Delaying berry ripening of Bobal and Tempranillo grapevines by late leaf removal in a semi-arid and temperate-warm climate under different water regimes

I. BUESA[1,2], G. CACCAVELLO[3], B. BASILE[3], M. C. MERLI[4], S. PONI[4], C. CHIRIVELLA[5] and D. S. INTRIGLIOLO[2]

[1] Instituto Valenciano de Investigaciones Agrarias (IVIA), Sustainable Agriculture Center, 46113, Moncada, Valencia, Spain; [2] Departamento de Riego, Centro de Edafología y Biología Aplicada del Segura (CSIC), 30100, Murcia, Spain; [3] Department of Agricultural Sciences, Division of Grape and Wine Sciences, Università degli Studi di Napoli Federico II, 83100, Avellino, Italy; [4] Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, 29100, Piacenza, Italy; [5] Instituto Tecnológico de Viticultura y Enología, Servicio de Producción Ecológica, Innovación y Tecnología, 46340, Requena, Valencia, Spain

Corresponding author: Dr Ignacio Buesa, email igbuepue@gmail.com

Abstract

Background and Aims: Climate change is advancing grape ripening and decoupling sugar and phenolic maturity, impacting wine typicity. The aim of this study was to test whether late leaf removal (LLR) under different watering regimes delayed harvest of two Spanish red cultivars in a semi-arid and temperate-warm climate.

Methods and Results: In two trials carried out in eastern Spain with the Bobal and Tempranillo cultivars, vines were partially defoliated above the bunch zone shortly before veraison under rainfed and deficit irrigation conditions during two seasons. The rate of grape ripening in both cultivars was significantly affected by LLR under either watering regime, consequently delaying harvest. Vine water status and leaf photosynthetic rate were improved by LLR. The reduction in leaf area-to-fruit ratio resulting from the LLR treatments was found to be more limiting for the accumulation of anthocyanin than for TSS. Consequently, LLR negatively affected wine colour intensity. In addition, yield was constrained by LLR in Tempranillo due to a reduction in bunch and berry mass.

Conclusions: The reduction in the rate of accumulation of grape TSS provoked by LLR did not necessarily result in a more balanced berry maturity. The effectiveness of the LLR technique appears to depend on its final impact on leaf area-to-fruit ratio and vine water status, the cultivar photosynthetic compensation capacity and the environmental conditions.

Significance of the Study: Late leaf removal might not be effective for coupling anthocyanin and TSS in berries under moderate water stress conditions, given the observed reductions in red wine colour.

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

Introduction

Climate type is a major component of winegrape terroirs (Castel et al. 2012, Hannah et al. 2013). Mediterranean viticulture could suffer from warmer and drier growing seasons in the coming decades (Lereboullet et al. 2014), as winegrapes are considered 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 temperature induces an increase in the rate of sugar accumulation in the berries (Petrie and Sadras 2008), leading to a higher alcohol concentration in wine or to an earlier harvest date (Jones et al. 2005, Koufos et al. 2014, Cook and Wolkovich 2016). Other authors have also documented the shortening of the 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 sugar and anthocyanin 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). For instance, Duchêne and Schneider (2005) observed an increasing trend in vine evapotranspiration demand after flowering over 30 years in Alsace, France. Furthermore, the higher probability of heat waves and drought events (Intergovernmental Panel on Climate Change 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. 2017), 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 reduction (Stoll et al. 2010, Palliotti et al. 2013) may delay ripening so that it occurs during cooler periods of the season. Practices, such as pruning, trimming and leaf removal, can be certainly employed to manipulate vine source–sink balance (Caccavello et al. 2017, Moran et al. 2017, Santesteban et al. 2017).

Late leaf removal (LLR), defined as leaf removal applied late in the season (near veraison), tends to have a limited

doi: 10.1111/ajgw.12368

effect on fruit yield but it can postpone grape ripening (Palliotti et al. 2013, Caccavello et al. 2017). For instance, Intrieri et al. (2017), after removing 30–40% of total vine leaf area (LA), achieved an increase in anthocyanin concentration in Sangiovese berries when the harvest date was delayed 7–8 days. Poni et al. (2013), after applying LLR at veraison in the same cultivar, delayed the technologically defined ripeness without affecting berry colour or the concentration of phenolic substances. Lanari et al. (2013) found that LLR negatively affected the concentration of anthocyanin and phenolic substances in Montepulciano grapevines, but not in Sangiovese. Bobeica et al. (2015) reported that sugar accumulation in berries of potted Sangiovese and Cabernet Sauvignon vines could be maintained at the expense of phenolic substances under LA removal. Moreover, the carbon limitation affected the anthocyanin profile in a cultivar-dependent manner. Therefore, it appears that the response to LLR may vary depending on the cultivar.

In semi-arid climates, the effect of LLR can vary depending on the irrigation regime, because of the significant effect of application of water 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 anthocyanin-to-sugar 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 phenolic substances and anthocyanin because it can stimulate anthocyanin biosynthesis in berry skin (Castellarin et al. 2007, Santesteban et al. 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 can promote a higher concentration of phenolic substances 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 water stress may be detrimental to anthocyanin accumulation (Girona et al. 2009, Romero et al. 2010). Salón et al. (2005) and Intrigliolo and Castel (2011) reported that maintaining midday stem water potentials (Ψ_{stem}) above a specific threshold during post-veraison (−1.2 and − 1.5 MPa in Bobal and Tempranillo, respectively) induced an increase in anthocyanin concentration and colour intensity in the must and wine.

In this trial, we assessed the effect of LLR on the ripening dynamics and yield components of Bobal and Tempranillo in a semi-arid and temperate-warm climate under different watering regimes (WR). To the best of our knowledge, all previous studies have in fact determined the effect of LLR within a given WR, and in cooler and more humid areas (Lanari et al. 2013, Palliotti et al. 2013, Poni et al. 2013, Bobeica et al. 2015, Caccavello et al. 2017, Intrieri et al. 2017). Our working hypothesis was that the LLR technique could delay fruit ripening, thereby alleviating grape heat stress, while irrigation effects could interact differently depending on the cultivar. In this context, we were interested on the photosynthetic compensatory response to LLR under different WR. Particularly, we investigated if LLR applied under both deficit irrigation and rainfed conditions could improve the concentration of grape and wine phenolic substances.

Materials and methods

Site and crop

The trials were conducted in 2014 and 2015 in a commercial vineyard (*Vitis vinifera* L.) located near Requena, Valencia, Spain (39°30′18.10″N, 1°13′54.30″W; elevation 700 m asl). Both trials were located in two adjacent plots planted, respectively, with Bobal (grafted onto 110 Richter) and 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 plot, respectively. Shoots were trained vertically with a pair of steel catch wires. The Bobal plot was planted in 2002 at a spacing of 2.5 × 1.4 m (2857 vines/ha), whereas the Tempranillo plot was planted in 1991 at 2.5 m × 2.45 m (1633 vines/ha). The vineyard rows were oriented north–south. The two plots were independently deficit irrigated with two drippers per vine for more than ten years before commencement of the present experiments.

The vineyard soil is a Typic Calciorthid with a clay-loam to light clay texture, highly calcareous and with low fertility. Soil depth was greater than 2 m and available water capacity was about 200 mm/m. The climate in this area is continental Mediterranean and semi-arid, the heliothermal index of Huglin (Huglin 1978) is 2291°C corresponding to a temperate-warm viticultural climate, with cool nights and moderately dry according to the classification system for grapegrowing regions proposed by Tonietto and Carbonneau (2004). At the experimental site, the annual average values (for the 2002–2013 period) of the reference evapotranspiration (ET_o) and the rainfall were 1127 and 380 mm, respectively. About 65% of the precipitation generally occurs in winter.

Sources of variation

Four treatments were tested in each trial, obtained by combining two types of WR (irrigated or rainfed) and two types of canopy management (CM, defoliated or undefoliated). The treatments applied were: irrigated–undefoliated (IU), irrigated–defoliated (ID), rainfed–undefoliated (RU); rainfed–defoliated (RD) (Table 1). The treatments had three replicates in the Bobal trial and four replicates in the Tempranillo trial. With both cultivars, the experimental design was a randomised block design, where WR was assigned to the main plot and CM to the subplots. Each subplot or experimental unit (EU) consisted of a row of ten vines in the Bobal trial and seven vines in the Tempranillo trial. The vines located in the surrounding perimeter of the plots were used as borders. Both trials utilised 30 vines per treatment.

Deficit irrigation was applied to maintain midday Ψ_{stem} above the threshold values of −1.15 and −1.40 MPa of pre- and post-veraison, respectively. Late leaf removal was applied 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 the photosynthetic capacity of the vine at the beginning of berry ripening. Defoliation consisted of removing all the mature apical leaves of the main shoots and removing lateral shoots starting from the second node above the bunches [only the leaves at the top of the shoot were retained (Figure S1)]. This was done because at around veraison, the leaves located in the apical two-thirds of the shoots are considered to be the most photosynthetically active (Poni et al. 1994). Shoot tips were preserved in order to allow for potential lateral shoot regrowth to compete for photoassimilates with grapes (McCarthy 1997). Bunch zones were left unchanged by the defoliation.

Table 1. Total amount of water received (rain plus irrigation) from 1 April to 30 September and total leaf area after application of late leaf removal in Bobal and Tempranillo vines in Requena, Valencia, Spain.

Cultivar/treatment	Water received (mm)		Total leaf area (m^2/vine)	
	2014	2015	2014	2015
Bobal				
IU	329	311	2.9a	5.7a
ID	329	311	2.3b [24]	3.9b [32]
RU	96	203	2.1b	5.6a
RD	96	203	1.7c [23]	3.7b [34]
Tempranillo				
IU	232	287	5.5a	4.4a
ID	232	287	4.4b [21]	3.4b [24]
RU	96	203	4.1c	4.3a
RD	96	203	3.0d [28]	3.1b [30]

Within the total leaf area column and for each cultivar, mean values followed by a different letter indicate a significant difference between treatments at $P < 0.05$. Values within the brackets indicate the proportion of leaf area reduction within watering regime and season. ID, irrigated, defoliated; IU, irrigated, undefoliated; RD, rainfed, defoliated; RU, rainfed, undefoliated.

Field determinations

During the experiment, weather data were measured hourly with an automated meteorological station, named Requena Cerrito, located at the vineyard. Reference evapotranspiration (ET_o) was calculated with the Penman–Monteith equation (Allen et al. 1998). Cumulative growing degree days (GDD) from 1 April until harvest was computed as the sum of the average daily temperature above a threshold 10°C (Amerine and Winkler 1944). The amount of water applied with irrigation was measured with on-line water meters. Midday Ψ_{stem} was measured, respectively, on six and four dates in 2014 and 2015 with a pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA) on bag-covered leaves from two representative vines per EU at midday (measurements were made between 1130 and 1230 solar time). Leaves 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 h prior to measurement. In addition, on the last three dates, on the same vines where Ψ_{stem} was measured, net CO_2 assimilation rate, transpiration rate and stomatal conductance were measured (between 1000 and 1300 solar time) on two basal, mature, sun-exposed leaves per vine with a portable gas exchange analyser (LCpro+, ADC BioScientific, Hoddeson, England).

Fruit yield, number of bunches per vine, average bunch mass and shoot fruitfulness (number of bunches per shoot) were determined at harvest on each experimental vine. Additionally, one bunch per vine was randomly sampled at harvest in order to determine the number of berries per bunch. In 2016, when the 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 weighed from samples from 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_{ext}) per vine was determined by photographic analysis by means of an image processing software (ImageJ 1.47v, National Institutes of Health, Bethesda, MD, USA) following the methodology described by Schneider et al. (2012). The pictures were taken once the shoot growth had ceased, just after the LLR, on one side of the canopy hedge with a background curtain using a visible light camera (Ixus 220 HS, Canon, Tokyo, Japan). Additionally, on one representative vine per EU and cultivar, total leaf area ($LA_{measured}$) was estimated using allometric relationships computed for each cultivar between shoot length and LA per shoot measured with a LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE, USA). These relationships were obtained by separating main and lateral shoots, using samples of 12 shoots of different vigour. The LA in each experimental vine was estimated with the significant LA_{ext} and $LA_{measured}$ regression equations. Leaf area index (LAI) was calculated as the LA per unit ground surface area. The LA removed of the selected vines was measured with a LI-3100 Area Meter.

Grape and wine composition

Berry ripening evolution was assessed approximately every 10 days, starting from the day before LLR until harvest, except for phenolic composition, which was determined only after veraison. Berry fresh mass was determined from a random sample of at least 50 berries per EU and date. Thirty berries were crushed and hand pressed through a metal screen filter to evaluate technologically defined maturity; 20 berries were homogenised with a blender (Ultraturrax T25, IKA-Werke, Staufen, Germany) for the determination of phenolic maturity. Must TSS was determined by refractometry (PR-101, Series Palette, Atago, Tokyo, Japan), pH and TA were measured with an automatic titrator (Metrohm, Herisau, Switzerland). Juice was titrated with 0.1 N NaOH to an end point of pH 8.2, and results were expressed in tartaric acid equivalents. In order to assess the effect of the treatments on berry ripening, the TSS-to-TA ratio was calculated as a maturity index, by dividing total sugar concentration (g/L) by the TA (g/L) at harvest (Al-Kaisy et al. 1981). The concentration of tartaric acid and malic acid was measured only at harvest with an infrared analyser (Bacchus II, Tecnología Difusión Ibérica, Barcelona, Spain) following the procedures described by García-Romero et al. (1993). Anthocyanin and phenolic substances (expressed in malvidin equivalents) were determined in duplicate by UV/VIS spectrophotometry (Iland et al. 2004). In addition, the phenolic substances were calculated on a per berry basis, as the ratio between berry concentration and its mass, in an attempt to separate the dilution effect of berry size on these compounds.

Each treatment was harvested when the TSS level reached a specific target that was defined for each cultivar and season, and therefore, harvest occurred at 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 of increasing 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, 12 vinifications for Bobal and 16 for Tempranillo. Grapes were mechanically crushed, destemmed and fermented at a temperature of approximately 22°C in 60 L stainless steel containers. Five grams of SO_2 were added to all the musts, and these were then inoculated with 20 g of commercial *Saccharomyces cerevisiae* yeast per 100 kg of grapes (FR Excellence, Lamothe-Abiet, Bordeaux, France). Skin contact time was 7 days and during this period fermentations were punched down daily. After alcoholic fermentation was completed, the wines were pressed and decanted into 30 L demijohns. All wines were stored for 6 months before analysis once the spontaneous MLF ended. Phenolic composition was determined by measuring the optical density (OD; nm) using spectrophotometric methods (Ati-Unicam UV-4, Thermo Spectronic, Cambridge, England) as described by Ribéreau-Gayon et al. (2000); anthocyanin in HCl media (OD_{520}–OD_{860}), phenolic substances index (TPI) (OD_{280}–OD_{860}), wine colour intensity (OD_{420} + OD_{520} + OD_{620}-OD_{860}) and hue (OD_{420}/OD_{520}). All analytical determinations in grape, must and wine were in duplicate.

Data analysis

Data from the Bobal and Tempranillo trials were analysed separately because the vines were of different ages, rootstocks and vine spacing. For each cultivar, the effect of WR, CM and WR × CM interaction on vine traits and grape composition was tested by a two-way ANOVA. If the ANOVA detected significant 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 from the Statgraphics Centurion XVI package (version 16.0.07) (Statgraphics Technologies, The Plains, VA, USA). Wine composition data were subjected to a two-way ANOVA using the alcohol concentration as a covariate, because significant linear relationships between alcohol concentration and colour intensity, anthocyanin and TPI were found.

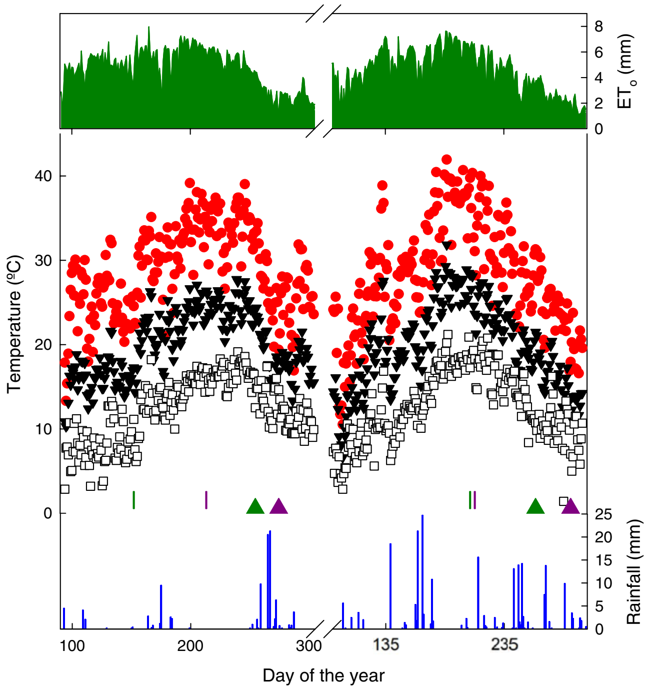

Figure 1. Seasonal patterns of the daily maximum air temperature (●), mean temperature (▼) and minimum temperature (□) in Requena, Valencia, Spain. Rainfall is represented with blue bars and reference evapotranspiration (ET_o) with green bars. The moment of occurrence of late leaf removal in Bobal (|) and in Tempranillo (|) and the latest harvest for Bobal (▲) and for Tempranillo (▲) are indicated for each season.

Results

Meteorological conditions and irrigation applications

During the experimental seasons, from 1 April to 30 September, 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 the spring was noticeably higher compared to that of 2014, 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.

During berry ripening the average maximum air temperature during July and August was 33.5°C in 2014 and 35°C in 2015. Moreover, during September, when the grapes of the retarded treatments were still ripening, the average was 30.0 and 26.7°C, respectively. 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 was up to 7°C. In contrast, the average minimum air temperature during the week before harvest was below 18°C for all treatments and seasons.

Vine phenology and vegetative growth

Vine phenology until veraison was similar among treatments within each trial. No difference among treatments at the date of budburst, flowering or veraison was found for both cultivars. In both trials, the number of shoots per vine did not differ among treatments, because the seasonal pruning strategy (dormant pruning and early shoot thinning) employed was the same (19 ± 2 and 10 ± 2 shoots per vine in Tempranillo and Bobal, respectively). In Bobal LLR was applied on the day of year (DOY) 212 in 2014 and 211 in 2015, and on DOY 210 in 2014 and 209 in 2015 in Tempranillo. This corresponded to 10–12 days before 50% veraison (BBCH 83), when the berry TSS values were around 9°Brix.

Pooling the data across seasons, the average LA removed per vine represented 28 and 25% of the total vine LA at that moment in Bobal and in Tempranillo, respectively (Table 1). After LLR was applied, vegetative growth was negligible, probably because the growth of the shoot tips, both on the main and lateral shoots, was limited by the moderate water stress experienced by the vines. Lateral shoot regrowth was not observed in the defoliated vines (LLR treatments) even under irrigation because this regrowth is dependent upon weather conditions (Poni et al. 2014). 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 application induced a significant increase in LAI in the driest year (2014). In contrast, the pruning fresh mass per vine was significantly increased by irrigation in both cultivars, while LLR did not induce any significant response from pruning fresh mass (Table 2).

Vine water status and leaf assimilation rates

In both trials and seasons, vine water status was significantly affected by WR and CM (Figure 2). Differences in Ψ_{stem} between irrigated and rainfed vines were more noticeable in Bobal than in Tempranillo. Interestingly, in both trials, the defoliated vines showed significantly less negative midday Ψ_{stem} values than the undefoliated vines independently of the WR.

In both trials, WR and CM treatments significantly affected leaf stomatal conductance (g_s), transpiration (E) and photosynthesis (A) (Figure 3). A significant reduction was observed in g_s, E and A in rainfed vines compared to

Table 2. Values of yield components and vegetative growth in the trials established during 2014 and 2015 on *Vitis vinifera* L., cvs Bobal and Tempranillo in Requena, Valencia, Spain.

Parameter	Cultivar	Year	Treatment				Significance of effects		
			IU	ID	RU	RD	WR	CM	WR × CM
Fruit yield (kg/vine)	Bobal	2014	2.0a	1.8a	0.6b	0.8b	***	NS	NS
		2015	6.4a	5.5a	4.0b	4.1b	***	NS	NS
	Tempranillo	2014	1.5a	1.3ab	1.1bc	0.8c	**	*	NS
		2015	8.0a	7.0a	7.1a	5.2b	*	*	NS
Bunches per vine (No.)	Bobal	2014	5.3	4.9	5.5	4.9	NS	NS	NS
		2015	11.5a	10.9ab	9.2b	10.0ab	*	NS	NS
	Tempranillo	2014	14.7	12.8	15.4	12.3	NS	NS	NS
		2015	26.1a	28.7a	24.6ab	21.2b	**	NS	NS
Bunch fresh mass (g)	Bobal	2014	329a	282a	105b	125b	***	NS	NS
		2015	583a	498ab	447b	407b	**	NS	NS
	Tempranillo	2014	94a	81ab	72bc	62c	**	*	NS
		2015	297a	238b	282a	227b	NS	***	NS
Berry fresh mass (g)	Bobal	2014	3.7a	3.6a	1.6b	1.6b	***	NS	NS
		2015	3.1a	3.0a	2.7ab	2.56b	**	NS	NS
	Tempranillo	2014	1.5ab	1.8a	1.4b	1.3b	*	NS	*
		2015	2.3a	1.9b	2.2a	2.0b	NS	***	NS
Shoot fruitfulness (No. bunches/shoot)	Bobal	2015	0.84a	0.80ab	0.70b	0.74ab	*	NS	NS
		2016	0.98a	0.97a	0.81b	0.94ab	*	NS	NS
	Tempranillo	2015	1.20a	1.27a	1.20a	0.99b	**	NS	**
		2016	1.29a	1.19a	1.02b	1.14a	NS	NS	**
Pruning mass/vine (g/vine)	Bobal	2014	507a	470ab	302bc	272c	**	NS	NS
		2015	620a	571ab	508ab	470b	*	NS	NS
	Tempranillo	2014	628a	675a	403b	543ab	**	NS	NS
		2015	–	–	–	–	–	–	–
LAI (m^2/m^2)	Bobal	2014	0.76a	0.62b	0.56b	0.45c	***	***	NS
		2015	1.52a	1.04b	1.50a	0.99b	NS	***	NS
	Tempranillo	2014	0.87a	0.76b	0.65c	0.50d	***	***	NS
		2015	0.72a	0.56b	0.70a	0.53b	NS	***	NS
LA-to-fruit ratio (m^2/kg)	Bobal	2014	1.6b	2.3b	3.9a	2.0b	*	NS	**
		2015	1.0ab	0.8b	1.6a	0.9b	**	***	*
	Tempranillo	2014	2.8b	2.6b	3.9a	2.8b	NS	NS	NS
		2015	0.7	0.6	0.7	0.6	NS	NS	NS

Data are the average treatment in 2014 and 2015, except shoot fruitfulness for where 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 WR, CM and their interaction is also indicated. *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. CM, canopy management; ID, irrigated, defoliated; IU, irrigated, undefoliated; LA, leaf area; LAI, leaf area index; NS, not significant; RD, rainfed-defoliated; RU, rainfed-undefoliated; WR, watering regime.

irrigated vines, and in undefoliated (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/g_s), whereas LLR did not have any significant effect on this physiological parameter (50–62 μmol CO_2/mol H_2O in Tempranillo and 46–60 μmol CO_2/mol H_2O in Bobal, on a seasonal average).

Yield components

The effect of the WR on fruit yield was similar in Bobal and Tempranillo, whereas the effect of 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 under study. In Tempranillo, both WR and CM affected fruit yield significantly, whereas in Bobal only WR significantly influenced 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 that of rainfed vines (Table 2). The number of berries per bunch was not affected by the WR or CM (77 ± 4 to 172 ± 12 in Bobal, and from 50 ± 9 to 117 ± 11 in Tempranillo, in 2014 and 2015, respectively).

Canopy management significantly affected bunch and berry fresh mass in the Tempranillo plot, whereas this was not the case for the Bobal trial. The pooling of yield data of Tempranillo vines across seasons within the same WR revealed a significant reduction of 19% in LLR vines compared to that of undefoliated vines. In both seasons, the LLR reduced bunch fresh mass, whereas berry fresh mass was affected only in 2015. In both trials, the seasonal pattern of berry fresh mass was generally more influenced by WR than CM (Figures 4,5). This was more evident in 2014, a drier 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 irrigation tended to significantly reduce the LA-to-fruit ratio. In the Tempranillo trial, however, 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 the WR and CM treatments (Figures 4,5) and this was translated into differences in berry composition at harvest (Tables 3,4). Despite our goal being the harvesting of grapes from all the treatments at similar TSS value, in the Bobal

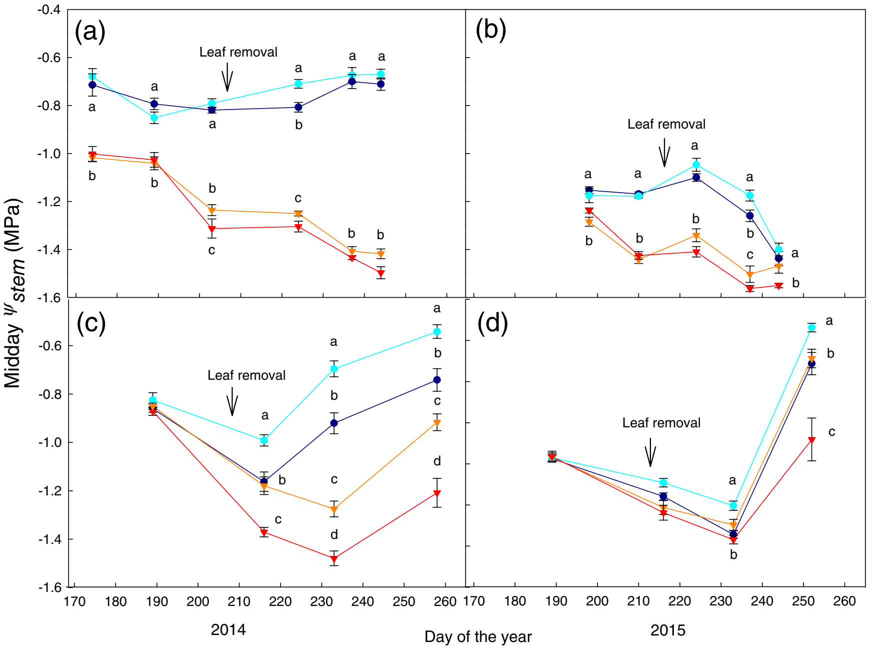

Figure 2. Effect of irrigated-undefoliated (●), irrigated-defoliated (●), rainfed-undefoliated (▼) and rainfed-defoliated (▼) treatments on the seasonal evolution of midday stem water potential (ψ_{stem}) of (a,b) Bobal and (c,d) Tempranillo trials in (a,c) 2014 and (b,d) 2015. Data are averages and SE of 16 leaves per treatment and date. The time of application of defoliation is indicated by an arrow.

trial this was not possible because of the occurrence of leaf senescence induced by the IU and ID treatments.

The WR affected TSS accumulation in the berries, but these effects were opposite in the two trials. In the Bobal trial, since the beginning of the ripening period, irrigation delayed the increase in berry TSS (Figure 4c,d), while in the Tempranillo trial, irrigation had a slightly opposite effect (Figure 5c,d). In both trials, rainfed treatments showed higher berry juice TA around veraison (Figures 4e, f,5e,f), 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. The relationship between TSS to TA during ripening was calculated to unravel the effect of the treatments on TA at similar TSS (Figure 6). This elucidates that irrigation and LLR had a tendency to reduce TA, although this effect became 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 concentration in berry juice was affected by the irrigation applied only in the first season, when berries from the IU and ID treatments did not reach TSS similar to that of the RU 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). For each WR in 2014 LLR did not affect the concentration of berry phenolic substances, whereas in 2015 the ID treatment induced an increase in the concentration of berry anthocyanin and phenolic substances. The opposite effect was found under rainfed conditions (Table 4). In contrast, in the Tempranillo trial in both seasons, the LLR treatment induced a significant decrease in anthocyanin concentration at harvest with the only exception being RD in 2014 (Table 4). In both trials, during ripening, the LLR treatment caused a decrease in the rate of anthocyanin accumulation compared to that of undefoliated vines (Figures 3g,h,4g,h). For both trials, however, the irrigated treatments significantly decreased the concentration of anthocyanin and phenolic substances in the berries compared to that in rainfed vines. These effects were more evident in Bobal than in Tempranillo.

The anthocyanin-to-TSS ratio tended to be higher in the grapes of rainfed than irrigated vines (Table 4). This effect was less evident in the Tempranillo trial in 2015. Leaf removal induced a decrease in the anthocyanin-to-TSS ratio

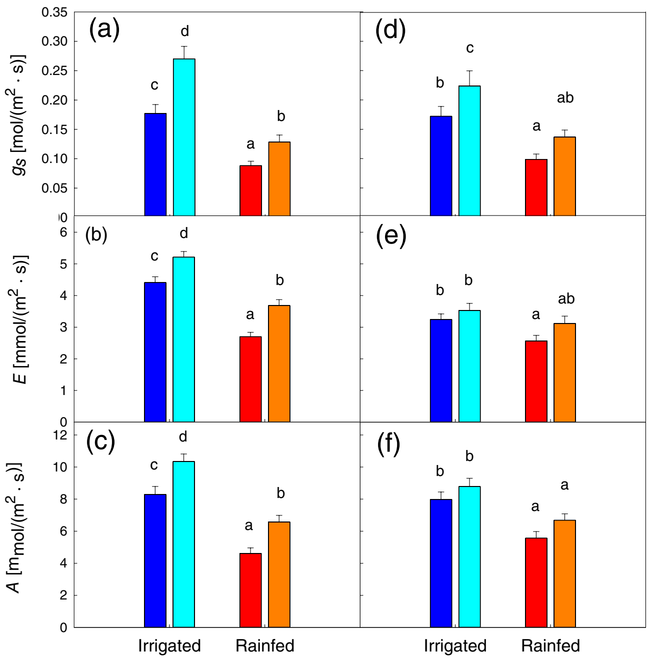

Figure 3. Effect of the irrigated-undefoliated (■), irrigated-defoliated (■), rainfed-undefoliated (■) and rainfed-defoliated (■) treatments on stomatal conductance (g_s), transpiration (*E*) and photosynthesis (*A*) leaf rates of (a,b, c) Bobal and (d,e,f) Tempranillo trials during 2014 and 2015. Data are averages and SE of 16 leaves per treatment and three dates after late leaf removal in each season. Different letters mean significant difference among treatments at $P < 0.05$.

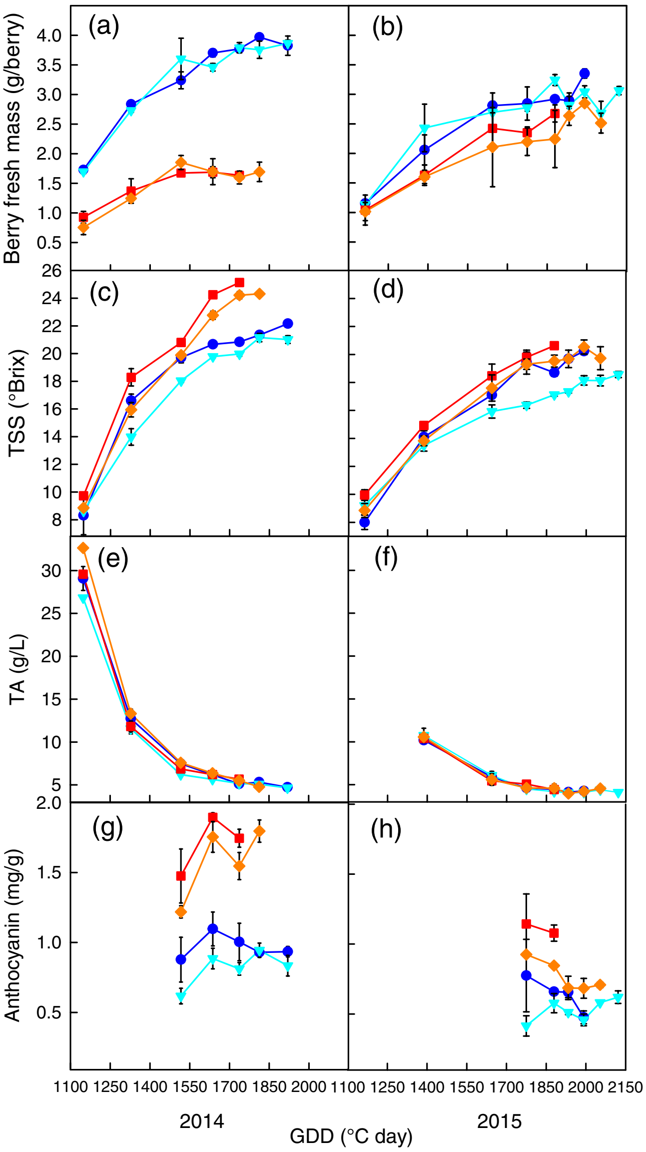

Figure 4. Effect of the (a,c,e,g) 2014 and (*b,d,f,h) 2015 seasons on berry (a,b) fresh mass, (c,d) TSS, (e,f) TA, and (g,h) anthocyanin concentration of irrigated-undefoliated (●), irrigated-defoliated (▼), rainfed-undefoliated (■) and rainfed-defoliated (◆) treatments of Bobal vines from the date of application of late leaf removal until harvest. Data are the average and SE of three replications per treatment for each date. GDD, growing degree days.

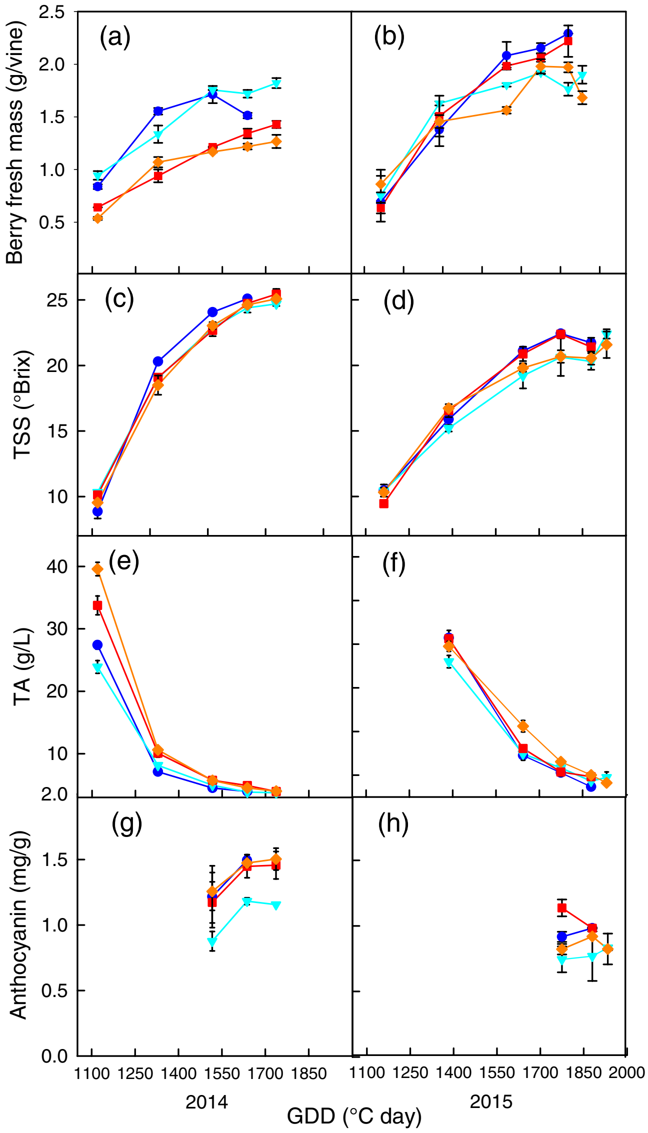

Figure 5. Effect of the (a,c,e,g) 2014 and (b,d,f,h) 2015 seasons on berry (a,b) fresh mass, (c,d) TSS, (e,f) TA, and (g.h) anthocyanin of the irrigated-undefoliated (●), irrigated-defoliated (▼), rainfed-undefoliated (■) and rainfed-defoliated (◆) treatments in Tempranillo vines from the date of application of late leaf removal until harvest. Data are the average of four replications per treatment for each date. GDD, growing degree days.

in the Tempranillo trial, without a significant effect in Bobal. Even if LLR vines were harvested later than the undefoliated vines, grape anthocyanin concentration was lower than that in the undefoliated vines. In 2015 in Tempranillo LLR reduced the concentration of berry anthocyanin and phenolic substances when they were calculated on a per berry basis (Table 4). In 2014, the effect of LLR on total berry anthocyanin and phenolic substances depended on the irrigation regime.

Wine composition

To assess treatment effects on wine composition, the alcohol concentration was used as a covariate aiming to analyse its influence on the extractability of phenolic substances (Table 5). In both the Bobal and Tempranillo trials, LLR did not cause any seasonally consistent effect on the TA. It should be highlighted that TA was higher in Bobal wines made with rainfed grapes compared to irrigated treatments. This effect was not observed in the Tempranillo wines. In both trials, wine pH was similar among treatments.

Late leaf removal tended to decrease wine colour intensity of both cultivars. Bobal wines made in 2014 were an exception, as an interactive effect was found due to the lower colour of RU wines compared to that of RD wines. There was no effect of WR on the colour of Tempranillo wines; however, irrigation significantly decreased colour intensity in wines made from Bobal grapes compared to those 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 being Bobal RU wines from 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 WR.

Table 3. Effect of watering regime and canopy management on the harvest date and on must composition at harvest of *Vitis vinifera* L. cv. Bobal and Tempranillo trials in 2014 and 2015 in Requena, Valencia, Spain.

			Treatment				Significance of effects		
Parameter	**Cultivar**	**Year**	**IU**	**ID**	**RU**	**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	***	***	NS
		2015	20.2a	18.6b	20.6a	19.7a	*	***	NS
	Tempranillo	2014	25.1	24.7	25.4	25.1	NS	NS	NS
		2015	21.7	22.4	21.4	21.6	NS	NS	NS
pH	Bobal	2014	3.5	3.4	3.4	3.4	NS	NS	NS
		2015	3.5ab	3.6a	3.4b	3.5b	**	NS	NS
	Tempranillo	2014	3.3b	3.5a	3.5a	3.5a	***	***	**
		2015	3.3b	3.7a	3.2b	3.7a	NS	***	NS
TA (g/L tartaric acid)	Bobal	2014	5.0a	4.8ab	4.5b	4.9ab	NS	NS	NS
		2015	4.9c	4.8c	5.9a	5.5b	***	*	NS
	Tempranillo	2014	4.2a	3.6c	4.0b	3.9b	NS	***	***
		2015	5.6	5.5	5.6	5.7	NS	NS	NS
Tartaric acid (g/L)	Bobal	2014	1.8c	1.6d	3.9b	4.7a	***	***	***
		2015	2.5c	2.4c	4.7a	4.0b	***	***	***
	Tempranillo	2014	4.0a	3.6b	4.0a	4.0a	***	***	***
		2015	4.1bc	4.0c	4.2ab	4.4a	***	NS	*
Malic acid (g/L)	Bobal	2014	3.0a	3.0a	1.9b	1.6b	NS	***	NS
		2015	2.7	2.6	2.7	2.5	NS	NS	NS
	Tempranillo	2014	2.0ab	1.9b	2.0ab	2.1a	NS	NS	NS
		2015	2.8	2.8	2.9	2.9	NS	NS	NS

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 $P < 0.05$. For data analysis between factors, the statistical significance effect of WR, CM and their interaction are also indicated. **, *** significant at $P < 0.01$ and $P < 0.001$, respectively. CM, canopy management; DOY, day of the year; ID, irrigated, defoliated; IU, irrigated, undefoliated; NS, not significant; RD, rainfed-defoliated; RU, rainfed-undefoliated; WR, watering regime.

Table 4. Effect of watering regime and canopy management on the composition of phenolic attributes at harvest of berries of *Vitis vinifera* L. cvs Bobal and Tempranillo in 2014 and 2015 in Requena, Valencia, Spain.

			Treatment				Significance of effects		
Parameter	**Cultivar**	**Year**	**IU**	**ID**	**RU**	**RD**	**WR**	**CM**	**WR × CM**
Maturity index	Bobal	2014	48.2c	47.5c	62.1a	54.4b	***	*	NS
		2015	37.9d	41.3c	48.3a	45.4b	***	NS	**
	Tempranillo	2014	65.4c	77.2a	70.9b	71.9b	NS	***	***
		2015	42.9	44.3	42.2	43.9	NS	NS	NS
Anthocyanin (mg/g)	Bobal	2014	0.93b	0.83b	1.74a	1.79a	***	NS	NS
		2015	0.48d	0.62c	1.08a	0.71b	***	**	***
	Tempranillo	2014	1.49a	1.16b	1.46a	1.50a	NS	NS	*
		2015	0.98a	0.83b	0.98a	0.82b	NS	**	NS
Phenolic substances (mg/g)	Bobal	2014	2.10c	1.91c	2.81b	3.43a	***	*	**
		2015	1.60c	2.13b	2.55a	2.41a	***	NS	**
	Tempranillo	2014	2.96a	2.47b	2.95a	3.09a	**	NS	**
		2015	2.70	2.46	2.62	2.63	NS	NS	NS
Anthocyanin (mg/berry)	Bobal	2014	3.50a	3.00ab	2.78b	2.98ab	NS	NS	NS
		2015	1.49c	1.90b	2.89a	1.78b	***	**	***
	Tempranillo	2014	2.10a	2.08a	2.10a	2.03a	NS	NS	NS
		2015	2.38a	1.66b	2.23a	1.49b	NS	***	NS
Phenolic substances (mg/berry)	Bobal	2014	7.88a	6.90ab	4.45c	5.68bc	**	NS	*
		2015	5.38b	6.52a	6.82a	6.06ab	NS	NS	**
	Tempranillo	2014	4.20a	4.45a	4.25a	4.15a	NS	NS	NS
		2015	6.56a	4.92c	5.93b	4.76c	*	***	NS
Anthocyanin-to-TSS ratio [(mg·g)/°Brix]	Bobal	2014	0.04b	0.04b	0.07a	0.07a	***	ns	ns
		2015	0.01c	0.03b	0.05a	0.04b	***	NS	***
	Tempranillo	2014	0.06a	0.05b	0.06a	0.06a	NS	NS	**
		2015	0.05a	0.04b	0.05a	0.04b	NS	***	NS

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 $P < 0.05$. For data analysis between factors, the statistical significance effect of WR, CM and their interaction is also indicated. *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. CM, canopy management; ID, irrigated, defoliated; IU, irrigated, undefoliated; NS, not significant; RD, rainfed-defoliated; RU, rainfed-undefoliated; WR, watering regime.

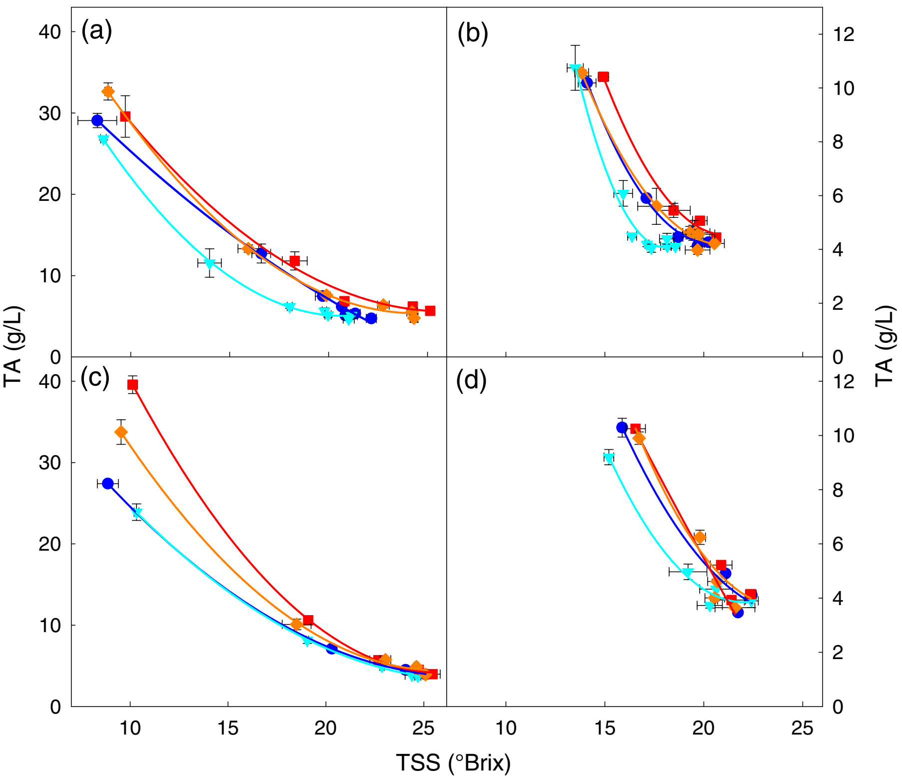

Figure 6. Effect of irrigated-undefoliated (●), irrigated-defoliated (▼), rainfed-undefoliated (■) and rainfed-defoliated (◆) treatments on the relationship between TSS accumulation and TA in berries and TA for (a) Bobal in 2014 and (b) in 2015, (c) Tempranillo in 2014 and (d) in 2015. Data are average of three replications per treatment in Bobal and four in Tempranillo for each date.

Table 5. Effect of watering regime and canopy management on the composition of Bobal and Tempranillo winegrapes in 2014 and 2015 in Requena, Valencia, Spain.

			Treatment				Significance of effects		
Parameter	Cultivar	Year	IU	ID	RU	RD	WR	CM	WR × CM
TA (g/L tartaric acid)	Bobal	2014	4.8b	4.9b	5.0b	6.0a	***	***	**
		2015	4.2b	4.4b	4.9a	5.2a	***	*	NS
	Tempranillo	2014	4.9ab	4.6b	5.3a	4.6b	NS	**	NS
		2015	4.0b	4.2a	4.1b	4.0ab	NS	*	NS
pH	Bobal	2014	3.7ab	3.7ab	3.8a	3.6b	NS	*	NS
		2015	3.7a	3.7ab	3.6ab	3.6b	*	NS	NS
	Tempranillo	2014	3.9b	4.0a	4.0ab	4.0a	NS	*	NS
		2015	3.8	3.8	3.9	3.9	NS	NS	NS
Colour intensity	Bobal	2014	8.88bc	8.86c	11.5b	13.99a	**	*	NS
		2015	5.10b	3.25c	8.59a	6.23b	***	**	NS
	Tempranillo	2014	12.69a	10.28c	11.67b	11.33b	NS	**	**
		2015	6.85a	6.44b	7.90a	6.42b	NS	***	NS
Anthocyanin (mg/L)	Bobal	2014	404.2a	423.3a	266.5b	459.7a	NS	**	**
		2015	281.6b	197.6c	347.0a	273.2b	***	***	NS
	Tempranillo	2014	396.7	332.2	406.6	380.3	NS	NS	NS
		2015	457.4a	325.3b	471.4a	315.7b	NS	***	NS
TPI (AU)	Bobal	2014	48.8a	47.9a	34.6b	46.9a	NS	**	**
		2015	44.6b	35.4c	50.5a	48.5a	***	***	**
	Tempranillo	2014	48.4ab	44.0b	50.5a	49.6ab	NS	NS	NS
		2015	53.9a	47.6b	51.9a	44.1c	***	**	***
Hue angle	Bobal	2014	0.63a	0.62a	0.60a	0.51b	***	***	***
		2015	0.70b	0.80a	0.59c	0.63ab	***	**	NS
	Tempranillo	2014	0.70b	0.71ab	0.69b	0.74a	NS	*	NS
		2015	0.64b	0.69a	0.64b	0.69a	NS	***	NS

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 WR, CM and their interaction is also indicated. *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. CM, canopy management; ID, irrigated, defoliated; IU, irrigated, undefoliated; NS, not significant; RD, rainfed-defoliated; RU, rainfed-undefoliated; TPI, phenolic substances index; WR, watering regime.

Discussion

In both the Tempranillo and Bobal trials, LLR 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 CM nor by the WR.

A delay in harvest did not imply an improvement in the balance between sugar and phenolic grape maturity per se. Furthermore, in Bobal, a mid-late season maturing cultivar, the delay in berry ripening induced by LLR, and particularly by irrigation, was detrimental because berries could not reach the same technological maturity of berries of the rainfed and undefoliated vines due to the occurrence of leaf senescence before reaching the target ripeness (Table 3). Indeed, under the experimental conditions of the present trials, the hypothesis of higher phenolic biosynthesis at a slightly cooler temperature 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 being above 33°C during the core of the ripening period (Figure 1), the air temperature in all treatments was below the temperature identified as detrimental to the accumulation of these compounds (Mori et al. 2007, Movahed et al. 2016). It is also possible, however, that the severe defoliation applied in our study limited the biosynthesis of phenolic substances to a major degree than TSS. That is, the vines prioritise the synthesis of primary rather than secondary metabolites. This is in agreement with the results reported by Bobeica et al. (2015) and could be supported by the fact that the increase in net CO_2 assimilation rate measured in both trials in defoliated vines did not appear to be sufficient to fully compensate for the reduction in total vine LA. On average, the defoliation treatment induced an increase in leaf net photosynthetic rate of 25 and 13% in Bobal and Tempranillo, respectively (Figure 3), whereas the reduction in total vine LA induced by LLR was always above 23 and 21% for Bobal and Tempranillo, respectively (Table 1). Nonetheless, net photosynthetic rate of young leaves retained in the undefoliated vines was expected to be much higher than the rate of their basal leaves (Poni et al. 1994).

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 was indeed an expected adaptive response (Petrie et al. 2003, Poni et al. 2013). Despite this improvement in vine water status (higher ψ_{stem}), none of the treatments stimulated the growth of shoot tips or laterals, which would have enhanced the competition for photoassimilates with the berries (McCarthy 1997, Baeza et al. 2007). The increase in leaf net photosynthetic rate, however, appears to have been sufficient, at least in the Bobal trial, for maintaining the yield level of the defoliated treatments at a value similar to that of the undefoliated vines (Table 2). This was observed even in the second experimental season, after 2 years of consecutive leaf pulling applications and under the higher crop levels in general registered in 2015. In the Tempranillo trial, however, the observed increase in leaf photosynthesis of defoliated vines did not offset the reduction in bunch and berry fresh mass. In contrast, berry number per bunch was unaffected by treatments in both trials, discarding possible carry-over effects due to carbohydrate depletion. This suggests that flower formation and fruitset was primarily controlled by the environment and not by the management of cultivation factors, such as CM or WR.

The experiment was not designed to elucidate the effect of irrigation regime on vine performance and grape composition, because these aspects have already 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). There are still some insights, however, that must be noted. As expected, vine water status and leaf gas exchange were improved in response to moderate irrigation. But more interestingly, the intrinsic water-use efficiency (WUE_i) 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 WUE_i in grapevines [0.05–0.15 mol/(m^2·s)]. Nevertheless, WUE_i determination from single leaf measurements could mask or alter conclusions made about the adaptive response of the whole canopy to water stress (Poni et al. 2014).

In both trials, even in the rainfed vines, midday ψ_{stem} values did not decrease below −1.5 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 found in the Bobal and Tempranillo trials (Figures 4,5). Severe water stress, as in the rainfed treatments, detrimentally affected berry sugar accumulation in Tempranillo, while the opposite effect was found for Bobal. The present results appear to confirm previous research carried out over a single season by Salón et al. (2004) reporting how supplemental post-veraison irrigation differentially affected grape ripening in these cultivars. Indeed, the effect of vine water stress on TSS depends on the cultivar and on the severity of the water stress. For instance, Schultz and Stoll (2010) found that rainfed conditions decreased sugar content in Grenache but not in Syrah. In our trials, vine water status and the meteorological conditions post-veraison influenced berry sugar accumulation by the interactive effect of sugar biosynthesis and berry growth. Thus, the significant mesocarp cell expansion of Bobal grapes in response to irrigation appears 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.

As we expected a different behaviour of water stress response among cultivars, we carried out the experiment in the two *V. vinifera* cultivars under different WR. In Tempranillo, the reduction in TSS accumulation due to defoliation was, at least in the first season, less evident under rainfed conditions than under irrigation (Figure 5). It is possible that the alleviation of water stress due to defoliation compensated for the reduction in the LA in this cultivar where the accumulation of berry sugars was detrimentally affected by water stress (Intrigliolo et al. 2012). In Bobal, however, the reduction in grape TSS accumulation due to LLR was clear under both WRs (Figure 4). In any case, in terms of final berry composition and wine quality, the effect of LLR was in general quite similar within each WR, and when an interactive effect was found, this was not consistent among seasons (Tables 3–5). In general, when a reduction in TSS accumulation and a decrease in the acid concentration occurred in response to LLR, a similar pattern was observed in terms of berry phenolic substances (Figures 4,5). In fact, in both cultivars, the rate of accumulation of phenolic substances during the last stage of the ripening process was not as steady as the increase in berry TSS (Figures 4,5). Consequently, leaf removal in both trials tended to decrease the anthocyanin-to-TSS ratio (Table 4).

The comparison of the concentration of anthocyanin and phenolic substances on a per berry basis showed that the effect of LLR on the concentration of phenolic substances was in one season clearly detrimental (Table 4). This could have occurred because LLR in general reduced the LA-to-fruit ratio, and in some cases, this ratio was decreased below the minimum threshold (0.8 and 1.2 m^2/kg) that is considered to be required for reaching proper grape ripeness

(Kliewer and Dokoozlian 2005). In our trials, this ratio was lower in 2015 than in 2014 due to the higher number of bunches per vine and greater bunch fresh mass in the second season (Table 2). The rate of grape ripening in Bobal in 2015 was slow (Figure 4d) and the ID treatment was unable to reach the target ripeness level even though this target had been reduced in 2015 in comparison with that in season 2014. In our trials, when the LA-to-fruit ratio was under 1.6 m^2/kg, the anthocyanin concentration was lower than 1 mg/g. It should be noted, however, that LA-to-fruit ratio is not the only physiological parameter influencing final concentration of berry phenolic substances, as recently reported for Tempranillo grapes in a CM and irrigation trial (Mirás-Avalos et al. 2017). For instance, in 2014, when the irrigated Bobal vines reached half of the anthocyanin concentration compared to that of rainfed vines, the LA-to-fruit ratio was not detrimental, but the increase in berry mass led to a significant reduction in skin-to-pulp ratio. This played a more important role compared to the LA-to-fruit ratio, as demonstrated by the non-limiting effect of irrigation on the concentration of phenolic substances when expressed on a per berry basis (Table 4).

Previous studies on the effect of leaf removal or shoot trimming on sugar accumulation in the berry reported conflicting results, probably due to differences in cultivar sensitivity or because of differences in the severity and timing of the application of defoliation. For instance, Palliotti et al. (2013) reported that LLR applied to reduce the LA-to-fruit ratio to 1.13 m^2/kg did not affect phenolic composition of Sangiovese grapes, but it delayed harvest for 2 weeks compared to that of the undefoliated vines. In contrast, Caccavello et al. (2017) found in Aglianico grapevines a negative impact on the wine sensory score when defoliation or shoot trimming induced a reduction of the LA-to-fruit ratio below 2 m^2/kg. This could be due to the response of different cultivars to LLR, as described by Lanari et al. (2013) for the colour of Montepulciano and Sangiovese berries. Besides the intensity of defoliation, another critical aspect of this technique is the timing of application. 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) defoliated at around 12°Brix. The level of ripeness when we performed LLR was slightly lower (≈9°Brix), and this may have been detrimental for the onset of synthesis of phenolic substances, 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 explained this effect not only through the role of sugars as an energy source but also through their role as signal in the transduction pathway involved in the induction of anthocyanin biosynthesis. This is in agreement with the results of our study, as a lower anthocyanin concentration was already found at veraison in all the LLR treatments compared to that of the undefoliated vines (Figures 4g,h,5g,h), with the only exception being Tempranillo RD compared to RU vines in 2014.

Wine composition was affected by LLR because of a decrease in the concentration of phenolic substances and in colour intensity. Therefore, the objective of higher colour-to-alcohol ratio was not achieved; however, this trend was clearer in Tempranillo than in Bobal wines. The reported difference in wine hue angle among CM and WR treatments (Table 5) suggests that the different timing of ripening could also affect the type of berry pigments synthesised. This effect was reported in detail in response to LLR for Sangiovese and Cabernet Sauvignon grapes (Bobeica et al. 2015) and in response to drought for Tocai Friulano (Savoi et al. 2016). In our trials, LLR tended to increase the violet tones (%blue). This might be an interesting modification as violet tones are 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 contain a higher concentration of co-pigments.

Our results highlight the complexity of the interaction between LA-to-fruit ratio, vine water status and the environmental conditions. Other adaptive techniques which can delay the onset of the ripening process without so much modifying this ratio should be tested. Among them, late pruning (Gatti et al. 2016, Moran et al. 2017) or forcing bud growth (Gu et al. 2012) appear to be promising.

Conclusions

Late leaf removal apical to the bunch zone was shown to be an effective technique to delay the ripening process. With the subsequent delay in harvest, however, 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 the same terms as the technological ripeness of the undefoliated treatments even under slightly cooler conditions. In addition, LLR constrained yield in Tempranillo vines due to a reduction in bunch and berry mass. Under our experimental conditions, in a temperate-warm climate, vine water status was the main driver of grape ripening and these responses were genotype-dependent, while ambient temperature appeared to play a minor role in berry ripening. Improving our knowledge on the physiological principles underlying the response of local cultivars to canopy and water management, namely LA-to-fruit ratio and vine water status, will allow a better adaptation of the winegrape typicity to climate change conditions.

Acknowledgements

This work received financial support of the FEDER: AGL2014-54201-C4-4-R project and was co-funded by the CajaMar and Lucio Gil de Fagoaga Foundation. Thanks are also due to Mr. F. Sanz, Mr. T. Yeves and Mr. D. Pérez for their help with field and analytical measurements. We are grateful to the Irrigation Technical Service (STR-IVIA) for the acquisition of climate data.

References

Alcalde-Eon, C., García-Estévez, I., Ferreras-Charro, R., Rivas-Gonzalo, J.C., Ferrer-Gallego, R. and Escribano-Bailón, M.T. (2014) Adding oenological tannin vs. overripe grapes: effect on the phenolic composition of red wines. Journal of Food Composition and Analysis **34**, 99–113.

Al-Kaisy, A.M., Sachde, A.G., Ghalib, H.A. and Hamel, S.M. (1981) Physical and chemical changes during ripening of some grape varieties grown in Basrah. American Journal of Enology and Viticulture **32**, 268–271.

Allen, R.G., Pereira, L.S., Raes, D. and Smith, M. (1998) Crop evapotranspiration. Guidelines for computing crop water requirements; FAO Irrigation and drainage paper no. 56 (Food and Agriculture Organization of the United Nations: Rome, Italy).

Amerine, M.A. and Winkler, A.J. (1944) Composition and quality of musts and wines of California grapes. Hilgardia **15**, 493–675.

Baeza, P., Ruiz, C., Cuevas, E., Sotés, V. and Lissarrague, J.R. (2005) Ecophysiological and agronomic response of Tempranillo grapevines to four training systems. American Journal of Enology and Viticulture **56**, 129–138.

Baeza, P., Sánchez-de-Miguel, P., Centeno, A., Junquera, P., Linares, R. and Lissarrague, J.R. (2007) Water relations between leaf water potential, photosynthesis and agronomic vine response as a tool for establishing thresholds in irrigation scheduling. Scientia Horticulturae **114**, 151–158.

Bobeica, N., Poni, S., Hilbert, G., Renaud, C., Gomès, E., Delrot, S. and Dai, Z. (2015) Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. Frontiers in Plant Science **6**, 382.

Buesa, I., Pérez, D., Castel, J., Intrigliolo, D.S. and Castel, J.R. (2017) Vine performance and grape composition responses of Muscat of Alexandria wine grapes to deficit irrigation. Australian Journal of Grape and Wine Research **23**, 251–259.

Caccavello, G., Giaccone, M., Scognamiglio, P., Forlani, M. and Basile, B. (2017) Influence of intensity of post-veraison defoliation or shoot trimming on vine physiology, yield components, berry and wine composition in Aglianico grapevines. Australian Journal of Grape and Wine Research **23**, 226–239.

Caravia, L., Collins, C., Petrie, P.R. and Tyerman, S.D. (2016) Application of shade treatments during Shiraz berry ripening to reduce the impact of high temperature. Australian Journal of Grape and Wine Research **22**, 422–437.

Castel, J.R., Valdés, M.E., Prieto, M.H., Uriarte, D., Mancha, L., Montoro, A., Mañas, F., López-Urrea, R., López-Fuster, P. and Yuste, J. (2012) Terroir effects on the response of Tempranillo grapevines to irrigation in four locations of Spain: agronomic performance and water relations. Proceedings of the IX International Terroir Congress (Acta IX International Terroir Congress) 25–29 June; Dijon/Reims, France (Vinidea: Porte dell'Olio, Italy) 5, pp. 10–13.

Castellarin, S.D., Matthews, M.A., Gaspero, G.D. and Gambetta, G. A. (2007) Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta **227**, 101–112.

Cifre, J., Bota, J., Escalona, J., Medrano, H. and Flexas, J. (2005) Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): an open gate to improve water-use efficiency? Agriculture, Ecosystems & Environment **106**, 159–170.

Cook, B.I. and Wolkovich, E.M. (2016) Climate change decouples drought from early wine grape harvests in France. Nature Climate Change **6**, 715–719.

Duchêne, E. and Schneider, C. (2005) Grapevine and climatic changes: a glance at the situation in Alsace. Agronomy for Sustainable Development **25**, 93–99.

Esteban, M.A., Villanueva, M.J. and Lissarrague, J.R. (2001) Effect of irrigation on changes in the anthocyanin composition of the skin of cv. Tempranillo (*Vitis vinifera* L.) grape berries during ripening. Journal of the Science of Food and Agriculture **81**, 409–420.

Gambetta, G.A. (2016) Water stress and grape physiology in the context of global climate change. Journal of Wine Economics **11**, 168–180.

García-Romero, E., Sánchez-Muñoz, G., Martín-Alvarez, P.J. and Cabezudo-Ibáñez, M.D. (1993) Determination of organic acids in grape musts, wines and vinegars by high-performance liquid chromatography. Journal of Chromatography A **655**, 111–117.

Gatti, M., Pirez, F.J., Chiari, G., Tombesi, S., Palliotti, A., Merli, M. C. and Poni, S. (2016) Phenology, canopy aging and seasonal carbon balance as related to delayed winter pruning of *Vitis vinifera* L. cv. Sangiovese grapevines. Frontiers in Plant Science **7**, 659.

Girona, J., Marsal, J., Mata, M., Del Campo, J. and Basile, B. (2009) Phenological sensitivity of berry growth and composition of Tempranillo grapevines (*Vitis vinifera* L.) to water stress. Australian Journal of Grape and Wine Research **15**, 268–277.

Gu, S., Jacobs, S.D., McCarthy, B.S. and Gohil, H.L. (2012) Forcing vine regrowth and shifting fruit ripening in a warm region to enhance fruit quality in 'Cabernet Sauvignon' grapevine (*Vitis vinifera* L.). Journal of Horticultural Science and Biotechnology **87**, 287–292.

Hannah, L., Roehrdanz, P.R., Ikegami, M., Shepard, A.V., Shaw, M. R., Tabor, G., Zhi, L., Marquet, P.A. and Hijmans, R.J. (2013) Climate change, wine, and conservation. Proceedings of the National Academy of Sciences of the United States of America **110**, 6907–6912.

Huglin, P. (1978) Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. Proceedings of the Symposium International sur l'ecologie de la vigne; 25-29 September 1978; Constança, Roumania (Ministère de l'Agriculture et de l'Industrie Alimentaire: Paris, France) pp. 89–98.

Iland, P., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) Chemical analysis of grapes and wine: techniques and concepts (Patrick Iland Wine Promotions: Campbelltown, SA, Australia).

Intergovermental Panel on Climate Change (2014) Climate change 2014: synthesis report. Pachauri R.K. and Meyer L.A., eds. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change (Intergovernmental Panel on Climate Change: Geneva, Switzerland).

Intrieri, C., Filippetti, I., Allegro, G., Valentini, G., Pastore, C. and Colucci, E. (2017) Results of five-year trial to delay ripening of cv. Sangiovese (*Vitis vinifera* L.) by late mechanical defoliation. Proceedings of the 20th International meeting GiESCO. Sustainable viticulture and winemaking in climate change scenarios; 5–10 November 2017; Mendoza, Argentina (GiESCO: Montpellier, France) pp. 73–77.

Intrigliolo, D.S. and Castel, J.R. (2008) Effects of irrigation on the performance of grapevine cv. Tempranillo in Requena, Spain. American Journal of Enology and Viticulture **59**, 30–38.

Intrigliolo, D.S. and Castel, J.R. (2010) Response of grapevine cv. 'Tempranillo' to timing and amount of irrigation: water relations, vine growth, yield and berry and wine composition. Irrigation Science **28**, 113–125.

Intrigliolo, D.S. and Castel, J.R. (2011) Interactive effects of deficit irrigation and shoot and cluster thinning on grapevine cv. Tempranillo. Water relations, vine performance and berry and wine composition. Irrigation Science **29**, 443–454.

Intrigliolo, D.S., Pérez, D., Risco, D., Yeves, A. and Castel, J.R. (2012) Yield components and grape composition responses to seasonal water deficits in Tempranillo grapevines. Irrigation Science **30**, 339–349.

Jackson, D.I. and Lombard, P.B. (1993) Environmental and management practices affecting grape composition and wine quality. American Journal of Enology and Viticulture **44**, 409–430.

Jones, G.V., Duchêne, E., Tomasi, D., Yuste, J., Braslavska, O., Schultz, H., Martinez, C., Boso, S., Langellier, F., Perruchot, C. and Guimberteau, G. (2005) Changes in European winegrape phenology and relationships with climate. Proceedings XIV International GESCO Viticulture Congress; 23–27 August, 2005; Geisenheim, Germany (Groupe d'Etude des Systemes de COnduite de la v\Vigne: Geisenheim, Germany) pp. 54–61.

Jones, G.V. and Webb, L.B. (2010) Climate change, viticulture, and wine: challenges and opportunities. Journal of Wine Research **21**, 103–106.

Kliewer, W.M. and Dokoozlian, N.K. (2005) Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. American Journal of Enology and Viticulture **56**, 170–181.

Kliewer, W.M., Lider, L.A. and Schultz, H.B. (1967) Influence of artificial shading of vineyards on the concentration of sugar and organic acid in grapes. American Journal of Enology and Viticulture **18**, 78–86.

Koufos, G., Mavromatis, T., Koundouras, S., Fyllas, N.M. and Jones, G.V. (2014) Viticulture–climate relationships in Greece: the impacts of recent climate trends on harvest date variation. International Journal of Climatology **34**, 1445–1459.

Lanari, V., Lattanzi, T., Borghesi, L., Silvestroni, O. and Palliotti, A. (2013) Post-veraison mechanical leaf removal delays berry ripening on 'Sangiovese' and 'Montepulciano' grapevines. Proceedings of the international workshop vineyard mechanization and grape and wine quality; 27–29 June 2013; Piacenza, Italy. Acta Horticulturae **978**, 327–334.

Lereboullet, A.-L., Beltrando, G., Bardsley, D. and Rouvellac, E. (2014) The viticultural system and climate change: coping with long-term trends in temperature and rainfall in Roussillon, France. Regional Environmental Change **14**, 1951–1966.

Lorenz, D.H., Eichhorn, K.W., Bleiholder, H., Klose, R., Meier, U. and Weber, E. (1995) Growth stages of the grapevine. Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)—Codes and descriptions according to the extended BBCH scale. Australian Journal of Grape and Wine Research **1**, 100–103.

McCarthy, M.G. (1997) The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research **3**, 102–108.

Mirás-Avalos, J.M., Buesa, I., Llacer, E., Jiménez-Bello, M.A., Risco, D., Castel, J.R. and Intrigliolo, D.S. (2017) Water versus source–sink relationships in a semiarid Tempranillo vineyard: vine performance and fruit composition. American Journal of Enology and Viticulture **68**, 11–22.

Mirás-Avalos, J.M. and Intrigliolo, D.S. (2017) Grape composition under abiotic constraints: water stress and salinity. Frontiers in Plant Science **8**, 851.
Moran, M.A., Sadras, V.O. and Petrie, P.R. (2017) Late pruning and carry-over effects on phenology, yield components and berry traits in Shiraz. Australian Journal of Grape and Wine Research **23**, 390–398.
Moratiel, R., Durán, J. and Snyder, R.L. (2010) Responses of reference evapotranspiration to changes in atmospheric humidity and air temperature in Spain. Climate Research **44**, 27–40.
Mori, K., Goto-Yamamoto, N., Kitayama, M. and Hashizume, K. (2007) Loss of anthocyanins in red-wine grape under high temperature. Journal of Experimental Botany **58**, 1935–1945.
Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., Cavallini, E., D'Incà, E., Tornielli, G.B. and Filippetti, I. (2016) The grapevine VviPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. Journal of Plant Research **129**, 513–526.
Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. and Deloire, A. (2002) Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. American Journal of Enology and Viticulture **53**, 261–267.
Palliotti, A., Panara, F., Silvestroni, O., Lanari, V., Sabbatini, P., Howell, G.S., Gatti, M. and Poni, S. (2013) Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines. Australian Journal of Grape and Wine Research **19**, 369–377.
Petrie, P.R. and Sadras, V.O. (2008) Advancement of grapevine maturity in Australia between 1993 and 2006: putative causes, magnitude of trends and viticultural consequences. Australian Journal of Grape and Wine Research **14**, 33–45.
Petrie, P.R., Trought, M.C.T., Howell, G.S. and Buchan, G.D. (2003) The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. Functional Plant Biology **30**, 711–717.
Pirie, A.J.G. (1977) Phenolics accumulation in red wine grapes (*Vitis vinifera* L.). PhD Thesis, University of Sydney, Sydney, NSW, Australia.
Poni, S., Gatti, M., Bernizzoni, F., Civardi, S., Bobeica, N., Magnanini, E. and Palliotti, A. (2013) Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. Australian Journal of Grape and Wine Research **19**, 378–387.
Poni, S., Intrieri, C. and Silvestroni, O. (1994) Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. I. Gas exchange. American Journal of Enology and Viticulture **45**, 71–78.
Poni, S., Zamboni, M., Vercesi, A., Garavani, A. and Gatti, M. (2014) Effects of early shoot trimming of varying severity on single high-wire trellised pinot noir grapevines. American Journal of Enology and Viticulture **65**, 493–498.
Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000) Phenolic compounds. Handbook of enology. Volume 2. The chemistry of wine stabilization and treatments (John Wiley: New York, NY, USA) pp. 129–187.
Risco, D., Pérez, D., Yeves, A., Castel, J.R. and Intrigliolo, D.S. (2014) Early defoliation in a temperate warm and semi-arid Tempranillo vineyard: vine performance and grape composition. Australian Journal of Grape and Wine Research **20**, 111–122.
Romero, P., Fernández-Fernández, J.I. and Martinez-Cutillas, A. (2010) Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. American Journal of Enology and Viticulture **61**, 300–312.
Sadras, V.O. and Moran, M.A. (2012) Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. Australian Journal of Grape and Wine Research **18**, 115–122.
Salón, J.L., Chirivella, C. and Castel, J.R. (2005) Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. American Journal of Enology and Viticulture **56**, 1–8.
Salón, J.L., Méndez, J.V., Chirivella, C. and Castel, J.R. (2004) Response of *Vitis vinifera* cv. 'Bobal' and 'Tempranillo' to deficit irrigation. Reynolds, A.G. and Bowen, P., eds. Proceedings of the XXVI international horticultural congress; 11–17 August 2004; Toronto, Canada. Acta Horticulturae **640**, 167–174.
Santesteban, L.G., Miranda, C. and Royo, J.B. (2011) Regulated deficit irrigation effects on growth, yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv.'Tempranillo'. Agricultural Water Management **98**, 1171–1179.
Santesteban, L.G., Miranda, C., Urrestarazu, J., Loidi, M. and Royo, J.B. (2017) Severe trimming and enhanced competition of laterals as a tool to delay ripening in Tempranillo vineyards under semiarid conditions. OENO One **51**, 191–203.
Savoi, S., Wong, D.C., Arapitsas, P., Miculan, M., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F. and Castellarin, S.D. (2016) Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). BMC Plant Biology **16**, 67.
Schneider, C., Rasband, W. and Eliciri, K. (2012) NIH image to image J: 25 years of image analysis. Nature Methods **9**, 671–675.
Schultz, H.R. (2016) Global climate change, sustainability, and some challenges for grape and wine production. Journal of Wine Economics **11**, 181–200.
Schultz, H.R. and Stoll, M. (2010) Some critical issues in environmental physiology of grapevines: future challenges and current limitations. Australian Journal of Grape and Wine Research **16**, 4–24.
Stoll, M., Lafontaine, M. and Schultz, H.R. (2010) Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis vinifera* L. Riesling. Progrès Agricole et Viticole **127**, 68–71.
Tonietto, J. and Carbonneau, A. (2004) A multicriteria climatic classification system for grape-growing regions worldwide. Agricultural and Forest Meteorology **124**, 81–97.
Vitrac, X., Larronde, F., Krisa, S., Decendit, A., Deffieux, G. and Mérillon, J.M. (1999) Sugar sensing and Ca 2+ calmodulin requirement in *Vitis vinifera* cells producing anthocyanins. Phytochemistry **53**, 659–665.

Manuscript received: 12 February 2018
Revised manuscript received: 29 June 2018
Accepted: 6 July 2018

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12368/abstract.

Figure S1. Appearance of Tempranillo irrigated-defoliated vines at full veraison in 2014.