

# Response of grapevine cv. ‘Tempranillo’ to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition

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Received: 4 February 2009 / Accepted: 8 June 2009 / Published online: 27 June 2009  
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**Abstract** The effects of several moderate irrigation regimes on vine water status, yield, and must and wine composition, were investigated during five seasons in a vineyard planted with *Vitis vinifera* cv. Tempranillo. Treatments consisted of non-irrigated vines and six differentially irrigated treatments with contrasting watering regimes during the pre-veraison and post-veraison periods. There were large differences in yield and grape and wine quality responses to irrigation among seasons, probably as consequence of the different environmental conditions and crop levels. It was, however, clear that vines benefit more of the irrigation supplied in years of high yield levels. Across seasons, yield increased in proportion to the amount of water applied mostly due to the larger berries of irrigated vines, and there was no clear response to the timing of irrigation supplied. In addition, there were no carry over effects due to irrigation on bud fertility. The post-veraison water application was necessary to increase must sugar level and wine alcohol content. However, water restrictions during the pre-veraison period lead to more concentrated berries in terms of total phenolic and anthocyanins. The only noticeable detrimental effect of irrigation, regardless of the timing of its application, on wine composition was an increase in wine pH.

## Introduction

Wine production is the major economic activity in the counties of Requena and Utiel of Spain, where there are about 44,000 ha planted to grapevines. The two main cultivars grown for red wine production are Bobal (of local origin), which occupies about 79% of the total cultivated area, and Tempranillo which is the variety more widely grown in Spain for high quality red wines. Until recently vineyards in this area were dry-farmed due to legal restrictions. After derogation of the European Community law that prohibited the use of irrigation for wine production in Spain, drip irrigation has been steadily increasing.

Soil water availability is a critical factor for vine performance and wine composition. Irrigation allows increasing yields (Williams and Matthews 1990), though a moderate water deficit is often desirable to improve wine composition (Jackson and Lombard 1993). Deficit irrigation has been suggested as a strategy to improve fruit composition for premium quality wines reducing canopy vigor, increasing fruit exposure to light and reducing berry growth to avoid dilution effects (McCarthy et al. 2000).

Deficit irrigation consists in applying water rates to replace only part of the potential vine evapotranspiration either during the whole season or only during some phenological periods previously established. Previous results determined that water stress during the period from fruit set to veraison heavily reduces fruit size (McCarthy 1997). This is because the detrimental effect of soil water deficit on early fruit growth (Ojeda et al. 2001) that cannot be recovered even if water supplies return at full dosage later in the season (Poni et al. 1994a). On the other hand, late season water restriction may reduce fruit cell enlargement and water accumulation (Smart and Coombe 1983), and in

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Communicated by J. Ayars.

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general has a less detrimental impact on final berry size than early season water stress (McCarthy 1997).

Irrigation might also indirectly affect berry quality because of increased and prolonged vegetative growth. After veraison, shoot growth may compete for the carbohydrates available for fruit ripening. Increased vegetative growth might also impair cluster microclimate, particularly fruit light exposure (Smart et al. 1985). In other cases, irrigation has led to a delay in obtaining the desirable sugar levels (Bravdo et al. 1984).

However, reports also show that severe water stress might be detrimental to fruit quality because of a poor canopy development and reduced leaf assimilation rate thus leading to an inadequate vine capacity to ripen the crop (Hardie and Considine 1976), particularly under high yield levels (Freeman and Kliewer 1983).

Regulated deficit irrigation can be applied as a strategy to reduce the possible negative impact of irrigation on wine quality. In the past, Sal3n et al. (2005) studied the response to irrigation of cv. Bobal. However, in Tempranillo, in this area, the effects of different irrigation regimes on vine performance and wine quality have not been yet reported. Under these circumstances it was considered important to test different irrigation amounts and times of application on a Tempranillo vineyard performance and fruit composition. The ultimate goal is to provide vine growers with information about the more appropriate volumes of irrigation to apply on each phenological period, depending on the desired yield levels and wine styles.

## Materials and methods

### Site description

The experiment was carried out during five consecutive seasons (2000–2004) in a ‘Tempranillo’ vineyard (*Vitis vinifera* L.) located near Requena (39°29′N, 1°13′W, elevation 750 m), Valencia, Spain. The vineyard was planted in 1991 on 161-49 rootstock at a spacing of 2.45 × 2.45 m (1,666 vines ha<sup>-1</sup>) and in 2000, a drip-irrigation system was installed and vines trained to a vertical trellis on a bilateral cordon system oriented in the North–South direction. Shoot thinning was carried out each year according to the vineyard manager goals. This led to a different number of shoots and hence different number of clusters collected among years. All treatments were fertilized at a rate of 30–20–60–16 kg ha<sup>-1</sup> of N, P, K, and Mg, respectively.

The soil at the site was a Typic Calcicorthid, with a clay loam to light clay texture, highly calcareous and of low fertility (0.66% of organic matter, and 0.04% of nitrogen). The soil has a deep soil profile (>2 m), available water

capacity is about 180 mm m<sup>-1</sup> and bulk density 1.43–1.55 ton m<sup>-3</sup>.

Budbreak for Tempranillo in this area usually occurs by mid April, flowering by early June; veraison is reached by early-August with harvest during late September and leaf fall at the beginning of November. Climate is continental and semiarid with average annual rainfall of 430 mm of which about 65% falls during the dormant period. Weather conditions during the experiment (Table 1) were measured with an automated meteorological station located in the plot and reference evapotranspiration (ET<sub>o</sub>) was calculated with hourly values by the Penman-Monteith formula as in Allen et al. (1998). Crop evapotranspiration (ET<sub>c</sub>) was estimated as a product of ET<sub>o</sub> and crop coefficient ( $K_c$ ). The  $K_c$  values employed were based on results obtained in previous irrigation trials located in the same vineyard (Sal3n et al. 2004) and in a nearby vineyard planted with cv. Bobal (Sal3n et al. 2005). The seasonal  $K_c$  used varied with the phenological period and the expected pattern of leaf area development. Thus, from June to July,  $K_c$  was gradually increased from 0.08 to 0.30. After veraison, the objective was to induce a moderate soil water deficit, therefore applied water amounts were 0.15 of ET<sub>o</sub>.

### Irrigation treatments and experimental design

Treatments consisted of a rain-fed control (T1) and six irrigation treatments, where water was applied at different levels from flowering until near harvest: T2 (0–66–0), T3 (0–100–0) in 2000, 2001 2003 and 2004 and (0–75–50) in 2002, T4 (100–33–00), T5 (100–66–0), T6 (100–100–33) and T7 (100–100–66). Numbers in parentheses are the percentage of the estimated ET<sub>c</sub> applied, respectively, in each of three periods: flowering to fruit set, fruit set to veraison, and veraison to maturity, as depicted in Fig. 1. In 2003 and 2004, treatments 0–66–0, 100–33–0, and 100–100–33 were used for a partial rootzone drying trial and those results are reported elsewhere (Intrigliolo and Castel 2009).

Each treatment had six replicates in a randomized complete block design. Each plot consisted of ten rows with nine vines per row and the surrounding perimeter vines used as buffers. Water was applied with two pressure-compensated emitters of 2.4 L h<sup>-1</sup> located 60 cm on either side of the vine. Frequency of water applications was the same for all irrigated treatments and varied from 3 to 5 days per week. Water meters measured the amount applied to each irrigated replicate.

### Field determinations

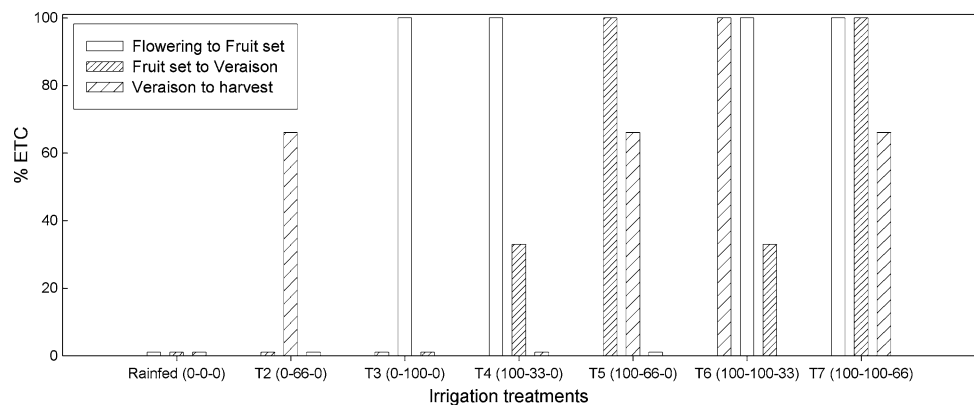
Determinations of plant water potential were performed with a pressure chamber (Soil Moisture Corp., Santa

**Table 1** Values of reference evapotranspiration (ET<sub>0</sub>), rainfall during the growing season (April to harvest) and annual rainfall of each year

Year	Growing season ET <sub>0</sub> (mm)	Growing season rainfall (mm)	Annual rainfall (mm)	Irrigation (mm)						
				0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
2000	883	127	254	0	44	59	39	58	64	77
2001	940	152	448	0	41	51	68	92	91	99
2002	836	187	386	0	18	44	26	38	29	53
2003	828	185	321	0	–	47	–	83	–	93
2004	798	228	521	0	–	39	–	67	–	82

Irrigation volumes applied to the different treatments are also shown

**Fig. 1** Schematic diagram of the irrigation treatments carried out. The percentage of water applied with respect to the estimated crop evapotranspiration (ET<sub>c</sub>) is shown for each phenological period



Barbara, USA) on five representative plants per treatment and two bag covered leaves per vine (stem water potential,  $\Psi_s$ ) performed at early morning (0700–0800 hours solar,  $\Psi_s^{em}$ ) and at midday (1130–1230 hours solar,  $\Psi_s^{md}$ ) at fortnightly intervals. In order to carry out all determinations within 1 h, water potentials were only measured in the rainfed and in the 0–66–0, 100–33–0 and 100–100–66 treatments.

Yield was determined at harvest on each of the seven internal rows (7 vines/row) of each replicate. The number of clusters per vine was determined in 12 vines per plot and average cluster weight determined from randomly selected samples of at least 20 clusters per plot. Berry weight was determined on random samples of about 200 berries per replicate.

Pruning weight (PW) and leaf area (LA) were determined in four vines per replicate. Leaf area was estimated after veraison when shoot growth had ceased. Leaf area per vine was estimated from a linear equation relating leaf area ( $Y$ , cm<sup>2</sup> per shoot) and total (main plus laterals) shoot length ( $X$ , cm). This relationship was obtained from samples of about 10 to 20 representative shoots of different lengths collected after veraison each year. Thus, leaf area per vine was calculated from the sum of each of the measured individual shoot lengths. Leaf area to yield ratio (LA/Y) and yield to pruning weight ratio (Y/PW) were also calculated in the four selected vines per replicate.

## Must and wine quality determinations

Must components were determined in the same samples collected for berry fresh weight determination, which were crushed with a small hand-press, and the juice centrifuged. Total soluble solids (Brix) were determined by refractometry. Juice pH and titratable acidity (TA) were determined by an automatic titrator. Organic acids (malic and tartaric) were analyzed by high-performance liquid chromatography following the procedures described by Romero et al. (1993). Ethanol in the wines was analyzed by gas chromatography. Wine color intensity (OD420 + OD520 + OD620) and total phenolics index (OD280) were determined by spectrophotometry in accordance with Ribereau-Gayon et al. (2000) and they were expressed in terms of absorbance units (AU). Anthocyanins (OD520 in HCl media) were also determined by spectrophotometry. All analytical determinations were duplicated.

## Microvinifications procedure

Grapes from the different treatments were harvested on the same day (or with 1 day difference), when a minimum 21° Brix was reached, and were transported to the experimental winery in field boxes. In 2000, non-irrigated vines could not reach the threshold Brix value and they had to be harvested

at a minimum Brix of 20°. Vinifications were performed at “Estación Viticultura y Enología Requena” separately on samples of about 30 kg from each plot, most often six vinifications per treatment. Grapes were mechanically crushed, de-stemmed, and fermented at about 25°C in stainless steel containers. All wine lots were inoculated with a commercial yeast strain (L-2056, Danstar Ferment AC, Zug Switzerland) at 100 mg kg<sup>-1</sup>. Skin contact time was 7 days and during this time they were punched down automatically every 4 h. After alcoholic fermentation they were racked off and malolactic bacteria (*Oenococcus oeni*) inoculated. They were again racked off, sulfited at 100 mg L<sup>-1</sup> K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, decanted and bottled. Analytical determinations in the wines were performed at the same time in both years just before inoculation with malolactic bacteria and about one month after grapes were crushed.

### Statistical analysis

Analysis of variance was performed using the MIXED procedures of the SAS statistical package (version 8.2; SAS Institute, Cary, NC, USA). Differences between treatment means were assessed by Dunnett’s *t* test against the non-irrigated (control) and by means of designed contrasts between pair of treatments. Across years, data were analyzed with irrigation treatment, year and their interaction as factors.

## Results and discussion

### Climatic conditions and soil and plant water relations

The first experimental season was the driest with only 254 mm of annual rainfall of which 127 occurred during the growing season (Table 1). In the other 4 years precipitation was closer to the average for this site. As a consequence the lowest values of  $\Psi_s^{\text{em}}$  and  $\Psi_s^{\text{md}}$  were recorded in rain-fed vines in 2000, with minimal values of -1.1 and -1.4 MPa for  $\Psi_s^{\text{em}}$  and  $\Psi_s^{\text{md}}$ , respectively (Figs. 2, 3). These values are indicative of a relatively severe water stress (Deloire et al. 2004). During the other seasons  $\Psi_s^{\text{em}}$  and  $\Psi_s^{\text{md}}$  values reached by the non-irrigated treatment were around -0.8 and -1.2, indicative of a milder water stress.

Differences of plant water status between irrigated treatments and the non-irrigated ones were in general clearer for the determination carried out early in the morning (Fig. 2) than at midday (Fig. 3). This probably reflects some degree of stomatal closure and growth reduction, both physiological processes that probably reduced plant transpiration. These results are in agreement with other findings obtained in the same plot and discussed with more detail by Intrigliolo and Castel (2006).

### Vegetative growth, yield and crop load

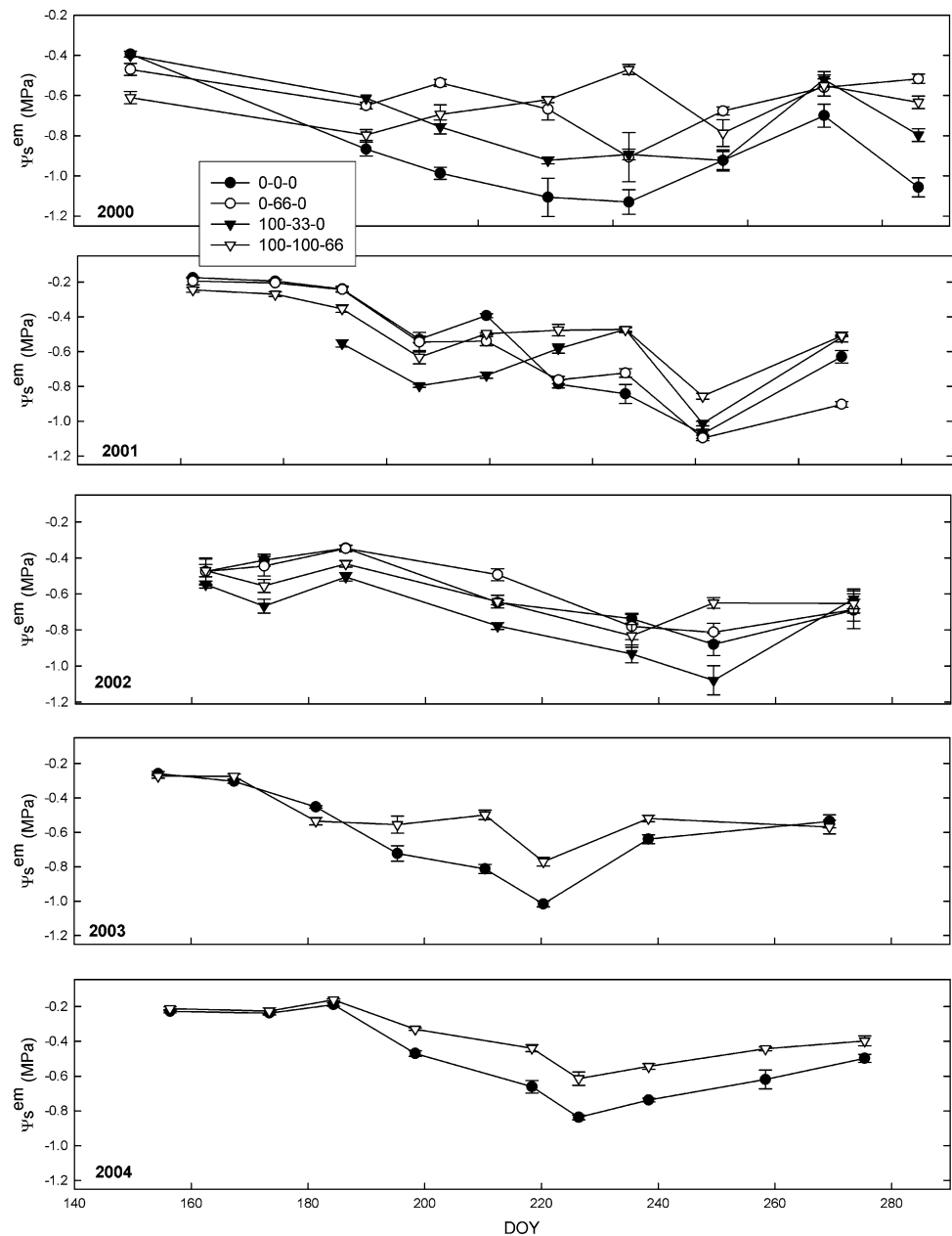
The season-by-season effects on all the vine growth and yield parameters studied were highly significant (Table 2). In addition for vine yield and for berry weight there was also a significant effect of the year by treatment interaction, suggesting that the effect of the irrigation regime on these parameters was different among seasons (Table 2). In fact, for vine vegetative growth there were large differences among years (Table 3). In the more watered (100–100–66) treatment leaf area was significantly higher than in the rainfed one only in 2003 and 2004. However, the highest leaf area values were obtained in the 0–100–0 treatment; despite they received only 39 mm of average water application but concentrated during the pre-veraison period, when most of the vegetative vine growth occurs (Williams 1997).

Similarly, compared with non-irrigated vines PWs were significantly higher in the 100–100–66 treatment in 2001, 2003 and 2004 (Table 3). Overall these results suggest that vine vegetative growth was stimulated by the water applied. However, while PWs appear to respond linearly to the total amount of water applications, leaf area was also affected by the timing of irrigation. In fact, when pooling average data across seasons for PW and for leaf area against the irrigation volumes applied there was a significant linear trend for PW ( $r^2 = 0.94$ ,  $P < 0.05$ ), but not for leaf area ( $r^2 = 0.24$ ,  $P > 0.05$ ).

The effects of irrigation on yield were mainly due to differences in berry weight among treatments (Table 4). In fact, when pooling data across seasons, there was a significant negative relationship between stem water potentials (averages from June to September) and berry fresh weight (Fig. 4). The relationship was slightly tighter with  $\Psi_s$  determinations carried out early in the morning than at midday.

In most of the years there were not significant differences among treatments in the number of clusters per vine collected and in the number of berries per cluster (Table 4). This suggests that the irrigation supplied did not have a carryover effect and it did not affect bud fertility. This finding agrees with reports on Shiraz and Carignane (Freeman et al. 1979; Kliewer et al. 1983), but differs from results on Tempranillo in central Spain (Esteban et al. 1999) where irrigation increased both berry size and number of clusters per vine. Considering the soil characteristics of our site that allowed high water retention, about 180 mm m<sup>-1</sup>, and the relatively low vine water use during the initial growing period, water storage in the soil would have been probably enough to ensure full canopy transpiration until July. This is why there was a very slow development of water stress (Figs. 2, 3), and rain-fed vines reached considerable lower plant water status than the

**Fig. 2** Seasonal variation of early morning stem water potential ( $\Psi_s^{em}$ ). Values are treatment mean values  $\pm$  SE of eight leaves determinations. *DOY* day of the year



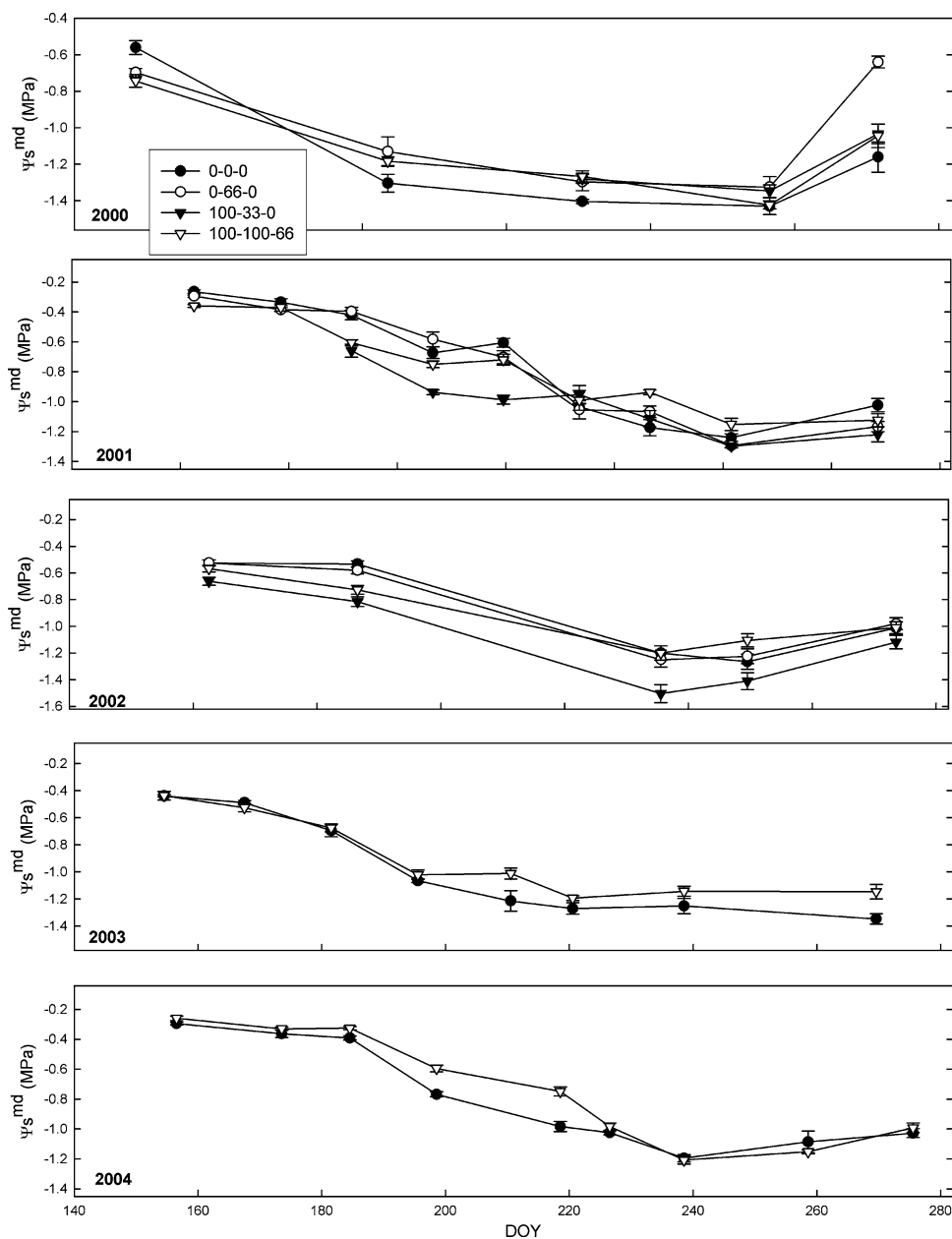
irrigated ones only late in the season (July–August) most likely after bud differentiation.

It should be noted that the yield response to irrigation was different among seasons. In the first experimental year, with very low precipitation rates and a high crop demand due to the large number of cluster per vine, yield was increased by up to a 22% by the 100–100–66 treatment when compared with rainfed vines. In 2003 instead, under a lower crop demand, irrigation did not increase yield in comparisons with the rain-fed vines. In fact, when pooling data over seasons and separated according to the number of clusters per vine collected (high  $>20$  and low  $<20$ ) there was a significant negative correlation between  $\Psi_s$  and yield

only for the “high group”, that is under a large crop demand (Fig. 5). The relation was tighter for  $\Psi_s$  determinations carried out at early morning than at midday. Given that differences between treatments were clearer for  $\Psi_s$  at early morning than at midday (Figs. 2, 3), overall these results suggest that, under our experimental conditions,  $\Psi_s^{em}$  seems to be a better water stress indicator than  $\Psi_s^{md}$ . However, at a commercial level,  $\Psi_s^{md}$  can be more easily implemented because there is more time available at midday to take the pressure chamber readings than at early morning, when environmental conditions change more quickly.

The balance between vine supply capacity and crop demand (i.e. crop load) expressed in terms of leaf

**Fig. 3** Seasonal variation of midday stem water potential ( $\Psi_s^{md}$ ). Values are treatment mean values  $\pm$  SE of eight leaves determinations. *DOY* day of the year



**Table 2** Effects of the irrigation treatment on the source variation among the different factors and their interactions

Variable	Treat	Block <sup>a</sup>	Year	Treat × block	Year × block	Treat × year
Leaf area	0.027	0.407	<0.001	0.0285	0.789	0.816
Pruning weight	<0.001	<0.001	<0.001	<0.001	0.058	0.100
Yield	<0.001	0.283	<0.001	0.080	0.071	0.005
Clusters per vine	0.370	0.904	<0.001	0.484	0.460	0.370
Berry weight	<0.001	0.005	<0.001	<0.001	0.001	<0.001
Berries per cluster	0.770	0.013	<0.001	0.623	0.132	0.820
Leaf area:yield	0.876	0.241	<0.001	0.012	0.927	0.375
Yield:pruning weight	0.007	<0.001	<0.001	0.013	0.003	0.059

<sup>a</sup> *P* value of Blocks and its interaction refers to the hypothesis of zero variance

**Table 3** Leaf area and pruning weight of the different treatments during each season

Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Leaf area (m <sup>2</sup> vine <sup>-1</sup> )	2000	4.3	4.0 <sup>X</sup>	5.9*	4.0	4.9 <sup>Y</sup>	4.2	3.7
	2001	5.2	6.0	6.7	5.6	6.2	6	5.7
	2002	6.1	6.9	8.2	6.8	6.3	6.5	6.2
	2003	4.8	–	5.9	–	5.6	–	6.2*
	2004	8.4	–	9.7	–	9.8	–	10.4*
Pruning weight (kg vine <sup>-1</sup> )	2000	0.52	0.52	0.61	0.59	0.54	0.48	0.58
	2001	0.66	1.03*	1.04*	0.80	0.94	1.05*	1.06*
	2002	0.80	1.03	1.01	0.92	0.98	1.0	0.95
	2003	0.85	–	1.12*	–	1.16*	–	1.25*
	2004	1.54	–	1.78*	–	1.81*	–	1.92*

\* Significant differences among irrigation treatments and the control (non-irrigated, 0–0–0) based on Dunnett's *t* test at  $P < 0.05$

<sup>X,Y</sup> Significant difference at  $P < 0.05$  in the 0–66–0 vs 0–100–0 and 100–66–0 vs 100–100–66 contrast, respectively

**Table 4** Yield and yield components of the different treatments during each season

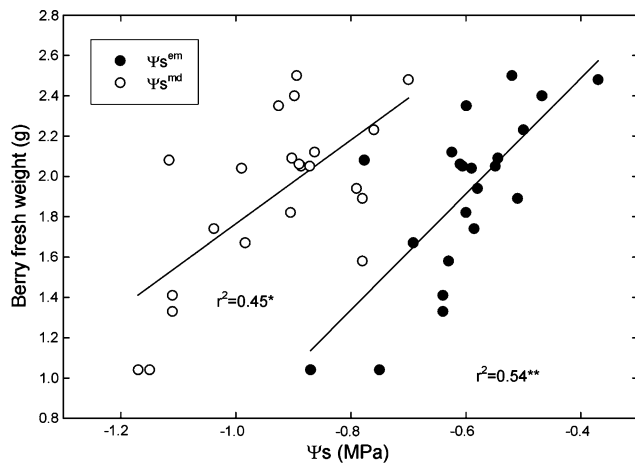
Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Yield (t ha <sup>-1</sup> )	2000	9.0	10.7*	10.2*	10.8*	10.5*	10.3*	11.0*
	2001	4.5	4.8 <sup>J</sup>	5.2	6.2*	6.3*	6.0*	6.3*
	2002	8.7	9.8* <sup>X,J</sup>	10.2*	9.7 <sup>W</sup>	11.7*	11.0*	11.0*
	2003	6.3	–	5.5	–	6.3	–	6.3
	2004	14.1	–	16.3*	–	18.7*	–	18.3*
Clusters per vine	2000	31	31	31	30	32	29	30
	2001	13	11 <sup>X</sup>	14	14	14	13	14
	2002	23	27	27	22 <sup>W</sup>	27	28*	29*
	2003	11	–	10	–	10	–	10
	2004	21	–	22	–	22	–	21
Berry weight (g)	2000	1.19	1.32*	1.34*	1.37*	1.36*	1.40*	1.41*
	2001	1.74	1.86	1.95	1.92* <sup>W</sup>	2.11*	2.05*	2.06*
	2002	2.07	2.09	2.07	2.06	1.97	1.94	1.98
	2003	2.16	–	2.22	–	2.54*	–	2.48*
	2004	2.07	–	2.33*	–	2.44*	–	2.47*
Berries per vine	2000	139	147	146	153	138	146	148
	2001	117	135	112	130	123	128	132
	2002	128	130	130	133	135	136	132
	2003	149	–	135	–	136	–	135
	2004	192	–	192	–	197	–	198

\* Significant differences among irrigation treatments and the control (non-irrigated, 0–0–0) based on Dunnett's *t* test at  $P < 0.05$

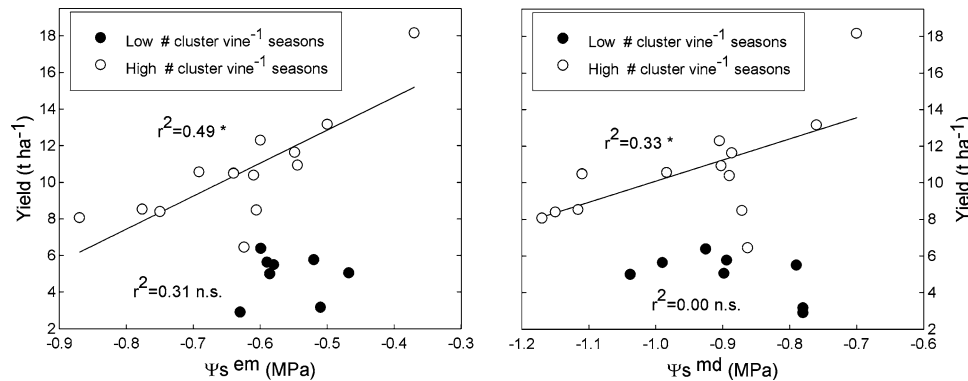
<sup>X,J,W</sup> Significant difference at  $P < 0.05$  in the 0–66–0 vs 0–100–0, 0–66–0 vs 100–66–0 and 100–33–0 vs 100–66–0 contrasts, respectively

area:yield or crop weight:pruning weight was not impaired by the supplemental irrigation applied (Table 5). This was because the increase in yield due to irrigation was in most part compensated by the higher vine growth of the irrigated vines. Nonetheless, there were considerable differences among years in these values. In 2000, LA:Y and Y:PW were around 0.6–1.0 and 10.8–14.5, respectively. These values according to Kliewer and Dokoozlian (2005) and Bravdo et al. (1984, 1985) are indicative of vines with a

limited source capacity. Season 2004 was another one with a large crop load; while 2001 and 2002 were years with crop load values that can be considered adequate for a proper grape ripening. In 2003, vines were instead under-cropped due to the very low yield. These large crop load differences among seasons will be considered in the next paragraph to explain temporal differences observed in the fruit and wine quality responses to the supplemental irrigation.



**Fig. 4** Relationships between berry fresh weight and the average June to September midday ( $\Psi_s^{md}$ ) or early morning ( $\Psi_s^{em}$ ) stem water potential. Values are average mean per treatment replicate pooling data across seasons. *Double asterisks and single asterisk indicate significant linear trend at  $P < 0.01$  or  $P < 0.05$ , respectively*



**Fig. 5** Relationships between vine yield and the average June to September midday ( $\Psi_s^{md}$ ) or early morning ( $\Psi_s^{em}$ ) stem water potential. Values are average mean per treatment replicate pooling data across seasons, separated in two groups according to the number of cluster per vine collected. High, when cluster collected were more

**Must and wine composition**

The effect of the year on all the must and wine composition parameters studied was highly significant (Table 6). In addition, for most of those parameters there was also a significant effect of the year by treatment interaction, suggesting that the effect of the irrigation regime on these parameters was different between seasons (Table 6).

In most of the seasons the lowest amount of must sugar concentration and alcohol content in wines was observed in the rain-fed treatment (Tables 7, 8). Particularly, the treatment 100–100–66, which received a moderate water application also after veraison, stimulated must sugar accumulation and as a consequence wine alcohol content, suggesting that the after-veraison water application probably increased the vine source capacity. These results are in agreement with previous findings also obtained in Tempuranillo in the north of Spain (García-Escudero et al. 1994;

than 20 (seasons 2000, 2002 and 2004); Low, when clusters per vine were less than 20 (seasons 2001 and 2003). *Double asterisks, single asterisk and n.s. indicate significant linear trend at  $P < 0.01$ ,  $P < 0.05$  or non significant, respectively*

**Table 5** Leaf area to yield and yield to pruning weight ratio of the different treatments during each season

Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Leaf area:yield ( $m^2 kg^{-1}$ )	2000	0.8	0.6	1.0	0.6	0.8	0.7	0.6
	2001	1.9	2.1 <sup>J</sup>	2.2	1.5	1.7	1.7	1.5
	2002	1.2	1.2	1.4	1.2	0.9	1.0	1.0
	2003	2.2	–	1.9	–	1.5	–	1.8
	2004	1.0	–	1.1	–	0.8	–	0.9
Yield:Pruning weight ( $kg kg^{-1}$ )	2000	10.8	12.5	11.3	11.9	13.6 <sup>V</sup>	14.5 <sup>Z</sup>	11.7
	2001	3.9	3.1	2.5*	4.2	3.6	3.6	3.3
	2002	8.1	7.2	7.8	7.1	7.8	8.6	7.8
	2003	4.2	–	3.2	–	3.7	–	3
	2004	5.7	–	5.5	–	7.2*	–	6.4

\* Differences among irrigation treatments and the control (non-irrigated, 0–0–0) based on Dunnett’s *t* test at  $P < 0.05$

<sup>J,V,Z</sup> Significant difference at  $P < 0.05$  in the 0–66–0 vs 100–66–0, 100–66–0 vs 100–100–33 and 100–100–33 vs 100–100–66 contrast, respectively



**Table 6** Effects of the irrigation treatment on the source variation among the different factors and their interactions

Variable	Treat	Block <sup>a</sup>	Year	Treat × block	Year × block	Treat × year
Must soluble solids	0.118	0.012	<0.001	0.381	0.010	<0.001
Must titratable acidity	<0.038	0.0156	<0.001	0.976	0.1766	<0.001
Must tartaric acid	<0.001	0.001	<0.001	0.9223	0.371	0.034
Must malic acid	<0.001	<0.001	<0.001	0.020	<0.001	<0.001
Wine alcohol	0.020	0.487	<0.001	0.412	0.026	0.002
Wine titratable acidity	0.205	0.092	<0.001	0.604	0.077	0.009
Wine pH	<0.001	0.142	<0.001	0.175	0.037	0.284
Wine tartaric acid	<0.001	0.263	<0.001	0.046	0.098	0.071
Wine malic acid	0.001	0.097	<0.001	0.255	0.023	0.002
Wine total phenolics	0.003	0.472	<0.001	0.174	0.025	0.004
Wine anthocyanins	0.011	0.172	<0.001	0.222	0.055	<0.001
Wine color intensity	<0.001	<0.001	<0.001	0.377	0.244	<0.001

<sup>a</sup> *P* value of Blocks and its interaction refer to the hypothesis of zero variance

**Table 7** Parameters of must quality of the different treatments during each season

Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Total soluble solids (°Brix)	2000	20.1	20.7	21.2*	20.4	21.9*	21.8*	21.9*
	2001	22.4	24.4* <sup>J</sup>	23.8*	23.5	22.9	23.0	23.2
	2002	21.9	21.5 <sup>W</sup>	21.5	21.5	20.4	19.8 <sup>Z</sup>	21.4
	2003	21.6	–	21.8	–	21.7	–	22
	2004	19.7	–	20.7*	–	20.2	–	21.4*
Titratable acidity (g l <sup>-1</sup> tartaric acid)	2000	4.8	4.1	3.9*	4.4	4.0*	3.9*	3.9*
	2001	3.6	3.8 <sup>J</sup>	3.8	3.8	3.9*	4.0*	4.1*
	2002	4.7	5.1* <sup>J,W</sup>	5.2*	5.2*	5.4*	5.3*	5.3*
	2003	4.7	–	4.2	–	4.5	–	4.5
	2004	5.7	–	6	–	6.3	–	6.3
Tartaric acid (g l <sup>-1</sup> )	2000	9.1	8.1*	7.7*	8.1*	7.6*	7.7*	7.3*
	2001	4.6	4.6	4.5	4.5	4.5	4.5	4.4
	2002	4.3	4.5	4.5	4.4	4.5	4.7* <sup>Z</sup>	4.4
	2003	4.7	–	4.5	–	4.5	–	4.5
	2004	6.3	–	6.1	–	6.3	–	6.4
Malic acid (g l <sup>-1</sup> )	2000	1.2	1.4	1.4	1.6* <sup>W</sup>	1.5*	1.5*	1.4*
	2001	1.0	1.2* <sup>X,J</sup>	1.2*	1.4*	1.4*	1.5*	1.5*
	2002	1.1	1.3 <sup>X,J</sup>	1.4*	1.5*	1.5*	1.6*	1.6*
	2003	1.0	–	1.2*	–	1.4*	–	1.4*
	2004	1.4	–	1.6	–	1.6	–	1.8*

\* Significant differences among irrigation treatments and the control (non-irrigated, 0–0–0) based on Dunnett's *t* test at *P* < 0.05

<sup>J,W,Z,X</sup> Significant difference at *P* < 0.05 in the contrasts between 0–66–0 vs 100–66–0, 100–33–0 vs 100–66–0, 100–100–33 vs 100–100–66 and 0–66–0 vs 0–100–0, respectively

Sipiora and Gutiérrez-Granda 1998) but they are in contrast with results obtained in our area with cv. Bobal, that showed that after veraison irrigation cutoff did not impair berry sugar accumulation (Salón et al. 2005).

The year-to-year effect of irrigation on must and wine acidity varied, probably because of the different environmental conditions and crop levels. For instance, in 2000, irrigation decreased must acidity, most likely

because the rain-fed berries were less ripen due to the very dry season and large crop demand. On the other hand, in 2001, and particularly in 2002, must from irrigated wines was more acid mainly because of the much larger concentration of malic acid. This organic acid is the main one contributing to changes of acidity (McCarthy et al. 1983; Romero et al. 1993), and temperature is the main environmental factor affecting its evolution and

**Table 8** Parameters of wine quality of the different treatments during each season

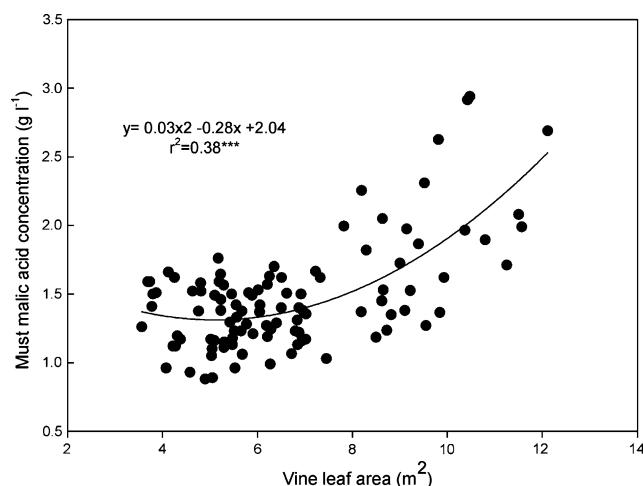
Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Wine alcohol (% Vol)	2000	10.8	11.2	12.5	11.4	12.4	12.3	12.8*
	2001	13.8	14.4 <sup>J</sup>	14.3	14.0	13.6 <sup>V</sup>	14.1	13.7
	2002	11.7	12.7	13.0	13.5 <sup>*W</sup>	12.4 <sup>Y</sup>	11.3 <sup>Z</sup>	12.9
	2003	12.4	–	12.4	–	12.3	–	12.7
	2004	10.9	–	11.0	–	10.6	–	11.5
Titrateable acidity (g l <sup>-1</sup> tartaric acid)	2000	5.5	5.5	5.4	5.5	5.3	5.3 <sup>Z</sup>	5.6
	2001	5.8	5.5	5.5*	5.7	5.6 <sup>V</sup>	5.3 <sup>*Z</sup>	5.7
	2002	6.5	6.1	5.9	6.1	5.8 <sup>*V,Y</sup>	7.3*	7.1*
	2003	5.0	–	4.9	–	5.1	–	4.8
	2004	6.8	–	6.6	–	6.8	–	6.8
Wine pH	2000	3.58	3.61	3.69	3.66	3.73	3.68	3.69
	2001	3.72	3.83*	3.82*	3.91*	3.91*	3.90*	3.80*
	2002	3.70	3.83	3.80	3.84	3.83	3.69 <sup>Z</sup>	3.84
	2003	3.76	–	3.89	–	3.85	–	3.94
	2004	3.26	–	3.40*	–	3.44*	–	3.47*
Tartaric acid (g l <sup>-1</sup> )	2000	2.4	2.0*	1.8*	2.1	2.0*	2.0*	2.0*
	2001	2.5	2.1*	2.2*	2.1*	2.0*	1.9 <sup>*Z</sup>	2.2*
	2002	2.8	2.4	2.3	2.2*	2.2*	2.6	2.2*
	2003	3.3	–	2.8*	–	2.7*	–	2.7*
	2004	3.8	–	3.1*	–	3.0*	–	2.7*
Malic acid (g l <sup>-1</sup> )	2000	1.4	1.4	1.6	1.7	1.8	1.7	2.0*
	2001	2.2	2.4	2.2	3.0*	2.9	–	2.9
	2002	1.7	2.1*	2.3*	2.3*	2.3*	2.2 <sup>*Z</sup>	2.5*
	2003	1.4	–	1.7	–	2.3	–	1.6
	2004	2.0	–	2.4	–	2.4	–	2.6

\* Significant differences among treatments and the control (non-irrigated, 0–0–0) based on Dunnett's *t* test at  $P < 0.05$

J,V,W,Y,Z Significant differences at  $P < 0.05$  in the 0–66–0 vs 100–66–0, 100–66–0 vs 100–100–33, 100–33–0 vs 100–66–0, 100–66–0 vs 100–100–66 and 100–100–33 vs 100–100–66 contrasts, respectively

concentration in berries (Hale 1977). Irrigated vines had more vegetative growth, which probably reduced cluster exposure to direct solar radiation and therefore cluster temperature-conditions favorable for the retention of malic acid. Pooling data across seasons there was a significant relationship between must malic acid concentration and vine leaf area (Fig. 6). Malic acid concentration increased when vine leaf area values were above 8 m<sup>2</sup>, equivalent to a leaf area index of 1.3. Overall these results are in agreement with previous reports (Buttrose et al. 1971; Smart et al. 1985; Sepúlveda and Kliever 1986) that related acid content with temperature, and with the higher rate of malic acid degradation in non-irrigated vines because of less cluster shading by leaves.

Similarly to what reported for the must organic acid concentrations, malic acid also increased in the wines of the more irrigated treatments. The opposite behavior was observed for the tartaric acid concentration in the wines that decreased with irrigation. Given that malic is a weaker acid than tartaric, the overall effect of irrigation on wine pH was to increase it. This has been also previously



**Fig. 6** Relationships between must malic acid concentration and vine leaf area. Values are average mean values per treatment replicate pooling data across seasons

reported in other studies (Freeman and Kliever 1983), and might be detrimental to sanitary and aging stability of the wines made from the irrigated vines.

**Table 9** Parameters of wine quality of the different treatments during each season

Parameter	Year	0–0–0	0–66–0	0–100–0	100–33–0	100–66–0	100–100–33	100–100–66
Total phenolics index (AU)	2000	46	44	52	46	50	48	46
	2001	74	77 <sup>J</sup>	75	73 <sup>W</sup>	66	67	63*
	2002	53	61 <sup>J</sup>	60	56 <sup>W</sup>	51 <sup>Y</sup>	47 <sup>Z</sup>	57
	2003	50	–	47	–	47	–	45
	2004	49	–	47	–	47	–	50
Anthocyanins (mg l <sup>-1</sup> )	2000	335	359	440	382	438	414	425
	2001	847	885 <sup>J</sup>	894	832 <sup>W</sup>	749	755	680*
	2002	507	528	605	640 <sup>W</sup>	503	450	523
	2003	487	–	428	–	419	–	409*
	2004	448	–	434	–	438	–	489
Color intensity (AU)	2000	8.9	8.5 <sup>X</sup>	11.6	9.3	10.6	10.3	10.0
	2001	14.6	14.6 <sup>J</sup>	13.3	11.8	11.0*	10.0*	10.9*
	2002	12.1	12	12.8	12.6 <sup>W</sup>	10.7	9.9	11.3
	2003	8.8	–	7.5	–	7.3	–	6.6
	2004	8.9	–	7.6	–	7.3	–	8.0

\* Significant differences among irrigation treatments and the control (non-irrigated, 0–0–0) based on Dunnett's *t* test at  $P < 0.05$

<sup>J,W,Y,Z,X</sup> Significant differences at  $P < 0.05$  in the 0–66–0 vs 100–66–0, 100–33–0 vs 100–66–0, 100–66–0 vs 100–100–66, 100–100–33 and 100–100–66 and 0–66–0 vs 0–100–0 contrasts, respectively

In most of the seasons irrigation, event at the highest rate, did not impair wine phenolic content, anthocyanin concentration and wine color (Table 9). Overall these results are in clear disagreement with previous observations in cv. Bobal growing in the same area (Salón et al. 2005). In that case, a clear detrimental effect of irrigation on wine phenolic content and color intensity was obtained, and wine color and anthocyanin concentration were closely and negatively related to the water stress integral. The contrasting response to irrigation observed for both cultivars may in part be attributed to their different area of origin and tolerance to drought. Bobal is a local cultivar well adapted to the area, while Tempranillo is originally from a cooler region in Spain and is reputed to be sensitive to water stress, and prone to early leaf senescence (Gómez del Campo et al. 2000). Nonetheless, we cannot rule out the possible influences of other factors imposed in our experiments, particularly the training method (open vase vs. vertical trellis; Smart 1985). These aspects merit future study.

In any case it should be noted that in 2000, when crop load and water stress experienced by rain-fed vines were high, it seems that irrigation helped to ripen the crop, as indicated by the increasing trend in wine anthocyanins concentration and color with irrigation. In 2001 and 2003 instead, when crop load was very low, irrigation, particularly at the highest level, had a somewhat detrimental effect on wine color and anthocyanins concentration. This suggests that the vine response to irrigation might well be different according to its crop level as has been reported for other grapevine cultivars (Bravdo et al. 1984; Poni et al. 1994b).

Interestingly, in 2001 and 2002, there were significant differences in wine phenolics between 100–33–0 and 100–66–0 treatments (Table 9) that only differed in their water application during the fruit set to veraison period. It seems that a more severe water restriction during this period increased wine total phenolics, anthocyanins concentration and color. At least in 2001, this could be the consequence of a dilution effect, but in 2002 berries from treatment 100–33–0 were not smaller than the 100–66–0 ones (Table 4). It seems then that the less water applied to the 100–33–0 treatment promoted the synthesis of phenolic pigments in berries, what is in agreement with recent reports (Castellarini et al. 2007) that showed that even before veraison water stress increased the expression of genes involved in the synthesis of anthocyanins in grape berries. Further research is needed to better investigate the suitability of applying before veraison water deficits under our soil and environmental conditions, as often recommended in the Australian viticulture (Dry et al. 2001).

## Conclusions

Much larger differences in vine performance and fruit and wine composition were obtained among seasons than among treatments within a season. This supports the need of conducting multi-year studies when analyzing the effects of irrigation practices under field conditions. Our results suggest that moderate irrigation supplies (50–85 mm) might benefit yield (+12–20%), without any severe detrimental effect on fruit and wine composition.

Particularly water application after veraison is beneficial to fruit ripening. Our results also show that in order to increase the concentration of phenolic substances in wines, water stress should be applied during the pre-veraison period. Crop level seems to be another major determinant of vine performance and wine quality to supplemental irrigation. In this sense vines with higher yield seem to benefit more of irrigation both in terms of productivity and of fruit composition. However, to further corroborate these findings, more genuine interactions between crop level and irrigation regimes should be studied in a multi-factor (deficit irrigation  $\times$  crop level) trial.

**Acknowledgments** This research was supported by funds from the Generalitat Valenciana, Consellería de Agricultura, Pesca y Alimentación, Project N° 2002TAHVAL0034, from CICYT, Project N° 1FD1997-1276 and Rideco-Consolider CDS2006-0067. We are grateful to the STR personnel for the meteorological data, to Caja Campo for help in the field determinations and to Dr. E. Carbonell and J. Pérez for help with the statistical analysis of data. Thanks are also due to the “Estación de Viticultura y Enología, Requena” for the vinifications and to the Fundación Lucio Gil de Fagoaga for allowing this research activity in its vineyard.

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