2008) in a vineyard of cv. Tempranillo located at Finca la Orden (Regional Government of Extremadura, Spain). The vineyard was planted in 2001 on Richter-110 rootstock at a spacing of 2.5 by 1.2 m (3 333 grapevines/ha) pruned to a double Royat cordon and rows with east-west orientation.

The experimental design was a split plot with four replicates; irrigation being the main factor, and crop load the second factor within each irrigation regime. The experimental plots had six rows with eighteen vines per row.

The irrigation regimes applied were as follows:

Deficit irrigation (DI): corresponding to 25% of crop evapotranspiration (ETc).

Full irrigation (FI): corresponding to 100% of ETc.

Crop evapotranspiration was estimated as the product of reference evapotranspiration (ETo), measured at a weather station located at the site, and crop coefficient (Kc), following the methodology of Allen *et al*. (1998). Irrigation was initiated when the stem water potential measured at midday reached a level of -0.9 MPa in the 2005 and 2006 seasons, and of -0.5 MPa in the 2007 and 2008 seasons. Irrigation was applied every day, except Sundays, and was terminated before harvest in mid-September. Drip irrigation was applied with pressure-compensated emitters of 4 L/h located in a single row 120 cm apart. The total water applied in each treatment, as well as values of ETo, ETc, Kc and average stem water potential for each season, are shown in Table 1.

Two cluster load levels were established for each irrigation regime:

- Control treatment (C) without thinning (7 to 9 clusters/m^2 of planting area).
- Thinning treatments (T), in which the load was adjusted to 4 to 5 clusters/m^2 of planting area by removing clusters at véraison.

Chemical composition of berries and wines

The chemical composition of the berries was determined in fresh samples of approximately 250 g of triturated berries per plot. Berries were collected at harvest by removing small portions of clusters in the early hours of the morning, and each berry was separated from its pedicel in the laboratory. The methodology used was as described by Valdés *et al*. (2009). Total soluble solids (TSS ºBrix) were determined by refractometry (Atago RX-1000 refractometer, Tokyo,

TABLE 1
Water balance variables in a Tempranillo vineyard, Extremadura (Spain). Seasonal: effective rainfall, reference evapotranspiration (ETo), crop evapotranspiration (ETc), average crop coefficient (Kc) from April to September and season average stem water potential Ψs. Irrigation: Water volumes applied in each treatment during 2005, 2006, 2007 and 2008 growing seasons. In 2007 and 2008, ETc values were obtained in a weighing lysimeter installed within the vineyard.

	Seasonal				Irrigation (mm)		Ψs (MPa)	
Year	Rainfall (mm)	ETo (mm)	ETc (mm)	Kc	DI	FI	DI	FI
2005	54	1039	373	0.35	56	222	-0.97	-0.80
2006	58	1024	354	0.31	81	323	-0.98	-0.84
2007	182	975	508	0.52	62	172	-0.66	-0.54
2008	129	1026	755	0.74	153	611	-0.81	-0.51

(1)DI = Deficit irrigation (25% ETc); FI = Full irrigation (100% ETc).

Japan). pH, titratable acidity (TA) and potassium (K) were determined following the official methods of the OIV (1990). A Crison Micro pH-meter (Barcelona, Spain) was used for the first two determinations and a Varian AA 240 FS spectrometer (California, USA) was used to determine K. Malic acid was quantified using the enzymatic method (EEC, 1990) and tartaric acid with the Blouin method (Blouin, 1992). Both acids were analysed in a SYSTEA Easychem automatic multidetector (Gomensoro, Madrid, Spain). All analyses were performed in triplicate.

Berry phenolic composition was analysed following the methodology of Valdés *et al*. (2009). In the extracts obtained, the phenol content of grapes was determined as total polyphenol potential (TPP) and extractable polyphenol potential (EPP), following the methodology described by Ribereau-Gayon *et al*. (2000); total anthocyanin potential and the extractable anthocyanin potential (TAnP & EAnP) were quantified in accordance with Di Stefano and Gentilini (2002). All measurements were taken with a Shimadzu UV-120 spectrophotometer (Kyoto, Japan).

The "cellular maturity" or "cellular extractability" (EA) and "phenolic maturity of the grapes" (Mp) were calculated using the following formulas, proposed by Saint-Cricq de Gaulejac *et al*. (1998):

1. EA%=[(TAnP-EAnP)/TAnP] x 100
2. Mp%={EPP-(EAnP x 0.04)]/EPP} x 100

TAnP and EAnP are expressed as mg of malvidin glucoside/L, and TPP and EPP as units of absorbance (u.a.)/L.

The phenolic content of the wines (total phenolic index – TPI) was measured by spectrophotometry at 280 nm (Ribereau-Gayon *et al*., 2000). Total anthocyanins, catechins and tannins were determined following the methodologies proposed by Di Stefano and Guidoni (1989), Broadhurst and Jones (1978) and Ribéreau-Gayon and Stonestreet (1966) respectively. The colorimetric parameters of colour intensity (CI), tonality (TC) and the % of red, blue and yellow and the proportion of red colour due to the flavylium cations (dA%) of the wines were determined following the method of Glories (1999). All colorimetric measurements were taken with a Shimadzu UV-120 spectrophotometer (Kyoto, Japan).

Microvinifications

Harvest was performed manually for the four seasons (2005 to 2008) when DIT reached 24ºBrix (common harvesting criterion for Tempranillo in this area). Grapes from the different experimental plots were transported to the INTAEX winery. The grapes were mechanically crushed and destemmed. Maceration-fermentation of the musts was conducted separately for each field replicate following standard oenological techniques in 50 L stainless steel tanks at 22ºC. SO_2 was added at 50 mg/kg, must pH was adjusted to 3.5 with tartaric acid, and fermentation was induced using the yeast strain *Saccharomyces cerevisiae* Viniferm from Agrovin (30 g/hL).

During fermentation, pumping over was performed twice daily, and the density and TPI were measured each day. End of maceration was reached when the values of TPI stabilised. Once fermentation was completed, the wines were settled at 4ºC and total sulphur content was adjusted to 75 mg/L, impeding malolactic fermentation. The wine was bottled (0.75 L bottles) and stored at 15ºC until its analysis three months later.

Statistical analysis

Data were analysed using a general linear model procedure, with the irrigation regime (two levels), the cluster thinning treatment (two levels) and their interaction as main factors. Year and its interaction with the other fixed factors as random factors were also included in the analysis. The difference between treatments was compared using Tukey's test for significant differences ($p \leq 0.05$). The analyses were performed using the SPSS v. 15.0 statistical software.

RESULTS

Physicochemical composition of the grapes at harvesting

Given that no statistically significant interaction was observed between the irrigation*thinning effects in all the parameters included under the description "pulp maturity", namely total soluble solids (TSS, ºBrix), pH, titratable acidity (TA), malic acid, tartaric acid and potassium (K), for the set of years of the study as a whole, the influence of the irrigation and thinning factors on these parameters was studied on an individual basis (Table 2). In contrast to irrigation regime FI, the application of DI decreased malic acid content (8%) and TA, but not tartaric acid, and there was no significant increase in pH.

Cluster thinning led to statistically significant higher values of TSS, tartaric acid and pH, but to lower malic acid content and TA in the T in respect to the control (C) grapes, except in K, where there were no differences. There were significant differences between treatments for TSS and TA, which was reflected in a 6.5% increase in TSS due to thinning in the DI plants, and a 4% increase in the FI ones.

Statistically significant differences were found in all parameters due to the year effect, except in total soluble solids (TSS), since the grapes were harvested with similar TSS values each year in accordance with harvesting criteria. The Thinning (T*) year was significant for all parameters except K, while the irrigation (Irr*) year was only significant for malic acid. No statistically significant irrigation*thinning interactions were observed in total potential anthocyanin (TAnP), extractable potential anthocyanin (EAnP), total polyphenols (TPP), extractable polyphenols (EPP), cell maturity (EA) or seed maturity (Mp) (Table 3).

Irrigation DI did not significantly affect berry phenolic content. However, cluster thinning significantly increased TPP, EPP, TAnP and EAnP, while it reduced EA and Mp. Between treatments, differences were found for TanP, TPP and EPP, showing that thinning always resulted in an increased concentration of phenolic compounds, although to a higher extent in DI grapevines. Statistically significant differences were observed in Mp. This related to the year and the effect interaction had on the thinning technique.

Physicochemical composition and chromatic characteristics of the wines

Although an identical winemaking process was employed in all four years of the study, the different composition of the grapes of the four crop seasons resulted in different wine composition. This also meant the year effect and the thinning*year interaction had statistical significance in most of the parameters analysed (Tables 4 and 5). As can be seen in Tables 4 and 5, the composition of the wines reflects the composition of the grapes from which they were made only to a certain degree. So, as occurred with the grapes, no statistically significant interactions were observed in irrigation*thinning or in irrigation*year, with the exception of the TA parameter. Likewise, in full concordance with the grapes from which they were made, the alcohol content and anthocyanin content were higher in the cluster thinning treatment (T) wines ($p < 0.05$) than in the control cluster (C) wines.

Variation in phenolic content due to irrigation regime was neither statistically significant nor quantitatively very high in the grapes, but was statistically significant and quantitatively higher in wines produced from thinning treatment (T) grapes. In addition, the thinning*year interaction was statistical significance in all phenolic parameters, which was not the case with the grapes. These findings corroborate the need to evaluate the effect of grape-producing practices on the resultant wines.

The results described above in relation to phenolic composition and, especially, the anthocyanin content and pH of the wines, have a direct effect on their chromatic characteristics (Table 6). Thus, in most of the chromatic parameters, the same statistically significant year*thinning interaction was observed as in the results for these parameters for the wines. The increase in CI in wines resulting from the cluster thinning treatment (T) was clear, with statistically significant differences ($p \leq 0.001$), and was observed independently of the irrigation regime. However, it should be noted that these differences are of equal range, although of higher statistical significance in the FI treatments ($p < 0.001$) than in the DI treatments ($p < 0.01$). When the four types of wine are compared to each other, two well-differentiated groups appear that are defined by the cluster

TABLE 2

Effect of deficit irrigation and thinning on total soluble solids (TSS), malic acid, tartaric acid, pH and K of Tempranillo grapes. Data are means for 2005, 2006, 2007 and 2008.

Treatments[(1)]	**TSS (ºBrix)**	**Malic acid (g/L)**	**Tartaric acid (g/L)**	**pH**	**Titratable acidity (g tartaric acid/L)**	**Potassium (g/L)**
FIC	22.81 ab[(3)]	2.04	5.90	3.76	5.11 a	1.34
FIT	23.83 a	2.26	6.31	3.83	4.34 ab	1.45
DIC	22.26 b	1.66	6.00	3.78	4.40 ab	1.23
DIT	23.82 a	1.98	6.45	3.89	3.68 b	1.47
Significance of effects:						
Irrigation	n.s. [(2)]	***	n.s.	n.s.	***	n.s.
Thinning	***	**	***	***	***	n.s.
Irrigation*Thinning	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year	n.s.	***	***	***	***	***
Irrigation*Year	n.s.	*	n.s.	n.s.	n.s.	n.s.
Thinning*Year	**	**	***	***	**	n.s.
Irrigation*Thinning*Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[(1)]FIC = Full irrigation (FI) with cluster control; FIT = Full irrigation with cluster thinning; DIC = Deficit irrigation (DI) with cluster control; DIT = Deficit irrigation with cluster thinning.

[(2)]Level of significance: n.s. = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

[(3)]Values followed by different letters indicate the existence of significant differences between them ($p \leq 0.5$).

TABLE 3
Effect of deficit irrigation and thinning on total potential anthocyanin (TAnP), extractable potential anthocyanin (EAnP), total polyphenols (TPP), extractable polyphenols (EPP), cell maturity (EA) and seed maturity (Mp). Data are means for 2005, 2006, 2007 and 2008.

Treatments[(1)]	TAnP (mg/L)	EAnP (mg/L)	TPP (u.a./L)	EPP (u.a./L)	EA	Mp
FIC	1433.67 b[(3)]	607.91	51.17 c	34.01 c	56.28	31.10
FIT	1723.34 ab	878.54	65.90 ab	44.01 ab	46.79	21.98
DIC	1496.04 b	622.55	54.07 bc	34.79 bc	58.06	29.90
DIT	1909.72 a	911.74	69.64 a	45.84 a	51.84	23.28
Significance of effects						
Irrigation	n.s. [(2)]	n.s.	n.s.	n.s.	n.s.	n.s.
Thinning	***	***	***	***	*	**
Irrigation*Thinning	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year	***	***	***	***	***	***
Irrigation*Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Thinning*Year	n.s.	n.s.	n.s.	n.s.	n.s.	*
Irrigation*Thinning*Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[(1)]FIC = Full irrigation (FI) with cluster control; FIT = Full irrigation with cluster thinning; DIC = Deficit irrigation (DI) with cluster control; DIT = Deficit irrigation with cluster thinning.
[(2)]Level of significance: n.s. = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.
[(3)]Values followed by different letters indicate the existence of significant differences between them ($p \leq 0.5$).

TABLE 4
Effect of deficit irrigation and thinning on alcohol content, pH, titrable acidity (TA) and sulphur dioxide (SO_2) of Tempranillo wines. Data are means for 2005, 2006, 2007 and 2008.

Treatments[(1)]	Alcohol content (% v/v)	pH	Titratable acidity (TA) (g tartaric acid/L)	SO_2 free (mg/L)	SO_2 total (mg/L)
FIC	12.76	3.74	7.03	18.06	54.19
FIT	13.46	3.63	6.70	16.63	49.86
DIC	12.94	3.63	7.09	19.28	57.84
DIT	13.45	3.48	7.30	15.47	46.41
Irrigation and thinning	n.s.	n.s.	n.s.	n.s.	n.s.
Significance:					
Irrigation	n.s. [(2)]	***	*	n.s	n.s
Thinning	***	**	n.s.	***	***
Irrigation*Thinning	n.s.	n.s.	*	n.s.	n.s.
Year	***	***	***	***	***
Irrigation*Year	n.s.	n.s.	n.s.	n.s.	n.s.
Thinning*Year	***	***	n.s.	n.s.	n.s
Irrigation*Thinning*Year	n.s.	n.s.	n.s.	n.s.	n.s.

[(1)]FIC = Full irrigation (FI) with cluster control; FIT = Full irrigation with cluster thinning; DIC = Deficit irrigation (DI) with cluster control; DIT = Deficit irrigation with cluster thinning.
[(2)]Level of significance: n.s. = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

load applied. On the one hand, DIC and FIC wines with CI values of around 8 and, on the other, DIT and FIT wines with CI values of around 11. The highest CI value was observed for the DIT treatment.

It is important to note the existence of a statistically significant irrigation*thinning interaction in the parameters of tonality (TC) and % Red (Table 6). So, the effect of thinning on wine colour was positive or negative depending on the amount of water stress, increasing TC and lowering % Red in FI wines, and had the opposite effects in DI wines. This situation was repeated in dA; when DI was applied with cluster thinning treatment (T), wines were obtained with the highest dA values, whereas wines resulting from FI regimes with cluster thinning treatments (T) had the lowest dA values. It is interesting to note that, when the wines from the four treatments are compared, it can be seen that deficit irrigation and thinning had synergic effects on IC, % Red and, as a consequence, on the TC of the wines.

TABLE 5
Effect of deficit irrigation and thinning on anthocyanins, total phenol index TPI), tannins and catechins in Tempranillo wines. Data are means for 2005, 2006, 2007 and 2008.

Treatments[(1)]	Anthocyanins (mg malvidin glucoside/L)	TPI (u.a./L)	Tannins (g catechin/L)	Catechins (mg catechin/L)
FIC	228.04	35.24[b(3)]	1.54	451.41
FIT	267.85	39.70[b]	1.75	476.86
DIC	231.13	36.59 [b]	1.57	497.15
DIT	272.19	50.36 [a]	1.58	401.22
Significance of effects:				
Irrigation	n.s. [(2)]	*	n.s.	n.s.
Thinning	***	n.s.	n.s.	n.s.
Irrigation*Thinning	n.s.	n.s.	n.s.	n.s.
Year	***	***	***	***
Irrigation*Year	n.s.	n.s.	*	n.s.
Thinning*Year	***	***	**	**
Irrigation*Thinning*Year	n.s.	n.s.	n.s.	n.s.

[(1)]FIC = Full irrigation (FI) with cluster control; FIT = Full irrigation with cluster thinning; DIC = Deficit irrigation (DI) with cluster control; DIT = Deficit irrigation with cluster thinning.
[(2)]Level of significance: n.s. = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.
[(3)]Values followed by different letters indicate the existence of significant differences between them ($p \leq 0.5$).

DISCUSSION

Effect of deficit irrigation

Several studies on the effect of water status on berry composition at harvest have shown that its impact depends to a large extent on the changes caused to berry weight (Ojeda *et al*., 2001). However, the response obtained generally is variable and depends on the phenological condition in which the water deficit occurs, as well as its duration and intensity (Ojeda *et al*., 2002; Keller *et al*., 2005, 2008; Intrigliolo & Castel, 2009).

As in this present study, most of the investigations undertaken on this wine variety, as well as others in different regions of Spain, found that a higher plant water status led to a significant increase in malic acid. This can be explained on the basis of the effects of the application of a larger amount of water on cluster microclimate. By provoking a cooler and more shaded microclimate induced by the larger leaf surface area and usually more compact canopies, less combustion of this acid occurs during maturation (Escalona *et al*., 2012). This increased malic acid concentration in FI grapes presumably was the reason for their rise in titratable acidity (TA), something also noted by Valdés *et al*. (2009). Moreover, other studies have shown a reduction in titratable acidity when the irrigation dosage is lowered (Santesteban *et al*., 2011).

As for pH, the results concur with those obtained in other studies carried out in climate conditions similar to those of the present study. De la Hera-Orts *et al.* (2005) and Yuste *et al*. (2004) studied the influence of irrigation and observed a trend towards lower pH values in plots with higher water irrigation volume. The fact that wines from FI grapes had higher pH and lower TA values may be due to a modified relationship between acids during the winemaking process. Rises in wine pH in response to irrigation have been published in previous studies (Intrigliolo & Castel, 2008).

Water stress regulation is a powerful tool that can be used to influence colour and astringency, which are important determinants for the composition of red wine (Kennedy *et al*., 2002). The small differences in wine composition observed in the present study suggest that, since water stress was induced post-véraison, it did not have the opportunity to have a major effect on the biosynthesis and accumulation of anthocyanins, tannins and catechins (Escalona *et al*., 2012; Romero *et al*. 2013). The absence of effects on anthocyanin concentration does not concur with the increase observed in values for colour tonality, % of Red and dA, so these changes may be due to the different pH values of the wines. The lower TC in DI wines could then be due to their lower pH, which results in a higher percentage of anthocyanins in ionised form and, consequently, higher absorbance at 520 nm. This result is concordant with the higher percentage of dA for these wines, but may also be due to a higher percentage of co-pigmented forms in these wines. These changes in wine colour composition concur with the results published for this variety by Intringliolo and Castel (2011).

Effect of cluster thinning

The results obtained for cluster thinning concur with those published by other authors, who have found that this practice enables the berry to mature in better physiological and sanitary conditions (González-Neves *et al*., 2002; Ojeda *et al*., 2002). The finding of statistically significant thinning*year interactions can be explained on the basis that cluster thinning was performed at véraison and, although the same percentage of clusters was removed each year, the number of clusters was different in each crop season, as occurred with other studies (Intrigliolo & Castel, 2011; Santesteban *et al*., 2011). On the other hand, hailstorms and pest attacks during the 2007 season resulted in an 'uncontrolled natural thinning', which resulted in a lower yield for the cluster control group

TABLE 6
Effect of deficit irrigation and thinning on colour intensity (CI), colour tonality (CT) % Red, % Blue, % Yellow and brilliance of a wine (dA). Data are means for 2005, 2006, 2007 and 2008.

Treatments(1)	CI	CT	% Red	% Blue	% Yellow	dA (%)
FIC	8.02 b(3)	0.60 ab	54.93 ab	12.05	33.02	58.79 ab
FIT	10.54 a	0.62 a	54.07 b	12.45	33.48	57.25 b
DIC	8.22 b	0.59 ab	55.76 ab	11.63	32.62	60.18 ab
DIT	10.61 a	0.56 b	57.62 a	10.53	31.85	62.71 a
Significance:						
Irrigation	n.s. (2)	***	***	n.s.	n.s.	***
Thinning	***	n.s.	n.s.	n.s.	n.s.	n.s.
Irrigation*Thinning	n.s.	*	*	n.s.	n.s.	*
Year	***	***	***	*	***	***
Irrigation*Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Thinning*Year	***	n.s.	n.s.	n.s.	n.s.	n.s.
Irrigation*Thinning*Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Contrasts:						
Irrigation on C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Irrigation on T	n.s.	*	*	n.s.	n.s.	*
Thinning on FI	***	n.s.	n.s.	n.s.	n.s.	n.s.
Thinning on DI	**	n.s.	n.s.	n.s.	n.s.	n.s.

(1)FIC = Full irrigation (FI) with cluster control; FIT = Full irrigation with cluster thinning; DIC = Deficit irrigation (DI) with cluster control; DIT = Deficit irrigation with cluster thinning.
(2)Level of significance: n.s. = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.
(3)Values followed by different letters indicate the existence of significant differences between them ($p \leq 0.5$).

C (7 156 kg/ha) compared to the cluster thinning group T (11 488 kg/ha). Consequently, the results obtained for these crop seasons were not attributable to the treatments imposed and did not follow the trend of the other seasons.

The influence of cluster thinning on TSS accumulation was more intense than the influence of the irrigation regime. In all years, cluster thinning brought forward the accumulation of sugars so that, at the same date, the content of these substances was higher in the T berries (Table 2). These results were similar to those obtained by Nuzzo and Matthews (2006) and Gil-Muñoz *et al*. (2009).

The response of the organic acids to thinning showed important seasonal variation, in correspondence with other studies (Santesteban *et al*., 2011). The lower temperatures in 2008 could have affected the accumulation of tartaric and malic acid and especially resulted in the lower combustion of the latter compared to the other seasons. This result may indicate that the effect of thinning on these acids is not conditioned so much by the number of clusters, but rather by the annual climatological conditions.

Thinning advanced grape maturity, giving rise to lower TA values ($p < 0.01$) at harvest. When this parameter was compared in grapes with similar TSS, the acidity of berries from cluster-thinned grapevines was higher (data not shown). Other studies on this subject have shown different responses of organic acids to thinning (Diago *et al*., 2010), with most of them having lower total acidity levels and higher pH values.

With regard to the above, it should be mentioned that, although these berries had the highest malic and tartaric acid content (T), they also had the lowest TA values. In addition, a fall in the tartaric/malic acid ratio was observed. This is not beneficial for the quality of the grape because, since tartaric acid has a higher buffer capacity, its importance is vital for the establishment and maintenance of a low pH in both the grape and must that come from warm environmental conditions (Etchebarne *et al*., 2010). From all these results it can be concluded that, as far as acidity is concerned, the practice of thinning in cv. Tempranillo grapevines would not be beneficial in our edapho-climatological conditions, given that this variety, due in part to its high potassium absorption capacity, is characterised by high pH and low acidity at harvest (Cuevas, 2001), factors that are accentuated by thinning. The addition of tartaric acid at the start of fermentation had different effects depending on the cluster treatment. The T grapes, with higher pH values, gave wines of lower pH than the C wines, an apparent contradiction that has also been reported in other studies (Santesteban *et al*., 2011).

The effect of thinning on phenolic composition has been studied by a number of authors: Prajitna *et al*. (2007) in cv. Chanbourcin, Guidoni *et al*. (2002) in cv. Nebbiolo, and Santesteban *et al*. (2011) and Valdés *et al*. (2009) in Tempranillo. However, the results obtained in these studies are not concordant, and vary depending on the test conditions and the variety used, although it is true that the general trend is for cluster thinning to increase the amount of accumulated phenols. The present study confirms this general trend, since the concentration of phenolic compounds of the T group grapes was higher than that of the C group, independent of the water status of the grapevine. This may be due to alterations to the sink-source balance (that is, the relationship between leaf surface area and fruit), resulting in an increase in concentrations of the substrates required for polyphenol synthesis (Prajitna *et al*., 2007), and to the higher sunlight exposure of the cluster-thinned grapevines (Matus *et al*., 2009) affecting the activity of the phenylalanine ammonia lyase enzyme, which is directly involved in the synthesis of these metabolites, or it may be due to a higher degree of synchronisation in terms of technological and phenolic maturity. Contributing factors to this last explanation are also the lower EA (cell maturity) values in the T plants compared to the C plants. This would also seem to point to a higher degree of maturity of the grapes, and its immediate consequence would be easier extraction of the skin anthocyanins during vinification as a result of greater degradation of the cell walls (Glories, 1999). Gil-Muñoz *et al*. (2009) obtained similar results for Tempranillo, but not for the variety Syrah, which may be because this response is determined by genetic factors. Statistically significant decreases in seed maturity (Mp) values were also observed in both irrigation regimes with cluster thinning. These same results were obtained when the effect of cluster thinning was studied in other grape varieties used for vinification (Gonzalez-Neves *et al*., 2002; Diago *et al*., 2010, Intrigliolo & Castel, 2011).

All the above results show that the application of cluster thinning at véraison affects the accumulation of anthocyanins and phenols and also brings forward the degradation of skin cells, facilitating the passage of these substances to the must. These results also have consequences on wine appearance, with significantly higher colour intensity (CI) values in the T group wines compared to the C group ones, which could also cause an increase in their organoleptic quality. This result may encourage commercial growers to thin clusters after yield estimation to intentionally reduce crop load, assuming that the lost revenue from lower yields will be recaptured through increased prices resulting from changes desired by the buyer, such as higher soluble solids or increased flavour (Preszler *et al*., 2010)

CONCLUSIONS

Our results show that, in semi-arid regions, the application of irrigation at dosages of 25% ETc as compared to 100% ETc of the water demand results in better grape quality, with the added benefit of water resource savings. Cluster thinning had a positive effect on grape and wine composition, synchronising pulp and berry peel maturity, which allows the harvesting of grapes with higher anthocyanin contents and enhances wine colour, although the results in terms of acidity were not as beneficial. Therefore we conclude that the strategy of applying moderate post-véraison water stress coupled with cluster thinning was most efficient in terms of have the potential to improve berry colour and wine, although the conditions of the crop season of course need to be taken into account when considering the practice of cluster thinning.

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