

**Delaying berry ripening of Bobal and Tempranillo grapevines by late leaf removal  
and irrigation in a semi-arid and temperate-warm climate**

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## ABSTRACT

**Background and Aims:** Climate change is advancing grape ripening, decoupling berry technological and phenolic composition and negatively impacting wine quality. The aim for this study was to test the usefulness of late leaf removal (LLR) under different watering regimes to delay harvest in two Spanish red cultivars in a semiarid and temperate-warm climate. The effects on vine physiology, yield, grape and wine composition were investigated.

**Methods and Results:** In two trials carried out in eastern Spain with Bobal and Tempranillo cultivars, vines were partially defoliated above the cluster zone shortly before veraison under rainfed and deficit irrigation conditions during two seasons. Grape ripening rate was significantly affected by the watering regime and canopy management in both cultivars. Vine water status and photosynthesis was improved by irrigation and LLR. However, the treatments harvested later, did not have an overall positive effect on grape nor on wine composition. The resultant leaf area-to-fruit ratios of the defoliated treatments appeared to be limiting for both sugars and anthocyanins accumulation. As a consequence, LLR negatively affected wine colour intensity. In addition, yield was constrained by LLR in Tempranillo due to reductions in cluster and berry mass.

**Conclusions:** A reduction in grape sugars accumulation provoked by LLR did not necessarily implies a better coupling between berry technological and phenolic maturity. The effectiveness of the LLR technique seems to depend on its final impacts on leaf area-to-fruit ratio and vine water status, the vine photosynthetic compensation capacity of the cultivar and the environmental conditions.

**Significance of the Study:** Under low vigour vine conditions, severe defoliation cannot be recommended as a strategy to balance grape sugars and phenolic composition, highlighting the importance of applying defoliation at the right time and intensity.

**Keywords:** global warming, technological and phenolic ripeness, vine yield, *Vitis vinifera*

## INTRODUCTION

Climate is a major component of wine grape terroirs (Castel et al. 2012, Hannah et al. 2013). Mediterranean viticulture could suffer from warmer and drier growing seasons over the coming decades (Lereboullet et al. 2013). Indeed, wine grapes are considered to be more vulnerable to climate change compared to other crops, because most of the added value of the final product is provided by the desired wine style that depends on grape composition at harvest (Jones and Webb 2010).

High air temperatures induces an increase in sugar accumulation rate in the berries (Petrie and Sadras 2008), leading to higher alcohol concentrations in wine or earlier harvest dates (Jones et al. 2005b, Koufos et al. 2014, Cook and Wolkovich 2016). Others authors have also documented the shortening of vine phenological cycle in response to an increase in ambient temperature (Duchêne and Schneider 2005). In addition, Sadras and Moran (2012) observed that high temperature decouples the synthesis of sugars and anthocyanins of Shiraz and Cabernet Franc grown in South Australia.

Global warming increases vapour pressure deficit and vine potential evapotranspiration, altering soil and plant water relations (Moratiel et al. 2010, Schultz 2016, Dr. Marcos Bonada et al. unpublished data, 2018). For instance, Duchêne and Schneider (2005) observed increasing trends in vine evapotranspiration demand after flowering over thirty years in Alsace, France. Furthermore, the higher probability of heat waves and droughts events (IPCC, 2014) will increase the frequency and severity of plant water stress (Gambetta 2016). In this regard, irrigation management (Salón et al. 2005, Buesa et al. 2016), canopy trellising systems (Baeza et al. 2005, Kliewer and Dokoozlian 2005), artificial shading (Kliewer et al. 1967, Caravia et al. 2016) and/or source-to-sink ratio reductions (Palliotti et al. 2013, Stoll et al. 2010) may delay ripening to occur during cooler periods of the year. Practices such as pruning, trimming and leaf removal can be certainly employed to manipulate vine source-sink balance (Santesteban et al. 2017, Caccavello et al. 2017, Moran et al. 2017).

Leaf removal applied late in the season (near veraison) tends to have limited effect on fruit yield while it postpones grape ripening (Palliotti et al. 2013, Caccavello et al. 2017). For instance, Intrieri et al. (2017) removing 30-40% of total vine leaf area achieved an increase in berry anthocyanin concentration in Sangiovese vines when harvest date was delayed of 7-8 days. Poni et al. (2013) in the same cultivar, applied LLR at veraison delaying the technological ripeness without affecting colour and phenolics. Lanari et al. (2013) found in Montepulciano and Sangiovese grapevines in terms of phenolic compounds. Therefore, it appears that the response to LLR may vary depending on the cultivar genotype.

In semi-arid climates, the effect of LLR can be different depending on the irrigation regime, because of the significant effects of watering applications on vine vigour and berry growth and development (Jackson and Lombard 1993, Risco et al. 2014). Moreover, irrigation management could also partially restore the disruption caused by high temperature on the anthocyanins-to-sugars ratio if a water deficit is applied shortly before veraison (Sadras and Moran, 2012). Pre-veraison water stress may lead to an increased must concentration of total phenolics and anthocyanins because it can stimulate anthocyanin biosynthesis in berry skin (Castellarin et al. 2007, Santesteban 2011) and it can induce an increase in the skin-to-pulp ratio (Ojeda et al. 2002, Intrigliolo and Castel 2010). Similarly, post-veraison water stress promotes higher concentrations of phenolics in berry skins and in addition, it may cause a decrease in berry sugar accumulation in Tempranillo (Esteban et al. 2001, Intrigliolo et al. 2012). Nevertheless, severe post-veraison may be detrimental to anthocyanin accumulation (Girona et al. 2009, Romero et al. 2010). Sal3n et al. (2005) and Intrigliolo and Castel (2011) reported that maintaining midday stem water potentials ( $\Psi_{\text{stem}}$ ) above specific thresholds (-1.2 and -1.5 MPa in Bobal and Tempranillo, respectively) induced an increase in total anthocyanin concentration and colour intensity in the must and wine. Nonetheless, its response to irrigation during post-veraison differed in terms of yield, berry growth and composition, and wine quality parameters. Hence, we hypothesised that the application of a moderate degree of vine water stress before veraison, namely midday  $\Psi_{\text{stem}}$  ranging from -0.95 to -1.15 MPa, would be effective in increasing the skin-to-pulp ratio, while avoiding overly severe water stress after veraison (midday  $\Psi_{\text{stem}} > -1.4$  MPa) may slow down sugar accumulation leading to higher anthocyanins-to-TSS ratio at harvest.

In this trial we assessed the effects of late leaf removal (LLR) on the ripening dynamics and yield components of these two Spanish red cultivars in a semiarid and temperate-warm climate under different watering regimes (WR). To the best of our knowledge, all previous studies have in fact determined the effects of LLR within a given watering regime, and under cooler and more humid areas (Pallioti et al. 2013, Poni et al. 2013, Lanari et al. 2013, Caccavello et al. 2017, Intrieri et al. 2017). Our working hypothesis was that LLR technique could delay fruit ripening alleviating grape heat stress, while irrigation effects could interact differently depending on the cultivar. In this context, we were concerned about the photosynthetic compensatory response to LLR under different WR. Particularly, we investigated on Tempranillo and Bobal cvs. if LLR applied under both deficit irrigation and rainfed conditions could improve grape and wine anthocyanins content at harvest.

## MATERIALS AND METHODS

## Site and crop

The trials were carried out in 2014 and 2015 in a commercial grapevine (*Vitis vinifera* L.) farm located near Requena, Valencia, Spain (39°30'18.10"N, 1°13'54.30"W; elevation 700 m a.s.l.). The experiments were conducted in two adjacent vineyards planted, respectively, with cv. Bobal (grafted onto 110 Richter) and cv. Tempranillo (grafted onto 161-49 Couderc) vines. Vines were trained to a bilateral cordon system leaving six or ten two-bud spurs per vine in the Bobal and Tempranillo vineyard, respectively. Shoots were trained vertically using \*\*\* pairs of steel catch wires. The Bobal vineyard was planted in 2002 at a spacing of 2.5 x 1.4 m (2857 vines ha<sup>-1</sup>), whereas Tempranillo vineyard in 1991 at 2.5 m x 2.45 m (1633 vines ha<sup>-1</sup>). In both vineyards, rows were oriented North-South. The two plots were independently deficit irrigated with two drippers per plant for more than ten years before the experiments started.

The soil of the vineyard was a Typic Calciorthid with a clay-loam to light clay texture, highly calcareous and of low fertility. Soil depth was higher than 2 m and available water capacity was about 200 mm m<sup>-1</sup>. The climate in this area is continental Mediterranean and semiarid, the Huglin heliothermal index (REF) is 2291 °C corresponding to a temperate warm viticultural climate, with cool nights and moderately dry according to the classification system for grape-growing regions proposed by Tonietto and Carbonneau (2004). At the experimental site, the annual average values (for the last 12 years) of the reference evapotranspiration (ET<sub>o</sub>) and the rainfall were 1127 and 380 mm respectively. About 65% of precipitation generally occurs in winter.

## Sources of variation

Four treatments were tested in each trial, obtained combining two types of watering regimes (WR, Irrigated or Rainfed) and two types of canopy managements (CM, Defoliated or Undeoliated). The treatments applied were: Irrigated-Undeoliated, IU; Irrigated-Defoliated, ID; Rainfed-Undeoliated, RU; Rainfed-Defoliated, RD (Table 1). Treatments had three and four replicates in the Bobal and Tempranillo trial, respectively. In both cases, the experimental design was a randomized block design, where WR was assigned to the main plot and CM to subplots. The vines located in the surrounding perimeter of the plots were used as borders. Each subplot or experimental unit (EU) consisted of a row of ten and seven vines in the Bobal and Tempranillo trial, respectively.

Deficit irrigation was applied to maintain midday  $\Psi_{\text{stem}}$  above the threshold values of -1.15 and -1.40 MPa in pre- and post-veraison, respectively. Late leaf removal was performed manually at the onset of berry ripening (Phase III of berry development), corresponding to phenological stage number 79-81 in the BBCH-scale (Lorenz et al. 1995). The goal was to reduce vine

photosynthetic capacity at the beginning of berry ripening process. Defoliation consisted of removing all the apical mature leaves of the main shoots and removing lateral shoots starting from the second node above the clusters (only the leaves at the top of the shoot were retained, Panel A). This was done because, around veraison, leaves located in the apical two-thirds of the shoots are considered to be the most photosynthetically active (Kliewer and Antcliff 1970, Poni et al. 1994). Shoot tips were preserved in order to allow vegetative competition for photoassimilates with grapes (McCarthy 1997). Clusters zone was left unchanged by the defoliation.

#### **Field determinations**

During the experiment, weather data were hourly measured with an automated meteorological station located at the vineyard. Reference evapotranspiration (ET<sub>o</sub>) was calculated with the Penman–Monteith equation (Allen et al. 1998), and the accumulation of growing degree days (GDD) was computed as the sum of the average daily temperature above a threshold 10 °C from 1 April until harvest (Amerine and Winkler 1944). The amount of water applied with irrigation was measured with an on-line water meters. Midday  $\Psi_{\text{stem}}$  was measured, respectively, on six and four dates in 2014 and 2015 with a pressure chamber (Model 600, PMS Instrument Company, USA) on bag-covered leaves from two representative vines per EU at midday (measurements were carried out between 11:30 and 12:30 solar time). Leaves used for these measurements were located on the west side of the row and were enclosed in hermetic plastic bags covered with aluminium foil for at least 1 hour prior to the measurements. In addition, on the last three dates, net CO<sub>2</sub> assimilation rate, transpiration rate and stomatal conductance were measured (between 10:00 and 13:00 solar time) on two basal, mature, sun-exposed leaves per vine with a portable gas exchange analyser (LCpro+, ADC BioScientific Ltd., England).

Fruit yield, number of clusters per vine, average cluster weight and shoot fruitfulness (number of clusters per shoot) were determined at harvest on each experimental vine. Additionally, one cluster per vine was randomly sampled and frozen at harvest in order to calculate the number of berries per cluster. In 2016, when treatments were not applied, vines were assessed to evaluate possible carry-over effects of the two consecutive experimental seasons on vine performance and shoot fruitfulness. Pruning fresh mass was weighted in samples of four vines per EU in Bobal and three in Tempranillo (in 2015 in the latter vineyard this measurement was not done because a mechanical pre-pruning was carried out by the vineyard owner).

External leaf area (LA<sub>ext</sub>) per vine was determined by photographic analysis by means of an image processing software (ImageJ 1.47v, National Institutes of Health, USA) following the methodology described by Schneider et al. (2012). The pictures were taken once shoot growth had ceased, just after the late leaf removal, in one side of the canopy hedge with a background curtain using a visible light camera (Ixus 220 HS, Canon Inc., Tokyo, Japan). Additionally, on

one representative vine per EU and cultivar, total leaf area ( $LA_{\text{measured}}$ ) was estimated using allometric relationships computed for each cultivar between shoot length and leaf area per shoot measured with LI-3100 Area Meter (LI-COR Inc. Lincoln, Nebraska, USA). These relationships were obtained separating main and lateral shoots, using samples of 12 shoots of different vigour. The significant  $LA_{\text{ext}}$  and  $LA_{\text{measured}}$  regression equations were used to estimate leaf area (LA) in each experimental vine. Leaf area index (LAI) was calculated as the LA per unit ground surface area. The leaf area removed of the selected vines was measured with LI-3100 Area Meter.

#### **Grape and wine composition**

Berry ripening evolution was assessed approximately every 10 days, starting from the day before LLR was performed until harvest, except for phenolic composition, which was determined only after veraison. Berry fresh mass was determined from random samples of 50 berries per EU. Thirty berries were crushed and hand pressed through a metal screen filter and used to evaluate technological maturity, whereas 20 berries were homogenised with a blender (Ultraturrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany) and used for phenolic maturity. Must total soluble solids (TSS) were determined by refractometry (PR-101, Series Palette, Atago Co, LTD, Japan), pH and titratable acidity (TA) were measured with an automatic titrator (Metrohm, Herisau, Switzerland). Juice was titrated with a 0.1 N solution of NaOH to an end point of pH 8.2, and results were expressed in tartaric acid equivalents. In order to assess the effects of the treatments on berry ripening, the TSS to TA ratio was calculated as a maturity index by dividing total sugars content ( $\text{g L}^{-1}$ ) by the TA ( $\text{g L}^{-1}$ ) at harvest (Al-Kaisy et al. 1981). Tartaric and malic acid concentrations were measured only at harvest with an infrared analyser (Bacchus II, Tecnología Difusión Ibérica S.L.). Total anthocyanins and polyphenols (expressed in malvidin equivalents) were determined in duplicate by ultraviolet/visible spectrophotometry (Iland et al. 2004).

Harvest was carried out when each treatment reached specific target TSS level that were defined in each cultivar and season, and therefore, it was performed on different dates depending on the treatment. In the first season the TSS target corresponded to full berry maturity for both cultivars (25 °Brix), whereas in 2015, with the aim to increase comparability among treatments, the TSS goal was set at 22 and 20 °Brix for Tempranillo and Bobal, respectively. The grapes of each EU were separately vinified at the experimental winery. Thus, on each season, 12 and 16 vinifications were performed for Bobal and Tempranillo, respectively. Grapes were mechanically crushed, destemmed and fermented at a temperature of approximately 22 °C in stainless steel containers of 60 L. All musts were added with five grams of  $\text{SO}_2$  and inoculated with 20 g of commercial *Saccharomyces cerevisiae* yeast per 100 kg of grapes (FR Excellence, Lamothe-Abiet). Skin contact time was seven days and during this period they were punched down daily.



After alcoholic fermentation was ended, the wines were pressed and decanted in 30 L demijohns. The length of wine storage was the same for all treatments and years. Wine composition analyses were carried out after the spontaneous malolactic fermentation ended (approximately six months after the grapes were crushed). Phenolic composition was determined measuring the optical density (OD; nm) by spectrophotometric methods (Ati-Unicam UV-4) as described by Ribereau-Gayon et al. (2000); anthocyanins in HCl media ( $OD_{520}-OD_{860}$ ), total polyphenols index (TPI) ( $OD_{280}-OD_{860}$ ), wine colour intensity ( $OD_{420}+OD_{520}+OD_{620}-OD_{860}$ ) and hue ( $OD_{420}/OD_{520}$ ). Wine technological composition was assessed by infrared analyser. All analytical determinations in grape, must and wine were done in duplicate.

## Data analysis

Data from the two trials (Bobal and Tempranillo) were analysed separately because vines had different ages, rootstocks and vine spacing. For each cultivar, two-way analysis of variance (ANOVA) was used to test the effect of WR, CM and WR×CM interaction on vine traits and grape composition. In case the ANOVA detected significance effects ( $P<0.05$ ), mean separation was assessed either by the Duncan multiple range test (when data followed a normal distribution) or the Kruskal-Wallis procedure of “Statgraphics Centurion XVI” package (version 16.0.07). For wine composition analysis, two-way ANOVA was undertaken using the alcohol content as a covariate, because significant linear relationships between alcohol content and colour intensity, anthocyanins and TPI were found.

## RESULTS

### *Climatic conditions and irrigation applications*

During the experimental seasons, from April 1<sup>st</sup> to September 30<sup>th</sup>,  $ET_o$  was 946 and 920 mm, the Winkler index was 1892 and 1939, and the rainfall was 96 and 203 mm in 2014 and 2015, respectively (Figure 1). In 2015, rainfall during spring was noticeably higher compared to the first season, and this retarded the start of irrigation in 2015. Over the two seasons, the average fraction of  $ET_o$  received by rainfall and irrigation in Bobal and Tempranillo vineyards was 34% and 28%, respectively (Table 1).

During the berry-ripening periods (August-September), the average maximum air temperatures were over 33 °C. In the Tempranillo trial, the maximum air temperature during the week prior to harvest for the treatments harvested later was 3 °C lower than in the treatment picked earlier (IU). In the Bobal trial, this difference reached up to 7 °C. On the other hand, the average



of minimum air temperatures during the week before harvest was for all treatments and seasons below 18 °C (Figure 1).

#### *Vine phenology and vegetative growth*

Vine phenology until veraison was similar among treatments within each trial. Indeed, no differences among treatments in the date of budburst, bloom or veraison were found in both cultivars. In both trials, the number of shoots per vine did not differ among treatments and between years, because the seasonal pruning strategy (dormant pruning and early shoot thinning) employed was the same ( $19 \pm 2$  and  $10 \pm 2$  shoots per vine in Tempranillo and Bobal, respectively). LLR was performed in Bobal on the day of year (DOY) 212 and 211 in 2014 and 2015, respectively; and on DOY 210 and 209 in Tempranillo in 2014 and 2015, respectively. This corresponded to 10-12 days before veraison (BBCH 83), when the berry TSS were around 9 °Brix.

Pooling the data across seasons, average LA removed per vine represented 30% and 27% of the total vine LA at that moment in Bobal and in Tempranillo, respectively (Table 1). After LLR was applied, vegetative growth was very negligible, probably because shoot tips growth (both on the main and lateral shoots) was limited by the moderate water stress experienced by the vines. Lateral shoot regrowth was not observed also in the defoliated vines (LLR treatments). In both cultivars, LAI after leaf removal was significantly lower in the defoliated vines compared to that of undefoliated vines independently of the WR treatment (Table 2). Irrigation induced a significant increase in LAI in the driest year (2014). On the other hand, the pruning fresh mass per vine was significantly increased by irrigation in both cultivars, while LLR did not induce any significant response on this parameter (Table 2). It should be reported that visual difference in basal leaf senescence was observed between treatments, it started earlier in the non-defoliated vines probably due to the higher proportion of basal-to-young leaves in undefoliated compared to in LLR vines.

#### *Vine water status and leaf assimilation rates*

Vine water status was significantly affected by watering regime (WR) and canopy management (CM) in both trials and seasons. The seasonal pattern of midday  $\Psi_{\text{stem}}$  revealed significant differences among irrigated and rainfed treatments in both cultivars (Figure 2). Bobal vines manifested higher differences between irrigated and rainfed vines compared to Tempranillo vines. In addition, in the Tempranillo trial in 2015, the observed differences in vine water status between WR treatments were not very noticeable. Interestingly, in both trials, the defoliated vines showed significantly less negative midday  $\Psi_{\text{stem}}$  values than the undefoliated vines independently of the WR, and therefore LLR improved vine water status. This effect was statistically significant in both cultivars for each WR on both years. Despite the severe water stress suffered by rainfed

vines in both trials and seasons, with minimum values of midday  $\Psi_{\text{stem}}$  of almost -1.5 MPa, no clear leaf abscission symptoms were observed, even in Tempranillo vines which are more prone to early basal leaf senescence in response to high soil water deficit.

In both trials WR and CM treatments significantly affected leaf stomatal conductance (gs), transpiration (E) and photosynthesis (A) (Figure 5). Significant reductions were observed in gs, E and A in rainfed vines compared to irrigated vines, and in un-defoliated (Control) vines compared to LLR vines. The observed effects were always statistically significant in the Bobal vineyard. In the Tempranillo plot, the differences among treatments were relatively smaller and, thus, in some cases, were not statistically significant. Irrigation negatively affected intrinsic water use efficiency (A/gs), whereas LLR did not have any significant effect on this physiological parameter (ranging from 50 to 62  $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$  in Tempranillo and 46 to 60  $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$  in Bobal, on seasonal average).

#### *Yield components*

The effect of the watering regime (WR) on fruit yield was similar in both trials (Bobal and Tempranillo), whereas the effect of canopy management (CM) varied depending on the trial (Table 2). In the two vineyards, the effect of the interaction between the two experimental factors (CM and WR) was not significant for most of the yield components. In Tempranillo, both WR and CM affected fruit yield significantly, whereas in Bobal only WR influenced significantly yield. In both trials, fruit yield increased significantly in response to irrigation, with seasonal average increments of 47% in Bobal and 24% in Tempranillo compared to rainfed vines. Yield increase was mainly due to increments of cluster and berry fresh mass, with the only exception of Tempranillo in 2015, when the effects of WR on these parameters were less pronounced. In 2015 in the Tempranillo trial, the yield increment in response to irrigation was mainly due to the positive effect of previous season irrigation application on the number of clusters per vine. In 2015 in the Bobal trial, similar increments due to irrigation were observed in the shoot fruitfulness. In addition, this effect was also observed in 2016, the year following the end of the trials (Table 2). The number of berries per cluster were not affected by the WR or CM (ranging from  $77 \pm 4$  and  $172 \pm 12$  in Bobal, and from  $50 \pm 9$  and  $117 \pm 11$  in Tempranillo, in 2014 and 2015, respectively).

CM significantly affected cluster and berry fresh mass in the Tempranillo plot, whereas this was not the case of the Bobal trial. Pooling yield data of Tempranillo vines across seasons within the same WR, a significant reduction of 19% was observed in LLR vines compared to that of undefoliated vines. In both seasons the LLR reduced cluster fresh mass, whereas berry fresh mass was affected only in 2015. The seasonal pattern of berry fresh mass was more influenced by WR than CM (Figures 3 and 4). This was more clear in 2014, a dryer season, than in 2015.

The LA-to-fruit ratio during the ripening period was significantly higher during the first season in both trials (Table 2). In the Bobal trial, the LLR and the irrigation tended to reduce significantly the LA-to-fruit ratio. However, in the Tempranillo trial, the effect of the experimental treatments on this ratio was not fully consistent because both yield and LA were significantly constrained compared to undefoliated vines.

#### *Grape ripening*

In both trials, berry ripening was significantly affected by WR and CM treatments (Figures 3 and 4) and this was translated in differences in berry composition at harvest (Tables 3 and 4). Despite our goal to harvest grapes of all treatments at similar TSS values, in the Bobal trial this was not possible because of the occurrence of leaf senescence induced by IU and ID treatments.

The WR affected the dynamics of TSS accumulation in the berries, but these effects were opposite in the two trials. In the Bobal trial, since the very beginning of the ripening period, irrigation delayed the increase in berry TSS concentration (Figures 3C and 3D), while in the Tempranillo trial, irrigation had a slight opposite effect (Figures 4C and 4D). In both trials, rainfed treatments showed higher berry juice TA around veraison (Figures 3E, 3F, 4E and 4F), but at harvest the effect of the CM and WR on TA was less clear (Table 3). Overall, a decreasing trend of TA in response to harvest delay was perceived, whereas the opposite effect was observed in must pH. These effects were more evident in Tempranillo than in Bobal grapes. Thus, the relationship between TSS to TA during ripening was calculated to unravel the effect of the treatments on TA at equal levels of TSS (Figure 6). This elucidates that irrigation and LLR had a tendency to reduce TA, although this effect becomes less clear near harvest.

In both trials, tartaric acid concentration at harvest tended to be higher in rainfed than in irrigated vines. This effect was particularly clear in the Bobal trial (Table 3). The effect of LLR on tartaric acid concentration was inconsistent in both cultivars. In Bobal, malic acid concentrations in berry juice was affected by the irrigation application only in the first season, when IU and ID berries did not reach sugar concentration similar to RC and RD berries. In Tempranillo, neither WR nor CM had a clear effect on berry malic acid concentration. In the Bobal trial, LLR did not affect berry malic acid concentration at harvest.

In the Bobal trial, the effect of CM on phenolic composition at harvest was not consistent between seasons (Table 4). Indeed in 2014, for each watering regime LLR did not affect berry phenolic concentration, whereas in 2015 the ID treatment induced an increase in berry anthocyanins and polyphenols. The opposite effects were found under rainfed conditions (Table 4). On the other hand, in the Tempranillo trial in both seasons, the LLR treatments induced a significant decrease in anthocyanin concentration at harvest with the only exception of RD in 2014 (Table 4). In both trials, during ripening, the LLR treatments caused a decrease in

anthocyanin accumulation rate compared to undefoliated vines (Figures 3G, 3H, 4G and 4H). On the other hand, for both trials, the irrigated treatments significantly decreased anthocyanin and polyphenol concentration in the berries compared to rainfed vines. These effects were more evident in Bobal than in Tempranillo grapevines. In 2015 in the Tempranillo trial, differences among irrigation treatments in this parameter were not significant.

Anthocyanins-to-sugars ratio tended to be higher in the grapes of rainfed than irrigated vines (Table 4). This effect was less evidently in the Tempranillo trial in 2015. Leaf removal induced a decrease in this ratio in Tempranillo trial, without a significant effect in Bobal. Even if LLR vines were harvested later than undefoliated vines, grape anthocyanin concentration was lower than in the undefoliated vines. In addition, in both cultivars the anthocyanin and polyphenol content calculated on a per berry basis was unaffected by WR or even increased (data not shown). On the other hand, the LLR slightly decreased the anthocyanin content per berry in both trials. The effect on polyphenol content per berry followed a trend similar to anthocyanins, as the latter represents more than half of total polyphenols.

#### *Wine composition*

To assess treatment effects on wine composition, the alcohol content was used as a covariate aiming to analyse its influence in the extractability of phenolic components. The effects of watering regime (WR) and canopy management (CM) in wine composition were interactive (Table 5). It could be highlighted that total acidity (TA) was higher in Bobal wines made from the rainfed compared to irrigated treatments. This effect was not observed in the Tempranillo wines. In both Bobal and Tempranillo trials, LLR did not cause any seasonally consistent effect on the TA. In both trials, wine pH was similar among treatments.

LLR tended to decrease wine colour intensity (Table 5). This effect was more clear in Tempranillo, but it was also significant in Bobal in 2015. Bobal wines made in 2014 were an exception, when the RU wine had significantly lower colour than RD wine, whereas no difference was found in wine colour between IU and ID treatments. There were no effect of WR in the colour of Tempranillo wines, however irrigation decreased significantly colour intensity in wines made from Bobal grapes compared to the ones made from rainfed vines. Similar differences were found in wine anthocyanin concentration and TPI in both trials. In addition, in both trials and seasons, the hue angle of wines increased in response to LLR, with the only exception of Bobal RU wines of 2014. Irrigation also increased the hue angle of Bobal wines, while in the wines made from the Tempranillo trial this parameter was not affected by the watering regime.

## **DISCUSSION**

In both the Tempranillo and Bobal trials, late leaf removal and irrigation affected berry composition at harvest influencing grape ripening rate rather than delaying the onset of ripening (veraison). In general, vine phenology was not modified neither by canopy management (CM) nor by the watering regime (WR).

In the present trials, a delay in harvest, did not imply *per se* an improvement in the balance between technological and phenolic grape ripeness. Furthermore, in Bobal, a mid-late season maturing cultivar, the delay in berry-ripening induced by LLR, and particularly by irrigation, resulted indeed detrimental because berries could not reach the same berry technological maturity than rainfed-undefoliated (Table 3). Indeed, under the experimental conditions of the present trials, the hypothesis of higher phenolic biosynthesis at slightly cooler temperatures was not confirmed. This could be explained because the thermal threshold above which anthocyanin biosynthesis is limited or even anthocyanin degradation is enhanced was not exceeded in our study. Despite the maximum temperature at the experimental site was above 33 °C during the core of the ripening period (Fig. 1), the day-night thermal difference was in all treatments below the upper limit of the threshold for optimum skin pigment biosynthesis (Iland and Gago 2002, Mori et al. 2007, Movahed et al. 2016). However, it is also possible that the severe defoliation applied in our study limited polyphenols biosynthesis, since the increase in net CO<sub>2</sub> assimilation rate that was measured in both trials in defoliated vines did not appear to be enough to fully compensate the reduction in total vine leaf area. Indeed, on average, the defoliation induced an increment in leaf net photosynthetic rates of 25 and 13% in Bobal and Tempranillo, respectively (Fig. 5), whereas the reductions in total vine leaf area induced by LLR were always above 27%. The physiological reasons behind the increase in leaf gas exchange activity in response to defoliation are probably related to the fact that LLR promoted an alleviation of water stress, which is indeed an expected adaptive response (Petrie et al. 2003, Poni et al. 2013). Despite this improvement in vine water status (higher  $\Psi_{\text{stem}}$ ), none of the treatments stimulated the growth of shoot tips or laterals, that would have enhanced the competition for photoassimilates with the berries (McCarthy 1997, Baeza et al. 2007). However, the increase in leaf net photosynthetic rate seems to have been sufficient at least in the Bobal trial to maintain the yield levels of the defoliated treatments at similar values than the undefoliated (Table 2). This was observed even in the second experimental season, after two years of consecutive leaf pulling applications and under the higher crop levels in general registered in 2015. In the Tempranillo trial, however, the photosynthesis compensation observed in the LLR treatments did not palliate the reductions in cluster and berry fresh mass which were the main yield components affected by defoliation. Berry number per cluster was unaffected by treatments in both trials, discarding possible carry-over effects due to carbohydrate depletion. This suggests that flower formation and fruit set was primarily controlled by the environment and not by management factors, such as CM or WR. Conversely, other authors

did observe significant reductions in flowering of Chardonnay because of the influence of defoliation on carbohydrate reserves reductions (Bennett et al. 2005). In addition, this was an expected result because postponed harvest might reduce vine reserves storage because the period from harvest until leaf fall is when most of non-structural carbohydrates accumulates in vine storage organs (Williams and Smith 1991, Zufferey et al. 2012).

The experiment was not designed to elucidate the effects of irrigation regime on vine performance and grape composition, because these aspects have been the focus of an extensive body of previous research in both Bobal and Tempranillo cultivars in the same area (Mirás-Avalos and Intrigliolo 2017). However, there are still some insights to highlight. As expected, vine water status, leaf transpiration, photosynthesis and stomatal conductance were improved in response to moderate irrigation. But more interestingly, the intrinsic water use efficiency was higher in the rainfed than in the irrigated vines. In fact, the observed stomatal conductance values in the rainfed vines of both trials were similar to the optimum values suggested by Cifre et al. (2005) for increasing water use efficiency in grapevines ( $0.05 - 0.15 \text{ mol m}^{-2} \text{ s}^{-1}$ ). In both trials, even in the rainfed vines, midday  $\Psi_{\text{stem}}$  did not decrease below  $-1.5 \text{ MPa}$ , a value considered as the physiological threshold for efficient deficit-irrigation management under similar conditions (Intrigliolo and Castel 2008, Romero et al. 2010, Castel et al. 2012). Another interesting aspect to consider is the different response to water availability of grape ripening dynamics we found in the Bobal and Tempranillo trials (Fig. 3 and 4). Severe water stress, as in the rainfed treatments, affected negatively berry sugar concentration in Tempranillo, while the opposite effect was found in Bobal. The present results seem to confirm previous research carried out over a single season by Salón et al. (2004) reporting how supplemental post-veraison irrigation differentially affects grape ripening in these cultivars. Indeed, vine water stress effect on TSS depends on the cultivar and on the severity of water stress. For instance, Schultz and Jones (2010) found that rainfed conditions decreased sugars content in Grenache but not in Syrah. In our trials, vine water status and the climatic conditions in post-veraison influenced berry sugar accumulation by the interactive effect of sugar biosynthesis and berry growth. Thus, the great mesocarp cell expansion of Bobal grapes in response to irrigation seems to delay TSS accumulation in berries due to a dilution effect, whereas irrigation caused a milder enlargement response of Tempranillo berries promoting an increase in TSS.

Since we expected a different behaviour in water stress responses among cultivars, we carried out the experiment in the two *V. vinifera* varieties under different watering regimes. In Tempranillo, in fact, the reduction in TSS accumulation due to defoliation, was at least in the first season less evident under rainfed conditions than under irrigation (Figure 4). It is possible that the alleviation in water stress due to defoliation compensated the reduction in the leaf area in this variety where berry sugars accumulation is detrimentally affected by water stress (Intrigliolo et



al. 2012). In Bobal, however, the reduction in grape TSS accumulation due to LLR was more clear under both watering regimes. In any case, in terms of final berry composition and wine quality, the effects of LLR were in general quite similar within each watering regime, and when an interactive effect was found, this was not consistent among seasons (Tables 3 to 5). In general, when a reduction in sugar accumulation occurred in response to LLR, a similar pattern was observed in terms of berry phenolics (Figure 3 and 4). But, in both cultivars, phenolic accumulation rate during the last stage of the ripening process was not as steady as the increase in berry TTS concentration (Figures 3 and 4). This is an expected trend in the synthesis of polyphenolic compounds, that reach a maximum generally 28 to 35 days after veraison and then decrease towards harvest (Delgado et al. 2004).

The comparison of anthocyanin and polyphenol content on a per berry basis showed that the effect of LLR on phenolic content was always detrimental. Therefore, the anthocyanins-to-sugars ratio tended to be decreased by leaf removal in both trials. This could have been occurred because LLR in general reduced the LA-to-fruit ratio. In some cases, this ratio was decreased below the minimum thresholds (0.8 and 1.2 m<sup>2</sup>/kg) that are considered to be required to reach proper grape ripeness (Kliewer & Dokoozlian, 2005). In our trials when the LA-to-fruit ratio was under 1.6 m<sup>2</sup>/kg, the anthocyanin concentration was lower than 1 mg/g. However, it should be noted that LA-to-fruit ratio is not the only physiological parameter influencing final berry phenolic concentration as recently reported for Tempranillo grapes in a canopy management and irrigation trial (Mirás-Avalos et al. 2017). For instance, in 2014, when the irrigated Bobal vines reached only half of the anthocyanin concentration compared to rainfed vines, the LA-to-fruit ratio was reduced in average from 3 to 3 m<sup>2</sup>/kg mostly because of the significant increase of berry fresh mass. But in this phenolic content reduction, the skin-to-pulp ratio played a more important role compared to the LA-to-fruit ratio, as demonstrated by the not limiting effect in biosynthesis of WR when expressing the phenolic content on a per berry basis.

Previous studies about the effect of leaf removal or shoot trimming on sugar accumulation in the berry reported contrasting results probably because of differences in cultivar sensitivity or because of differences in the severity and timing of defoliation application. For instance, Palliotti et al. (2013) reported that LLR applied to reduce the LA-to-fruit ratio to 1.13 m<sup>2</sup>/kg did not affect phenolic composition of Sangiovese grapes, but it delayed harvest of two weeks compared to the undefoliated vines. On the other hand, Caccavello et al. (2017) found in Aglianico grapevines a negative impact on the wine sensory score when defoliation or shoot trimming induced reductions of LA-to-fruit ratio below 2 m<sup>2</sup>/kg. This could be due to the different cultivar response to LLR, as described by Lanari et al. (2013) for the Montepulciano and Sangiovese berry colour. Besides the intensity of defoliation, another critical aspect of the application of this technique is the timing when it is performed. Lanari et al. (2013), Palliotti et al. (2013) and Intrieri et al. (2017) removed



leaves when grape TSS was 14-17 °Brix, whereas Poni et al. (2013) and Caccavello et al. (2017) applied defoliation at around 12 °Brix. The level of ripeness when we performed LLR was slightly lower ( $\approx 9$  °Brix), and this may result detrimental for the onset of phenolic synthesis, because carbohydrate availability during the first week after the onset of veraison affects the synthesis of anthocyanin and other phenolic substances (Pirie 1977, Vitrac et al. 1999). These authors did explain this effect by the role of sugars as a source of energy, but by their role as signals in the transduction pathway involved in the induction of anthocyanins biosynthesis. This is in agreement with the results of our study, because lower anthocyanin concentrations were found already at veraison in all the LLR treatments compared to that of the undefoliated vines (Figures 3G, 3H, 4G and 4H), with the only exception of Tempranillo RD compared to RU vines in 2014.

Wine composition was affected by LLR mainly decreasing phenolic content and colour intensity in of both trials, but this trend was more clear in Tempranillo than in Bobal wines. The reported difference in wine hue angle (Table 5) suggests that the different timing of ripening could also affect the type of berry pigments synthesized. Late leaf removal tended to increase the violet tones (%blue), conversely to the irrigation effect. This might be an interesting modification since it is a probable indicator of aptitude for wine ageing (Alcalde-Eon et al. 2014). These more intense purple hues are usually obtained from more mature grapes, which usually contains a higher quantity of co-pigments. In future scenarios, a more interventionist winemaking style involving water additions, acid adjustments and alcohol reductions may be possible solutions for a better balance among wine composition parameters (Jones and Davis, 2000). For instance, Martínez de Toda and Balda (2011) produced low alcoholic wines with good acidity level by blending the wine made with Tempranillo grapes harvested immediately after veraison with the wine produced from the grapes harvested when the phenolic maturity had reached the highest level. However, the use of these oenological techniques may compromise wine typicity, because it is very likely that characteristic flavours and aromas will be modified (Schultz 2000, Lebon 2002). Thus, any adaptive technique to this climate shifts which could ensure the sustainability of traditional cultivars, well adapted to their original terroirs, is worth being tested. In fact, under the predicted warming scenarios (IPCC, 2014), in the area of study during the 21<sup>st</sup> century air temperatures are forecasted to overpass the reported thresholds that are considered to be detrimental for phenolic biosynthesis pathways. Therefore, LLR may become an interesting technique to be evaluated under less severe defoliation intensity or in more vigorous vines and postponing its timing of application. Our results highlight the complexity of the interaction between leaf area-to-fruit ratio, vine water status and the environmental conditions. Other adaptive techniques which can delay ripening process without modifying so much this ratio should be tested. Among them, late pruning (Gatti et al. 2016, Moran et al. 2017) or vine bud regrowth (Gu et al. 2012) appear to be very promising.

## CONCLUSIONS

Late leaf removal apical to the cluster zone under regulated deficit irrigation was shown as an effective technique to slow down the ripening process. However, with this consequent delayed harvest, the composition of Bobal and Tempranillo grapes and wine was not improved. Although defoliated treatments alleviated water stress resulting in a photosynthetic compensatory mechanism, this was insufficient to match grape phenolic composition in equality to the technological ripeness of undefoliated treatments. In addition, LLR constrained yield in Tempranillo vines due to reductions in cluster and berry mass. Under our experimental conditions, vine water status was the main driver of grape ripening and these responses were genotype dependent, while ambient temperature played a minor role in berry ripening in temperate-warm climate. Improving our knowledge on the physiological principles underlying the response of local cultivars to canopy and water management, namely leaf area-to-fruit ratio and vine water status, will allow a better adaptation of the winegrape tipicity to climate change conditions.

## ANKNOWLEDGMENTS

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**Tables**

**Table 1.** Total amount of water received (rain plus irrigation) from April 1<sup>st</sup> to September 30<sup>th</sup> and amount of leaf removed with late defoliation in Bobal and Tempranillo vines exposed to the following four treatments: IU, irrigated-undefoliated; ID, irrigated-defoliated; RU, rainfed-undefoliated; RD, rainfed-defoliated.

Cultivar	Treatment	Water received (mm)		Leaf area removed (m <sup>2</sup> vine <sup>-1</sup> )	
		2014	2015	2014	2015
Bobal	IU	329	311	2.9a	5.7a
	ID	329	311	2.3b	3.9b
	RU	96	203	2.1b	5.6a
	RD	96	203	1.7c	3.7b
Tempranillo	IU	232	287	5.5a	4.4a
	ID	232	287	4.4b	3.4b
	RU	96	203	4.1c	4.3a
	RD	96	203	3.0d	3.0b

Within each row, mean values followed by a different letter are significantly different at  $P < 0.05$

**Table 2.** Values of yield components and vegetative growth in the trial established during 2014 and 2014 on *Vitis vinifera* L., cv. Bobal and Tempranillo trials at Requena, Valencia, Spain.

Parameter	Cultivar	Year	Treatment				Significance of effects		
			IU	ID	RU	RD	WR	CM	WR× CM
Fruit yield (Mg ha <sup>-1</sup> )	Bobal	2014	5.32a	4.78a	1.70b	2.03b	<0.0001	0.8386	0.3827
		2015	17.02a	14.57a	10.68b	10.88b	<0.0001	0.2893	0.2121
	Tempranillo	2014	2.48a	2.08ab	1.87bc	1.38c	0.0030	0.0413	0.8450
		2015	13.04a	11.50a	11.51a	8.52b	0.0186	0.0178	0.4461
Number of clusters per vine	Bobal	2014	5.3	4.9	5.5	4.9	0.7452	0.3304	0.8454
		2015	11.5a	10.9ab	9.2b	10.0ab	0.0191	0.8847	0.2551
	Tempranillo	2014	14.7	12.8	15.4	12.3	0.9213	0.1373	0.6414
		2015	26.1a	28.7a	24.6ab	21.2b	0.0055	0.8187	0.0694
Cluster fresh mass (g cluster <sup>-1</sup> )	Bobal	2014	329a	282a	105b	125b	<0.0001	0.4711	0.0782
		2015	583a	498ab	447b	407b	0.0027	0.0922	0.5454
	Tempranillo	2014	94a	81ab	72bc	62c	0.0001	0.0247	0.7738
		2015	297a	238b	282a	227b	0.2368	0.0000	0.8554
Berry fresh mass (g berry <sup>-1</sup> )	Bobal	2014	3.7a	3.6a	1.6b	1.6b	<0.0001	0.8388	0.5838
		2015	3.1a	3.0a	2.7ab	2.56b	0.0098	0.6646	0.6807
	Tempranillo	2014	1.5ab	1.8a	1.4b	1.3b	0.0414	0.1449	0.0257
		2015	2.3a	1.9b	2.2a	2.0b	0.8025	0.0002	0.2291
Shoot fruitfulness (number of clusters per shoot)	Bobal	2015	0.84a	0.80ab	0.70b	0.74ab	0.0304	0.9704	0.3945
		2016	0.98a	0.97a	0.81b	0.94ab	0.0451	0.2184	0.1423
	Tempranillo	2015	1.20a	1.27a	1.20a	0.99b	0.0160	0.2374	0.0163
		2016	1.29a	1.19a	1.02b	1.14a	0.2794	0.0562	0.0005
Pruning mass/ vine (g vine <sup>-1</sup> )	Bobal	2014	507a	470ab	302bc	272c	0.0042	0.6189	0.9613
		2015	620a	571ab	508ab	470b	0.0152	0.3133	0.9080
	Tempranillo	2014	628a	675a	403b	543ab	0.0027	0.4093	0.1028
		2015	-	-	-	-	-	-	-
LAI (m <sup>2</sup> /m <sup>2</sup> )	Bobal	2014	0.76a	0.62b	0.56b	0.45c	<0.0001	0.0001	0.6420
		2015	1.52a	1.04b	1.50a	0.99b	0.4363	<0.0001	0.9146
	Tempranillo	2014	0.87a	0.76b	0.65c	0.50d	<0.0001	0.0002	0.5111
		2015	0.72a	0.56b	0.70a	0.53b	0.5874	<0.0001	0.5531
LA-to-fruit ratio (m <sup>2</sup> /kg)	Bobal	2014	1.6b	2.3b	3.9a	2.0b	0.0166	0.157	0.0031
		2015	1.0ab	0.8b	1.6a	0.9b	0.0001	0.0000	0.0336
	Tempranillo	2014	2.8b	2.6b	3.9a	2.8b	0.0819	0.0834	0.3025
		2015	0.7	0.6	0.7	0.6	0.5844	0.1329	0.7063

Data are the average treatment in 2014 and 2015, except for shoot fruitfulness which averages are for 2015 and 2016. Within each row, mean values followed by a different letter are significantly different at  $P<0.05$ . For data analysis between factors, the statistical significance effect of watering regime (WR), canopy management (CM) and their interaction are also indicated. LAI means leaf area index. Treatments are: IU, irrigated-undefoliated; ID, irrigated-defoliated; RU, rainfed-undefoliated; RD, rainfed-defoliated.

**Table 3.** The harvest date and must technological composition attributes at harvest of *Vitis vinifera* L., cv. Bobal and Tempranillo trials in the two seasons under different watering regime (WR) and canopy management (CM) in Requena, Valencia, Spain.

Parameter	Cultivar	Year	Treatment				Significances of effects		
			IU	ID	RC	RD	WR	CM	WR*CM
Harvest date (DOY)	Bobal	2014	274 [62]	274 [62]	254 [42]	259 [47]			
		2015	272 [61]	294 [83]	260 [49]	280 [69]			
	Tempranillo	2014	246 [36]	254 [44]	254 [44]	254 [44]			
		2015	254 [55]	264 [65]	254 [55]	264 [65]			
TSS (°Brix)	Bobal	2014	22.1c	21.0d	25.1a	24.3b	<0.0001	0.0009	0.5627
		2015	20.2a	18.6b	20.6a	19.7a	0.0243	0.0008	0.2373
	Tempranillo	2014	25.1	24.7	25.4	25.1	0.0721	0.2153	0.6718
		2015	21.7	22.4	21.4	21.6	0.2932	0.4324	0.6413
pH	Bobal	2014	3.5	3.4	3.4	3.4	0.0990	0.7457	0.0683
		2015	3.5ab	3.6a	3.4b	3.5b	0.0052	0.0588	0.4939
	Tempranillo	2014	3.3b	3.5a	3.5a	3.5a	0.0001	0.0002	0.0011
		2015	3.3b	3.7a	3.2b	3.7a	0.3840	<0.0001	0.1038
TA (g/L tartaric acid)	Bobal	2014	5.0a	4.8ab	4.5b	4.9ab	0.2102	0.3579	0.0536
		2015	4.9c	4.8c	5.9a	5.5b	0.0000	0.0250	0.0561
	Tempranillo	2014	4.2a	3.6c	4.0b	3.9b	0.2900	0.0000	0.0000
		2015	5.6	5.5	5.6	5.7	0.2646	0.9889	0.4460
Tartaric acid concentration (g/L)	Bobal	2014	1.8c	1.6d	3.9b	4.7a	<0.0001	0.0002	<0.0001
		2015	2.5c	2.4c	4.7a	4.0b	<0.0001	0.0000	<0.0001
	Tempranillo	2014	4.0a	3.6b	4.0a	4.0a	<0.0001	0.0000	<0.0001
		2015	4.1bc	4.0c	4.2ab	4.4a	0.0004	0.8051	0.0362
Malic acid concentration (g/L)	Bobal	2014	3.0a	3.0a	1.9b	1.6b	0.0900	<0.0001	0.2301
		2015	2.7	2.6	2.7	2.5	0.5241	0.0824	0.3074
	Tempranillo	2014	2.0ab	1.9b	2.0ab	2.1a	0.0598	0.5878	0.1244
		2015	2.8	2.8	2.9	2.9	0.0697	0.9648	0.5671

Data are the average values for 2014 and 2015 (n=6 in Bobal; n=8 in Tempranillo). Values between brackets means ripening duration expressed in days from veraison to the harvest date. Within each row, mean values followed by a different letter are significantly different at P<0.05. TSS, total soluble solids, TA, titratable acidity. Treatments are: IU, irrigated-undefoliated; ID, irrigated-defoliated; RU, rainfed-undefoliated; RD, rainfed-defoliated.

**Table 4.** Berry phenolic composition attributes at harvest of *Vitis vinifera* L., cv. Bobal and Tempranillo trials in the two seasons under different watering regime (WR) and canopy management (CM) in Requena, Valencia, Spain.

Parameter	Cultivar	Year	Treatment				Significance of effects		
			IC	ID	RU	RD	WR	CM	WR*CM
Maturity index	Bobal	2014	48.2c	47.5c	62.1a	54.4b	<b>0.0000</b>	<b>0.0273</b>	0.0593
		2015	37.9d	41.3c	48.3a	45.4b	<b>0.0000</b>	0.8002	<b>0.0016</b>
	Tempranillo	2014	65.4c	77.2a	70.9b	71.9b	0.934	<b>0.0000</b>	<b>0.0001</b>
		2015	42.9	44.3	42.2	43.9	0.732	0.2976	0.919
Anthocyanins (mg/g)	Bobal	2014	0.93b	0.83b	1.74a	1.79a	<b>&lt;0.0001</b>	0.6278	0.1614
		2015	0.48d	0.62c	1.08a	0.71b	<b>&lt;0.0001</b>	<b>0.0063</b>	<b>&lt;0.0001</b>
	Tempranillo	2014	1.49a	1.16b	1.46a	1.50a	0.0674	0.0824	<b>0.0278</b>
		2015	0.98a	0.83b	0.98a	0.82b	0.9600	<b>0.0080</b>	<b>0.9600</b>
Polyphenols (mg/g)	Bobal	2014	2.10c	1.91c	2.81b	3.43a	<b>&lt;0.0001</b>	<b>0.0386</b>	<b>0.0009</b>
		2015	1.60c	2.13b	2.55a	2.41a	<b>&lt;0.0001</b>	0.0709	<b>0.0040</b>
	Tempranillo	2014	2.96a	2.47b	2.95a	3.09a	<b>0.0093</b>	0.1028	<b>0.0082</b>
		2015	2.70	2.46	2.62	2.63	0.6270	0.1942	0.1529
Anthocyanins- to-sugars ratio (mg/g/°Brix)	Bobal	2014	0.04b	0.04b	0.07a	0.07a	<b>0.0000</b>	0.6713	0.1193
		2015	0.01c	0.03b	0.05a	0.04b	<b>0.0000</b>	0.7134	<b>0.0000</b>
	Tempranillo	2014	0.06a	0.05b	0.06a	0.06a	0.0942	0.1187	<b>0.0222</b>
		2015	0.05a	0.04b	0.05a	0.04b	0.6305	<b>0.0005</b>	0.9676

Data are the average values for 2014 and 2015 (n=6 in Bobal; n=8 in Tempranillo). Within each row, mean values followed by a different letter are significantly different at P<0.05. TSS, total soluble solids, TA, titratable acidity. Treatments are: IU, irrigated-undefoliated; ID, irrigated-defoliated; RU, rainfed-undefoliated; RD, rainfed-defoliated.

**Table 5.** Seasonal values of wine composition parameters made of Bobal and Tempranillo winegrapes subjected to different watering regime and canopy management factors in Requena, Valencia, Spain.

Parameter	Cultivar	Year	Treatment				Significance of effects		
			IU	ID	RU	RD	WR	CM	WR*CM
TA (g/L tartaric acid)	Bobal	2014	4.8b	4.9b	5.0b	6.0a	<b>0.0003</b>	<b>0.0004</b>	<b>0.0068</b>
		2015	4.2b	4.4b	4.9a	5.2a	<b>&lt;0.0001</b>	0.045	0.7487
	Tempranillo	2014	4.9ab	4.6b	5.3a	4.6b	0.3073	<b>0.0088</b>	0.2506
		2015	4.0b	4.2a	4.1b	4.0ab	0.691	<b>0.0121</b>	0.4664
pH	Bobal	2014	3.7ab	3.7ab	3.8a	3.6b	1	<b>0.0222</b>	<b>0.0778</b>
		2015	3.7a	3.7ab	3.6ab	3.6b	<b>0.0231</b>	0.3591	0.7831
	Tempranillo	2014	3.9b	4.0a	4.0ab	4.0a	0.0576	<b>0.0382</b>	0.5744
		2015	3.8	3.8	3.9	3.9	0.3724	0.0571	0.2797
Colour intensity	Bobal	2014	8.88bc	8.86c	11.5b	13.99a	<b>0.0056</b>	<b>0.0255</b>	<b>0.1158</b>
		2015	5.10b	3.25c	8.59a	6.23b	<b>&lt;0.0001</b>	<b>0.0003</b>	0.6974
	Tempranillo	2014	12.69a	10.28c	11.67b	11.33b	0.9642	<b>0.0002</b>	<b>0.0022</b>
		2015	6.85a	6.44b	7.90a	6.42b	0.9569	<b>&lt;0.0001</b>	0.9168
Anthocyanins (mg/L)	Bobal	2014	404.2a	423.3a	266.5b	459.7a	0.3227	<b>0.0001</b>	<b>0.0003</b>
		2015	281.6b	197.6c	347.0a	273.2b	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.7353
	Tempranillo	2014	396.7	332.2	406.6	380.3	0.3602	0.1279	0.503
		2015	457.4a	325.3b	471.4a	315.7b	0.8197	<b>&lt;0.0001</b>	0.2433
TPI (AU)	Bobal	2014	48.8a	47.9a	34.6b	46.9a	0.0640	<b>0.0025</b>	<b>0.0003</b>
		2015	44.6b	35.4c	50.5a	48.5a	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0093</b>
	Tempranillo	2014	48.4ab	44.0b	50.5a	49.6ab	0.0692	0.1554	0.3415
		2015	53.9a	47.6b	51.9a	44.1c	<b>&lt;0.0001</b>	<b>0.0042</b>	<b>&lt;0.0001</b>
Hue angle	Bobal	2014	0.63a	0.62a	0.60a	0.51b	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0005</b>
		2015	0.70b	0.80a	0.59c	0.63ab	<b>&lt;0.0001</b>	<b>0.0094</b>	0.2606
	Tempranillo	2014	0.70b	0.71ab	0.69b	0.74a	0.2345	<b>0.0427</b>	0.0943
		2015	0.64b	0.69a	0.64b	0.69a	0.8261	<b>&lt;0.0001</b>	0.7179

Data are the average values for 2014 and 2015 (n=3 in Bobal; n=4 in Tempranillo). Within each row, mean values followed by a different letter are significantly different at  $P<0.05$ . For data analysis between factors, the statistical significance effect of watering regime (WR), canopy management (CM) and their interaction are also indicated. TA, titratable acidity; TPI, total polifenols index. Treatments are: IU, irrigated-undefoliated; ID, irrigated-defoliated; RU, rainfed-undefoliated; RD, rainfed-defoliated.



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## **Figures captions**

**Figure 1.** Seasonal patterns of the daily maximum air temperature (●), mean temperature (●) and minimum temperature (▼) in Requena, Valencia, Spain. The day of year (DOY) follows a continuous time scale starting on 01/01/2014. Rainfall is represented with blue bars and reference evapotranspiration (ET<sub>o</sub>) with green bars. The moment of occurrence of late leaf removal in Bobal (★) and in Tempranillo (★) and the latest harvest for Bobal (✚) and for Tempranillo (✚) are indicated for each season.

**Figure 2.** Effect of irrigated-undefoliated (IU; ●), irrigated-defoliated (ID; ●), rainfed-undefoliated (RU; ▼) and rainfed-defoliated (RD; ▼) treatments on the seasonal evolution of midday stem water potential ( $\Psi_{\text{stem}}$ ) of Bobal [(a) and (b)] and Tempranillo [(c) and (d)] vineyards in 2014 [(a) and (c)] and 2015 [(b) and (d)]. Data are averages and standard errors of 16 leaves per treatment and date. The date of application of defoliation is indicated by an arrow.

**Figure 3.** Bobal trial: grape effects on the seasonal evolution of berry fresh weight in 2014 (a) and in 2015 (b), total soluble solids (TSS) in 2014 (c) and in 2015 (d), titratable acidity in 2014 (e) and in 2015 (f), and anthocyanins concentration in 2014 (g) and in 2015 (h) in irrigated-undefoliated (IU; ●), irrigated-defoliated (ID; ●), rainfed-undefoliated (RU; ▼) and rainfed-undefoliated (RD; ▼) vines from the date of late leaf removal was applied until harvest. Data are the averages and standard errors of three replications per treatment for each date. GDD, growing degree days.

**Figure 4.** Tempranillo trial: grape effects on the seasonal evolution of (a) berry fresh weight in 2014 and (b) in 2015, (c) total soluble solids (TSS) in 2014 and (d) in 2015, (e) titratable acidity in 2014 and (f) in 2015, and (g) anthocyanins concentration in 2014 and (h) in 2015 of the irrigated-undefoliated (IU; ●), irrigated-defoliated (ID; ●), rainfed-undefoliated (RU; ▼) and rainfed-defoliated (RD; ▼) treatments from the date of late leaf removal was applied until harvest. Data are the average of four replications per treatment for each date. GDD, growing degree days.

**Figure 5.** Effect on stomatal conductance ( $g_s$ ), transpiration (E) and photosynthesis (A) leaf rates of the irrigated-undefoliated (IU; ■), irrigated-defoliated (ID; ■), rainfed-undefoliated (RU; ■) and rainfed-defoliated (RD; ■) treatments of Bobal [(a), (b) and (c)] and Tempranillo [(d), (e) and (f)] vineyards during the two experimental seasons. Data are averages and standard errors of 16 leaves per treatment and 3 dates per season. Different letters mean significant difference among treatments at  $P < 0.05$ .

**Figure 6.** Effect of irrigated Control (IU; ●), irrigated defoliated (ID; ●), rainfed control (RU; ▼) and rainfed defoliated (RD; ▼) treatments on the relationship between total soluble solids (TSS) accumulation in berries and titratable acidity (TA) for (a) Bobal plot in 2014 and (b) in 2015, (c)

838 Tempranillo plot in 2014 and (d) in 2015. Data are averages of three replications per treatment in  
839 Bobal and four in Tempranillo for each date.

840 **Supplemental panel A.** Tempranillo ID vines appearance at the beginning of veraison during  
841 2014.