Effect of delaying winter pruning of Bobal and Tempranillo grapevines on vine performance, grape and wine composition

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Short title: Effect of late pruning in Bobal and Tempranillo

ABSTRACT

Background and Aims: Climate change is advancing grapevine phenology, decoupling berry technological and phenolic maturity. The aim of this study was to assess the usefulness of late winter pruning for delaying grape ripening.

Methods and Results: In two Bobal and Tempranillo vineyards located in eastern Spain, vines pruned at dormancy were compared with a delayed winter pruning (late pruning) carried out just before the basal buds burst (BBCH 05). In both cultivars, late pruning delayed grape ripening, resulting in grapes with higher anthocyanin concentration for similar TSS at harvest. In general, the resulting wines had a higher colour intensity and lower hue angle. These effects were more noticeable in Tempranillo, an earlier ripening cultivar, than in Bobal. Despite late pruning improving vine water status, the yield was steadily reduced by 10%.

Conclusions: Late pruning is a useful strategy for delaying grape ripening, thus adapting grapevine production to climate change. The small detrimental effects on yield and compression of pruning dates within a few days may limit the application of this practice.

Significance of the Study: Delaying winter pruning can be used to improve grape and wine composition when facing changes in the meteorological conditions experienced during berry ripening.

***Keywords**: alcohol concentration, global warming, technological and phenolic maturity, vine yield*, Vitis vinifera

Introduction

In the Mediterranean-like climate, viticulture is a farming system that is particularly sensitive to the potential effects of climate change, which can affect both vine performance and grape and wine composition, challenging the overall sustainability of the entire grape and wine value chain (Intergovernmental Panel on Climate Change 2014, Cramer et al. 2018). The suitability of winegrape growing regions is expected to shift upward in elevation and/or poleward (Ollat et al. 2016), but climate is not the only factor accounting for regional suitability to viticulture. For instance, the soil characteristics and the local cultivars, as well as other socio-economic features, must be considered when assessing the feasibility of a given region for grapevine cultivation (Keller 2010, van Leeuwen et al. 2019).

The most remarkable aspect of the vine that is affected by climate change is phenology, because it is directly influenced by temperature (Parker et al. 2011, Sadras and Moran 2013, Molitor et al. 2014). In fact, the period between budburst and harvest is becoming shorter and earlier worldwide (Jones et al. 2005, Petrie and Sadras 2008, Webb et al. 2012). The main impact of the advancement and compression of vine phenology is that grape ripening is occurring under increasingly warm conditions. In warm viticulture areas, the main consequences of an advancement in phenology is the decoupling between the accumulation of berry sugar and grape phenolic maturity, resulting in grapes harvested at a high sugar level in order to reach a complete phenolic maturity (Sadras and Moran 2012, van Leeuwen and Destrac-Irvine 2017).

Viticulture adaptation techniques can be applied to mitigate the effects of global warming on the development of grapevine phenology (van Leeuwen et al. 2019). These techniques include the modification of the vine water and source:sink relations (Parker et al. 2016, Santesteban et al. 2017). For instance, improving vine water status by increasing irrigation regimes has been shown to increase vegetative growth and yield, often resulting in a delay in the harvest date (Mirás-Avalos and Intrigliolo 2017). In contrast, reducing the vine source capacity by defoliation should result in a delay in the capacity of the vine to produce carbohydrates, therefore reducing the accumulation of grape sugar. Several studies with many cultivars have shown that ripening rates can be reduced after defoliation, but with contradictory results in terms of grape composition (Stoll et al. 2010, Poni et al. 2013, Intrieri et al. 2017). Buesa et al. (2019) reported a concomitant decrease in grape anthocyanin accumulation parallel with a decrease in berry sugar concentration when late leaf removal was applied in Bobal and Tempranillo vines in eastern Spain. Therefore, additional practices need to be explored to counteract the effect of increasing temperature on vine phenology and the reduction of the ratio of grape phenolic substances-to-technological ripeness.

It is well known that winter pruning applied later in the season, at the swelling bud stage, is effective in diminishing the risk of spring frost damage because it delays the date of budburst by a few days (Coombe 1964, Palliotti et al. 2014). Therefore, it is possible to postpone the entire phenological cycle as an alternative to shifting the ripening process to a cooler period (Friend and Trought 2007). With this goal in mind, the interest in 'late pruning' has been renewed as an alternative adaptation technique against climate change (Frioni et al. 2016, Gatti et al. 2016, Petrie et al. 2017, Zheng et al. 2017, Allegro et al. 2020). Delaying winter pruning until the shoot apical nodes initiate budburst (spring) postpones the development of basal nodes and all the subsequent phenological stages of the vine (Martin and Dunn 2000). Previous studies related to late pruning have reported delays in budburst, ranging from 24 to 32 days in Carignan and Grenache cultivars (Vergnes 1981) to 5 days in Cabernet Sauvignon (Martin and Dunn 2000). The yield response to the late pruning technique has had discordant effects as compared with normal (winter) pruning. Some authors observed a significant decrease in yield (Gatti et al. 2016, Palliotti et al. 2017, Zheng et al. 2017, Gatti et al. 2018, Allegro et al. 2020), while others reported an increase (Moran et al. 2017) or even inconsistent results (Friend and Trought 2007, Petrie et al. 2017). Yield losses may depend on pruning time, and when it is late, it has caused a yield loss of up to 100 and 71% in Sangiovese and Merlot, respectively (Frioni et al. 2016, Allegro et al. 2020). This could be attributed to the depletion of reserves that were first mobilised for the budburst of distal buds, which impairs the correct development of inflorescences from inflorescence primordia (Lebon et al. 2008, Levin et al. 2020). Nevertheless, late pruning has the potential of improving grape composition due to shifts in environmental conditions. Titratable acidity can be increased, as can the concentration of anthocyanin and phenolic substances (Parkin and Turkington 1980, Frioni et al. 2016, Moran et al. 2017, Palliotti et al. 2017, Gatti et al. 2018). Occasionally, the delay provoked in budburst can be offset at harvest because of the lower crop load of the late-pruned vines (Gatti et al. 2016). This could prevent ripening from occurring during cooler periods, but could attain an effect on grape composition similar to that of bunch thinning (Intrigliolo and Castel 2011). On other occasions, delay in the harvest date can have neutral effects on grape composition (Petrie et al. 2017, Allegro et al. 2020). This occurs because grape ripening depends on the complex interaction between source-to-sink ratio and the environmental conditions resulting from late winter pruning and the subsequent phenological delay (Buesa et al. 2019). Moreover, environmental conditions affect grape ripening differently depending on the cultivar (de Oliveira et al. 2015).

Overall, late winter pruning appears to be a promising technique for delaying grape ripening. Further studies, however, are needed to clarify its repeatability and the consistency of its effects under a wide array of cultivars and environments. The goal of our present research study was to assess the effects of delaying winter pruning until spring in two deficit-irrigated vineyards of Bobal and Tempranillo in the semi-arid and temperate-warm environment of eastern Spain. A comprehensive assessment was carried out over three seasons including aspects of vine performance and grape and wine composition.

Materials and methods

Site and crop

The trial was carried out from 2015 to 2017 in a commercial vineyard (*Vitis vinifera* L.) located in Requena, Valencia, Spain (39º30´18.10´´N, 1º13´54.30´´W; elevation 746 masl). The trial was conducted in two adjacent plots planted with Bobal (grafted onto 110 Richter) and Tempranillo (grafted onto 161-49 Couderc) vines. For both vineyards, vines were pruned, leaving six or ten two-bud spurs per vine in Bobal and Tempranillo, respectively. The shoots were trained to a bilateral cordon system in a north-south orientation, and were positioned vertically with a pair of steel catch wires. Canopy management practices included manual shoot thinning before the onset of flowering and no shoot hedging. The Bobal vineyard was planted in 2002 at a spacing of 2.5 x 1.45 m (2759 vines/ha), whereas the Tempranillo vineyard was planted in 1991 at a spacing of 2.5 m x 2.5 m (1600 vines/ha). The two vineyards were independently deficit-irrigated with 3.2 L/h drippers spaced every 1.25 m for more than 10 years before the present experiment.

The vineyard's soil was a Typic Calciorthid according to the Soil Taxonomy classification (United States Department of Agriculture 1999), with a clay-loam to light clay texture, highly calcareous (37%) and with low fertility (0.66% in organic matter and 0.04% in nitrogen). Soil depth was greater than 2 m with 200 mm/m of available water capacity. The climate in this area is classified as semi-arid hot-summer Mediterranean (Rodríguez-Ballesteros 2016), the heliothermal index of Huglin (Huglin 1978) was 2291°C corresponding to a temperate-warm viticultural climate, with cool and moderately dry nights according to the classification system for grapegrowing regions proposed by Tonietto and Carbonneau (2004). At the experimental site, the annual average values (for the 2002–2014 period) of the reference evapotranspiration (ET_o) and the rainfall were 1095 and 398 mm, respectively. Deficit irrigation was applied to maintain midday stem water potential (Ψ_{stem}) above the threshold values of -1.15 and -1.40 MPa in pre- and post-veraison, respectively.

Experimental treatments

In both cultivars, two pruning times were assessed: Winter pruning (WP) and late pruning (LP). The two treatments applied had four replicates (blocks). In both plots, the experimental design was a randomised block design. Each block consisted of 20 vines (*n* = 20). In both vineyards, 80 vines per treatment were used.

Winter pruning was applied manually when buds were dormant, at the phenological stage number 00 in the BBCH-scale (Lorenz et al. 1995), whereas the vines were late pruned when the two basal buds were swelling or at 'wool stage' (BBCH 05). The phenological stages of the basal buds corresponded to clearly visible green tips or already unfolding first leaves (BBCH 09–13) in the apical shoot buds (see Figure S1).

Field measurements

Weather data were recorded at an automated meteorological station located within the vineyard perimeter. Reference evapotranspiration (ET_o) was calculated with the Penman–Monteith equation (Allen et al. 1998). Thermal time for a specific period was computed as the sum of the average daily temperature above a threshold 10°C (Amerine and Winkler 1944). In both plots, the amount of water irrigated was measured with in-line water meters. Time periods were measured as days of the year (DOY). Midday Ψ_{stem} was determined on four dates per season with a pressure chamber (Model 600, PMS Instrument, Albany, OR, USA) on bag-covered leaves from two representative vines per block at midday (measurements were carried out between 1130 and 1230 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 h prior to measurement (Choné et al. 2001).

Vine phenology was monitored at the main phenological stages, such as budburst, flowering and veraison. Visual observations were carried out in two shoots per vine in each experimental vine on the same day for both treatments in each cultivar. Each treatment was harvested when fruit reached approximately 22°Brix (commercial harvest), and therefore, fruit was harvested at different dates depending on the treatment and cultivar. Grape 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. In 2018, when the treatments were not applied, vines were assessed to evaluate possible carry-over effects of the consecutive experimental seasons on shoot fruitfulness. Pruning fresh mass was recorded for each experimental vine (2015–2017) with the exception of Tempranillo in 2015 as the vineyard owner had previously carried out mechanical pre-pruning.

Grape and wine composition

Ripening of berries was assessed approximately every 10 days, starting from shortly before veraison until harvest, except for phenolic composition, which was determined only after veraison. Berry fresh mass was determined on two random samples of 100 berries per replicate. Seventy berries were crushed and hand pressed through a metal screen filter and used to evaluate technologically-defined maturity, whereas the remaining 30 berries were homogenised with an Ultra-Turrax T25 blender (IKA-Werke, Staufen, Germany) and used for phenolic maturity. Must TSS was determined by refractometry (PR-101, Series Palette, Atago, Tokyo, Japan), and pH and 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 equiv. The concentration of anthocyanin and phenolic substances (expressed as malvidin equiv.) was determined in duplicate by UV/Vis light spectrophotometry (Iland et al. 2004). The phenolic substances at harvest were also calculated on a per vine basis as the product between the anthocyanin concentration in the berries and the average vine yield for each replicate.

Grapes from each replicate were separately vinified at the experimental winery at the Technological Institute of Viticulture and Oenology of Requena, Valencia, Spain. Thus, in each season, 16 wines were produced, eight for Bobal and eight for Tempranillo. Grapes were mechanically crushed, destemmed, and fermented at a temperature of approximately 22ºC in 60

L stainless steel containers. Potassium metabisulfite ($K_2S_2O_5$) was added to all the musts, at a ratio of 10 g/100 kg of grapes, after which they were inoculated with 20 g of commercial *Saccharomyces cerevisiae* yeast which were previously hydrated at 37ºC for 30 min (FR Excellence, Lamothe-Abiet, Bordeaux, France). Skin contact time was 7 days, and during this period the fermentations were punched down twice-daily. After maceration, the wines were pressed and decanted into 30 L demijohns, where they completed the alcoholic fermentation. All the wines were stored at approximately 20ºC for 3–4 months before analysis. The composition of the wines was analysed once the spontaneous MLF ended. Fourier-transform infrared spectroscopy (BACHUS II, TDI, Barcelona, Spain) was used for determining alcohol concentration, TA, pH and glycerol concentration. Phenolic composition was determined by measuring the optical density (OD; nm) by spectrophotometry (Ati-Unicam UV-4, Thermo Fisher Scientific, MA, USA), as described by Ribéreau-Gayon et al. (2000): anthocyanin (OD520–OD860), phenolic substances (OD280-OD860), wine colour intensity (OD420+OD520+OD620–OD860) and hue (OD420/OD520). All analytical determinations in grape, must and wine were in duplicate.

Data analysis

Data from the two cultivars (Bobal and Tempranillo) were analysed separately because vines were of different ages, on different rootstocks and with different vine spacing. For each cultivar, a two-way ANOVA was used to test the effect of the treatment (pruning time) and seasons and treatment per season interaction (T*S) on vine traits and grape and wine composition attributes. In case the ANOVA detected significant differences (P<0.05), mean separation was assessed either with the LSD 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). Additionally, linear and exponential regressions were calculated using SigmaPlot (version 11.0) (Systat Software, San Jose, CA, USA). Differences between the regression's residuals were used to test the effects of the treatment on each cultivar

(Sadras and Moran 2012). The regression equations for each cultivar were fitted to the grape composition data and the effect of the treatments on residuals was tested with an ANOVA ($P<0.05$). The null hypothesis is that for each cultivar, the residuals of LP versus WP are statistically undistinguishable.

Results

Meteorological conditions and vine phenology

The climatic conditions during the trial were indicative of two standard seasons (2015 and 2016) while 2017 was drier (Figure S2). During the three growing seasons, from 1 April to 30 September, ET_o was 917, 924, and 925 mm (Table S1), and the rainfall was 207, 166, and 119 mm in 2015, 2016 and 2017, respectively (Figure S2). Irrigation volumes applied in each season during those periods are shown in Table S1. Over the seasons, the average fraction of ET_o received through rainfall and irrigation in the Bobal and Tempranillo plots was 34, 25, and 20% and 26, 24, and 20%, respectively.

The timing for the main phenological periods is summarised in Table 1. In the WP treatments, budburst took place during the first half of April in Tempranillo (DOY ≈ 100) and during the second half in Bobal (DOY ≈ 110), which corresponded to a thermal time from budburst of 85 and 130ºC day, respectively. In both cultivars, late pruning (LP) significantly delayed budburst, about 15–20 days on average as compared to WP. Flowering in WP vines occurred in late May or early June (DOY ≈ 140–150), with veraison occurring in late July or early August (DOY ≈ 205–225), whereas in LP these phenological stages were delayed by about 10 days. During the grape ripening period (from veraison to harvest), the average maximum air temperature was 33.1, 33.4, and 32.8ºC in 2015, 2016 and 2017, respectively (Figure S2). The delay in grape ripening due to LP resulted in an increase in diurnal temperature variation by 0.5ºC and 0.2ºC in Tempranillo and Bobal, respectively. In both cultivars, harvesting took place during the first half of September in the WP treatments, whereas LP vines were harvested approximately 1 week after their respective WP treatment (Table 1). This resulted in a reduction in the temperature during the last week of ripening of 1.1ºC in Tempranillo and 2.0ºC in Bobal when WP was applied.

Vine performance

In both cultivars, vine water status followed a similar seasonal pattern, as the growing conditions were similar in both vineyards (Figure S3). In 2015, there was no significant difference in Ψ_{stem} between WP and LP vines either in Bobal or in Tempranillo. Interestingly, in both cultivars, on some dates in 2016 and 2017, the LP vines showed a significantly less negative Ψ_{stem} value than that of the WP vines.

The effects of pruning time on vine yield were consistent among seasons in both cultivars (Table 2). Nonetheless, there was a significant interaction between treatment and season for some of the parameters evaluated. In addition, the season had a significant effect on most of the vegetative and production parameters. In both cultivars, pooling data across seasons, yield for the LP treatments was significantly reduced by 10% (Table 2). The number of bunches per vine was not affected in the 3-year average in either cultivar. This parameter was significantly affected only in Bobal in 2015, where LP caused an increase which could not be explained by the treatment applied. Late pruning treatments significantly reduced bunch mass by 20 and 8% in Bobal and Tempranillo, respectively. This effect was more noticeable in Bobal than in Tempranillo, because in Bobal the LP treatment had significantly lighter bunches than WP in every single season. In both cultivars, however, there was an interactive effect of the treatments and season on bunch mass. The number of berries per bunch was not significantly affected by pruning time in any cultivar (Table 2). In both cultivars, however, berry mass was significantly reduced by 12% in LP compared to WP. Nonetheless, this effect was of different magnitude depending on the season as shown in Figure 1, which depicts the berry mass changes.

Vegetative growth, assessed by means of pruning mass, was significantly reduced in LP treatments in two seasons in Bobal, whereas in Tempranillo, it increased significantly in 2016 (Table 2). The number of shoots per vine was not significantly affected by pruning time (data not shown), while shoot fruitfulness (number of bunches per shoot) decreased significantly in LP compared to that of WP. This effect was significantly noticeable only in 2017 and in 2018 but not in previous seasons.

Grape ripening

In both cultivars, berry ripening was significantly affected by pruning time (Figure 1) and this translated into differences in berry composition at harvest (Table S2). Harvest took place when the TSS reached 21.5 and 22.4ºBrix on average in Bobal and Tempranillo grapes, respectively. Despite the goal being to harvest the grapes from the two treatments at a similar TSS value each season, there were significant differences between WP and LP for Bobal berries (Table S2). Thus, the results of grape composition are shown as the ratios between TSS and TA, pH, anthocyanin and phenolic substances, respectively (Table 3). At harvest time, both TSS-to-TA and TSS-to-pH ratios did not show a clear pattern between treatments on either of the two cultivars, as evidenced by the significant T x S interaction (Table 3). Nevertheless, TA in LP tended to be higher for the same date than in WP in both cultivars (Figure 1). Moreover, in Tempranillo grapes, the relationship between TSS and TA during ripening indicates that LP tended to cause a slight increase in TA in comparison to that of WP (Figure 2a), although this effect became less clear near harvest time (at higher TSS concentration). In Bobal grapes, no difference was observed in the relationship of TSS and TA between treatments throughout the ripening period, as shown in the adjacent bar chart (Figure 2c).

After pooling the data across seasons, the concentration of phenolic substances at harvest significantly increased in response to LP in both cultivars as compared to WP (Tables 3, S2). In both cultivars, the ratio of phenolic substances-to-TSS and of anthocyanin-to-TSS at harvest

(Table 3) tended to be higher in the grapes from the LP treatment compared to the that of the WP treatment. But, pooling data across seasons, this effect was significant only for the first ratio in Bobal grapes (+9%) and for the anthocyanin-to-TSS ratio in Tempranillo (+26%). In Bobal grapes, the anthocyanin accumulation was dissimilar throughout the ripening process (Figure 1). Regardless of this, in both cultivars, the content of anthocyanin synthesised per vine increased by 31 and 36% in response to LP when compared to WP (Table S2). In fact, LP increased grape anthocyanin at equal TSS as compared to WP berries (Figure 2b). In both cultivars, this increase was noticeable from the start of the ripening process (veraison). Regarding the relationship between anthocyanin and TSS, the differences between the regression equations found for WP and LP were significantly different only for Tempranillo, as depicted in the regression residuals bar chart (Figure 2d).

Wine composition

The composition of the wines made from Bobal and Tempranillo grapes subjected to different pruning times has been split into Table 4 and Table S3. In general, for both cultivars, the wine data reported partially confirmed the results observed for the grapes of both cultivars. Regardless of the alcohol concentration, there were no differences in wine pH between treatments. Late pruning caused an 18% increase in colour intensity as compared to that of WP (Table 4), however, this effect was significant only for the Tempranillo wines. The concentration of phenolic substances and anthocyanin showed a similar trend as colour intensity. That is, both cultivars increased their values in LP wines as compared to the WP wines, but without statistically significant differences in Bobal wines. In contrast, in both cultivars, the hue angle of the wines was reduced by 6% in response to the LP with respect to the WP.

Discussion

Delaying winter pruning has been shown to be effective in modifying vine phenology in both grapevines cultivars tested (Table 1). The shifts in phenology influenced grape composition during the ripening period, for both the primary (TSS and TA) and the secondary metabolites (phenolic substances) (Figure 1). The delay was attenuated as the phenological cycle progressed due to the greater thermal time accumulation per unit of time as day length increased (Parker et al. 2011, Molitor et al. 2014), resulting in a harvest delay of 1 week on average (Table 1). These effects were in agreement with findings from other authors with cvs Grenache, Carignan, Shiraz, Cabernet Sauvignon, Sangiovese, Maturana, Pinot Noir and Merlot (de Oliveira et al. 2015, Frioni et al. 2016, Moran et al. 2017, Palliotti et al. 2017, Petrie et al. 2017, Zheng et al. 2017, Gatti et al. 2018, Silvestroni et al. 2018, Allegro et al. 2020).

The effectiveness of the late pruning technique for improving grape composition was confirmed in both Bobal and Tempranillo (Tables 3 S2 and). Grapes from the LP treatments had a higher concentration of phenolic substances for the same sugar concentration and lower pH, which is in line with what was observed in other cultivars (Moran et al. 2017, Palliotti et al. 2017, Zheng et al. 2017, Gatti et al. 2018, Silvestroni et al. 2018). It is worth mentioning that the significant effect of the interaction of treatment and season on some of grape composition parameters at harvest could be attributed to the effect that environmental conditions also exert on grape ripening. For this reason, a study of the dynamics of ripening is needed to better understand the berry composition data reported. In Tempranillo, for instance, the exponential decline in TA relative to TSS was significantly greater in LP than in the WP from the start of ripening (Figure 2a). In spite of this, the TSS-to-TA ratio at harvest was not consistent between seasons (Table 3). Nevertheless, the greatest effect of the pruning time on the composition of the grape was on the concentration of phenolic substances (Tables 3, S2). In both cultivars, the LP treatments tended to increase the anthocyanin accumulation against TSS as compared to WP (Figure 2b), confirming the potential of the LP technique to decouple the ripening dynamics of sugars and phenolic substances (Sadras and Moran 2012, Frioni et al. 2016). Nonetheless, in Bobal grapes, the increase in the anthocyanin-to-TSS ratio was less noticeable than in Tempranillo, mainly at a high concentration of TSS. The greater effect found in Tempranillo as compared to Bobal points to a different cultivar sensitivity, perhaps linked to the earlier period of ripening. In fact, Tempranillo is an early ripening cultivar while Bobal is a mid-late season cultivar.

Wine composition partially confirmed the effect of the pruning treatments observed on the grapes, which is consistent with what other authors found (Moran et al. 2018b). Differences between treatments were augmented in Bobal and slightly attenuated in Tempranillo wines (Table 5). In this sense, the overall effects of pruning time on wine composition were in line with what was intended. In both cultivars, the higher colour intensity, phenolic substances and anthocyanin pigments found in response to LP as compared to WP are desirable attributes, and it is in line with what Moran et al. (2018b) observed in Shiraz wines. The effect of pruning times on wine hue angle suggests that the different timing of ripening could also affect the type of berry pigments synthesised. Bobeica et al. (2015) reported this effect in response to late pruning for Sangiovese and Cabernet Sauvignon. In our trial, in both cultivars, LP tended to increase the violet tones (%blue). This is an interesting modification, as violet tones are more commonly obtained from more mature grapes, which usually contain a higher concentration of co-pigments, thereby indicating an aptitude for wine ageing (Alcalde-Eon et al. 2014).

Among the different factors that may have influenced grape and wine composition, the combination of temperature and light intensity was the most likely influence of the differences between treatments. In both cultivars, the difference between WP and LP in average temperature during the last week of ripening was greater in Bobal than in Tempranillo (Figure S2), but it is in the latter in which the effect on composition was more marked (Figure 1). That is because a harvest delay did not imply an improvement in the anthocyanin-to-sugar ratio per se if the photosynthetic capacity of the vine or the environmental conditions were not substantially improved (Buesa et al 2019). Late pruning can be effective only when the environmental conditions may limit the achievement of the desired grape ripeness (Mori et al. 2007, Sadras and

Moran 2012). In fact, Moran et al. (2018a) found an interaction between timing of pruning and temperature, whereby late pruning enhanced grape phenolic substances-to-sugars ratio in heated vines but not in the unheated Control. Thus, as the maximum temperature during ripening was lower in Bobal than in Tempranillo, the synthesis of phenolic substances was supposed to be greatly favoured in Tempranillo as compared to Bobal. This is due to the strong response of phenolic substances to temperature, as opposed to the stability of sugars (Sadras et al. 2007).

Other factors, such as vine crop load, berry size (Table 2) or vine water status (Figure S3), could also influence grape ripening. The effect of the source-to-sink ratio on grape composition has been widely studied (Kliewer and Dokoozlian 2005, Salón et al. 2005, Intrigliolo and Castel 2011, Parker et al. 2016). The yield-to-pruning mass ratio obtained here did not show a clear pattern in response to the pruning time (Table 1). According to the vine balance obtained here, our vines could be considered to be in the optimum range for producing high quality grapes. For this reason, we considered that the effect of pruning time on vine balance was not the main influence of the final effects on grape composition observed here. Nevertheless, in both cultivars, anthocyanin synthesised on a vine basis tended to increase in response to LP (Table S2). The 12% reduction in berry mass may in part explain this effect because of its implications on the skin-to-pulp ratio (Table 2). Regarding vine water status, it is well known that vine water stress promotes the synthesis of phenolic substances (Castellarin et al. 2007, Mirás-Avalos and Intrigliolo 2017), but if excessive, it can be detrimental (Intrigliolo and Castel 2010, Romero et al. 2010). Within the range of Ψ_{stem} value of both cultivars during the three seasons (Figure S3), the slight improvement in LP water status as compared to WP could have enhanced the photosynthetic rates, resulting in a greater synthesis of grape metabolites. The reduction in Ψ_{stem} in response to LP, at least in Bobal, could be due to the reduction in vegetative growth (Table 2), but may also be due to differences between treatments in soil water availability related to the evaporative demand at the time of measurement. This would warrant further analysis in the future, as it might have implications for counteracting the soil water deficits experienced by vines under semi-arid climates.

Having in mind the climate projections which predict global warming for the end of the 21st century ranging from 0.3ºC to 4.8ºC globally relative to the 1986–2005 period, it is probable that for correct grape ripening, greater phenological delays will be required than those obtained here with LP (leading to a 1.1ºC to 2.0ºC reduction during ripening). In this sense, late pruning could be used in combination with other adaptation techniques that could also delay the ripening process in order to achieve a greater delay if necessary (Santesteban et al. 2017). For instance, combining late pruning with pre-veraison irrigation may be beneficial in semi-arid environments, because this irrigation strategy itself can delay the onset of ripening (Castellarin et al. 2007, Buesa et al. 2017). But also, because avoiding pre-veraison water stress can increase main shoot vigour, which may be interesting for the application of LP in the following season. It should be noted that due to apical dominance, the shoot length determines the delay in the pruning time, namely the number of buds per shoot, and therefore phenological delay (Palliotti et al. 2017, Gatti et al. 2018, Silvestroni et al. 2018). Nevertheless, techniques that delay grape ripening further may be needed.

The late pruning technique assessed here reduced yield by only 10%, without a clear cumulative effect (Table 2). Similar effects on yield components were observed in Sangiovese and Merlot (Frioni et al. 2016, Allegro et al. 2019). Other authors, however, observed an important reduction in berries per bunch when winter pruning was delayed (Gatti et al. 2016, Zheng et al. 2017, Silvestroni et al. 2018), which could be associated with a reduction in the proportion of fruitset due to meteorological conditions (May 2000). In our trial, it is possible that the improvement in vine water status observed in LP treatments compared to WP (Figure S3) buffered the yield losses (Levin et al. 2020). Regardless, it is worth noting that the carry-over effects on shoot fruitfulness found in both cultivars of LP were more noticeable in Bobal than in Tempranillo (Table 2). This effect may be due to a depletion of the vine's internal reserves due to their mobilisation for the development of the distal buds (Lebon et al. 2008, Zufferey et al. 2012).

Reductions in shoot fruitfulness in response to late pruning were also observed in Sangiovese and Pinot Noir (Palliotti et al. 2017, Gatti et al. 2018, Silvestroni et al. 2018). In Bobal, this effect may be greater in vines subjected to LP than in Tempranillo because of a shorter period from postharvest to leaf senescence. Nonetheless, this effect did not result in a statistically significant decrease in the number of bunches per vine in any of the two cultivars under study. This suggests that under our conditions, late pruning did not notably affect inflorescence development nor fruitset in either Bobal or Tempranillo. This may be because adult vines are less susceptible to production losses across multiple seasons (Levin et al. 2020) or because the postharvest period was sufficient for the accumulation of reserves.

Conclusions

In a semi-arid and temperate-warm climate, delaying winter pruning until spring (BBCH 09-13) improved grape composition in both Bobal and Tempranillo cultivars. Moreover, these results were mostly magnified in the wines. In both cultivars, yield losses due late pruning (LP) were 10% due to reduction in both bunch mass and berry mass. Therefore, LP appears effective as a field technique for adapting to climate change in semi-arid, hot-summer Mediterranean winegrowing regions. Nonetheless, the increase found in the TA-to-TSS and anthocyanin-to-TSS ratios in grapes was dependent on the cultivar and the environmental conditions, as they were more pronounced in the early-maturing cultivar (Tempranillo) than in the late-maturing (Bobal). This was because the effect on grape composition was mainly due to temperature reduction during ripening as a result of the phenological delay caused by LP rather than to vine water status or source-to-sink ratios. Nevertheless, if global warming exceeds an increase of 1–2°C, further research will be needed for adapting to environmental conditions in order to preserve grape typicity. In addition, the practical application of LP in vineyards that are mainly focused on the production of high-quality wines is limited by the logistical difficulties of carrying out all the pruning operations in a short period of time.

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Tables

Table 1. Effect of winter pruning and late pruning in two vineyards of the cultivars Bobal and Tempranillo (*Vitis vinifera* L.) on crop phenology during three seasons in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Day of year	Treatment: Winter pruning	Treatment: Late pruning
Budburst (%)	Bobal	2015	114	87.8 b	2.9 a
		2016	118	94.2 b	5.6 a
		2017	108	90.2 b	2.2 a
	Tempranillo	2015	97	87.9 b	7.8 a
		2016	109	77.8 b	3.4 a
		2017	100	93.6 b	4.3 a
Flowering (%)	Bobal	2015	148	98.9 b	23.7 a
		2016	158	84.5 b	3.2 a
		2017	151	90.0 b	12.5 a
	Tempranillo	2015	141	67.4 b	2.0 b
		2016	159	75.6 b	24.7 a
		2017	158	93.6 b	31.9 a
Veraison (%)	Bobal	2015	211	33.7 b	3.2 a
		2016	222	24.7 b	1.9 a
		2017	206	55.4 b	5.1 a
	Tempranillo	2015	204	18.1 b	2.1 a
		2016	216	22.3 b	5.8 a
		2017	206	81.9 b	22.3 a
Harvest date (DOY)	Bobal	2015		258	272
		2016		258	265
		2017		247	257
	Tempranillo	2015		252	258
		2016		250	258
		2017		247	247

Data are the average for each treatment in 2015, 2016 and 2017. Within each row, mean values followed by a different letter are significantly different at $P<0.05$. DOY, day of the year.

Table 2. Effect of winter pruning and late pruning in two vineyards of the cultivars Bobal and Tempranillo (*Vitis vinifera* L.) on yield and its components during three seasons in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Treatment		Significance of effects		
			Winter pruning	Late pruning	Treatment	Season	T*S
Yield (t/ha)	Bobal	2015	14.6	14.1	0.683		
		2016	8.1 b	6.4 a	**0.002**		
		2017	8.0 b	6.7 a	**0.027**		
		Average	10.1 b	9.0 a	**0.033**	**0.000**	0.550
	Tempranillo	2015	12.0	10.6	0.230		
		2016	6.7	6.5	0.678		
		2017	9.8	9.3	0.461		
		Average	9.6 b	8.7 a	**0.021**	**0.000**	0.367
Bunches/vine	Bobal	2015	10.1 a	12.5 b	**0.005**		
		2016	13.2	12.3	0.152		
		2017	7.4	7.4	0.962		
		Average	10.2	10.7	0.156	**0.000**	**0.001**
	Tempranillo	2015	24.1	25.0	0.655		
		2016	26.8	25.2	0.180		
		2017	24.7	24.6	0.939		
		Average	25.8	24.1	0.060	0.335	0.450
Bunch mass (g)	Bobal	2015	511 b	388 a	**0.000**		
		2016	232 b	190 a	**0.000**		
		2017	382 b	324 a	**0.001**		
		Average	374 b	301 a	**0.000**	**0.000**	**0.007**
	Tempranillo	2015	293 b	240 a	**0.001**		
		2016	160	154	0.379		
		2017	245	235	0.340		
		Average	231 b	212 a	**0.003**	**0,000**	**0,001**
Berries/bunch	Bobal	2015	169	137	0,068		
		2016	151	147	0,927		
		2017	120	124	0,547		
		Average	147	136	0,432	0,.147	0.523
	Tempranillo	2015	128	143	0.172		
		2016	111 a	117 b	**0.031**		
		2017	118	118	0.940		
		Average	119	126	0.061	**0.000**	0.257
Berry mass (g)	Bobal	2015	3.0	2.8	0.091		
		2016	1.5 b	1.3 a	**0.001**		
		2017	3.2 b	2,6 a	**0.004**		
		Average	2.6 b	2.2 a	**0.000**	**0.000**	**0.086**

	Tempranillo	2015	2.3 b	1.7 a	**0.000**		
		2016	1.4	1.4	0.087		
		2017	2.1	2.0	0.410		
		Average	1.9 b	1.7 a	**0.000**	**0.000**	**0.000**
Pruning mass (t/ ha)	Bobal	2015	1.59 b	1.31 a	**0.001**		
		2016	0.99	1.05	0.561		
		2017	2.32 b	1.55 a	**0.000**		
		Average	1.63 b	1.31 a	**0.000**	**0.000**	**0.000**
	Tempranillo	2015	-	-			
		2016	1.07 a	1.36 b	**0.019**		
		2017	2.34	2.58	0.199		
		Average	-	-			
Yield-to-pruning mass	Bobal	2015	9.5	11.2	0.059		
		2016	3.5	4.1	0.246		
		2017	9.5 b	7.0 a	**0.003**		
		Average	7.4	7.4	0.908	**0.000**	**0.011**
	Tempranillo	2015	-	-			
		2016	6.90	6.14	0.318		
		2017	4.86	4.36	0.456		
		Average	-	-			
Shoot fruitfulness	Bobal	2016	1.00	0.92	0.074		
		2017	0.43 b	0.38 a	**0.046**		
		2018	0.57 b	0.45 a	**0.000**		
		Average	0.66 b	0.58 a	**0.000**	**0.000**	**0.449**
	Tempranillo	2016	1.35	1.27	0.154		
		2017	0.72 b	0.65 a	**0.037**		
		2018	0.73	0.72	0.778		
		Average	093 b	0,87 a	**0.005**	**0.000**	0.370

Data are the average treatment in 2015, 2016 and 2017, except for shoot fruitfulness, with averages only for 2016, 2017 and 2018. Within each row, mean values followed by a different letter are significantly different at *P*<0.05. For data analysis, the statistical significance effect of the treatment, season and their interaction are also indicated. Bold values indicate significant effects of a given factor. Letters on a given parameter and season indicate significant differences between treatments.

Table 3. Effect of winter pruning and late pruning in two vineyards of the cultivars Bobal and Tempranillo (*Vitis vinifera* L.) on the berry composition ratios at harvest during three seasons in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Treatment		Significance of effects		
			Winter pruning	Late pruning	Treatment	Season	T*S
TSS-to-TA	Bobal	2015	0.19 a	0.23 b	**0.000**		
		2016	0.32 b	0.31 a	**0.000**		
		2017	0.24	0.23	0.428		
		Average	0.25	0.25	0.728	**0.000**	**0.004**
	Tempranillo	2015	0.13 a	0.16 b	**0.049**		
		2016	0.20 b	0.18 a	**0.000**		
		2017	0.16	0.18	0.278		
		Average	0.17	0.17	0.332	**0.000**	**0.001**
TSS-to-pH	Bobal	2015	5.44 a	5.59 b	**0.006**		
		2016	7.33 b	6.72 a	**0.000**		
		2017	7.33 b	6.07 a	**0.000**		
		Average	6.28	6.21	0.331	**0.000**	**0.000**
	Tempranillo	2015	5.55	5.86	0.122		
		2016	6.27 a	7.35 b	**0.045**		
		2017	5.94	5.94	0.974		
		Average	5.92 a	6.38 b	**0.037**	**0.000**	0.064
Phenolic substances-to-TSS	Bobal	2015	0.11 b	0.10 a	**0.012**		
		2016	0.15 a	0.20 b	**0.001**		
		2017	0.22 a	0.23 b	**0.005**		
		Average	0.16 a	0.18 b	**0.000**	**0.000**	**0.000**
	Tempranillo	2015	0.04 a	0.06 b	**0.001**		
		2016	0.14	0.14	0.750		
		2017	0.21 a	0.23 b	**0.008**		
		Average	0.16	0.17	0.103	**0.000**	**0.025**
Anthocyanin-to-TSS	Bobal	2015	0.05 b	0.03 a	**0.000**		
		2016	0.06	0.07	0.314		
		2017	0.05 a	0.06 b	**0.000**		
		Average	0.06	0.06	0.985	**0.000**	**0.001**
	Tempranillo	2015	0.05 a	0.06 b	**0.001**		
		2016	0.05 a	0.06 b	**0.003**		
		2017	0.05 a	0.06 b	**0.000**		
		Average	0.05 a	0.06 b	**0.000**	0.165	0.288

Data are the average values for 2015, 2016 and 2017 (*n*=4). Within each row, mean values followed by a different letter are significantly different at *P*<0.05. For data analysis, the statistical significance effect of the treatment, season and their interaction are also indicated. Bold values indicate significant effects of a given factor. Letters on a given parameter and season indicate significant differences between treatments.

Table 4. Effect of winter pruning and late pruning in two vineyards of the cultivars Bobal and Tempranillo (*Vitis vinifera* L.) on the phenolic parameters of wine made from winegrapes during three seasons in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Treatment: Winter pruning		Treatment: Late pruning		Significance of effects: Treatment	Significance of effects: Season	Significance of effects: T*S
Colour intensity	Bobal	2015	7.8		7.0		0.580		
		2016	20.8		21.2		0.937		
		2017	12.3	a	20.3	b	**0.049**		
		Average	13.6		16.1		0.257	**0.000**	0.236
	Tempranillo	2015	7.8	a	10.5	b	**0.021**		
		2016	7.6		7.9		0.854		
		2017	9.1	a	10.4	b	**0.008**		
		Average	8.2	a	9.6	b	**0.022**	**0.032**	0.225
Phenolic substances (AU)	Bobal	2015	46.0		48.2		0.523		
		2016	53.6		55.3		0.675		
		2017	54.2		62.2		0.156		
		Average	51.2		55.2		0.108	**0.004**	0.476
	Tempranillo	2015	53.7		54.9		0.412		
		2016	26.6		27.3		0.664		
		2017	52.7	a	55.9	b	**0.043**		
		Average	44.3	a	46.0	b	**0.040**	**0.000**	0.356
Anthocyanin (mg/L)	Bobal	2015	339.1		333.3		0.887		
		2016	749.7		817.7		0.537		
		2017	713.9		894.1		0.065		
		Average	600.9		681.7		0.172	**0.000**	0.422
	Tempranillo	2015	454.6		447.3		0.839		
		2016	255.7	a	292.8	b	**0.049**		
		2017	618.5	a	697.5	b	**0.008**		
		Average	443.2	a	479.2	b	**0.017**	**0.000**	0.061
Hue angle	Bobal	2015	0.60		0.59		0.675		
		2016	0.39		0.37		0.432		
		2017	0.44	b	0.37	a	**0.006**		
		Average	0.48	b	0.44	b	**0.019**	**0.000**	0.181
	Tempranillo	2015	0.60		0.64		0.092		
		2016	0.63		0.59		0.279		
		2017	0.46		0.45		0.172		
		Average	0.58	b	0.54	a	**0.026**	**0.000**	0.7186

Data are the average values for 2015, 2016 and 2017 (*n*=4). Within each row, mean values followed by a different letter are significantly different at $P<0.05$. For data analysis, the statistical significance effect of the treatment, season and their interaction are also indicated. Bold values indicate significant effects of a given factor. Letters on a given parameter and season indicate significant differences between treatments.

Figure captions

Figure 1. Effect of winter pruning (●,▲) and late pruning (●,▲) of the cultivars Bobal (●,●) and Tempranillo (▲,▲) on the seasonal evolution of (a–c) berry fresh mass, (d–f) TSS, (g–i) TA and (j–l) the concentration of anthocyanin in (a,d,g,j) 2015, (b,e,h,k) 2016 and (c,f,i,l) 2017. Data are the average and SE of four values per treatment and cultivar for each date.

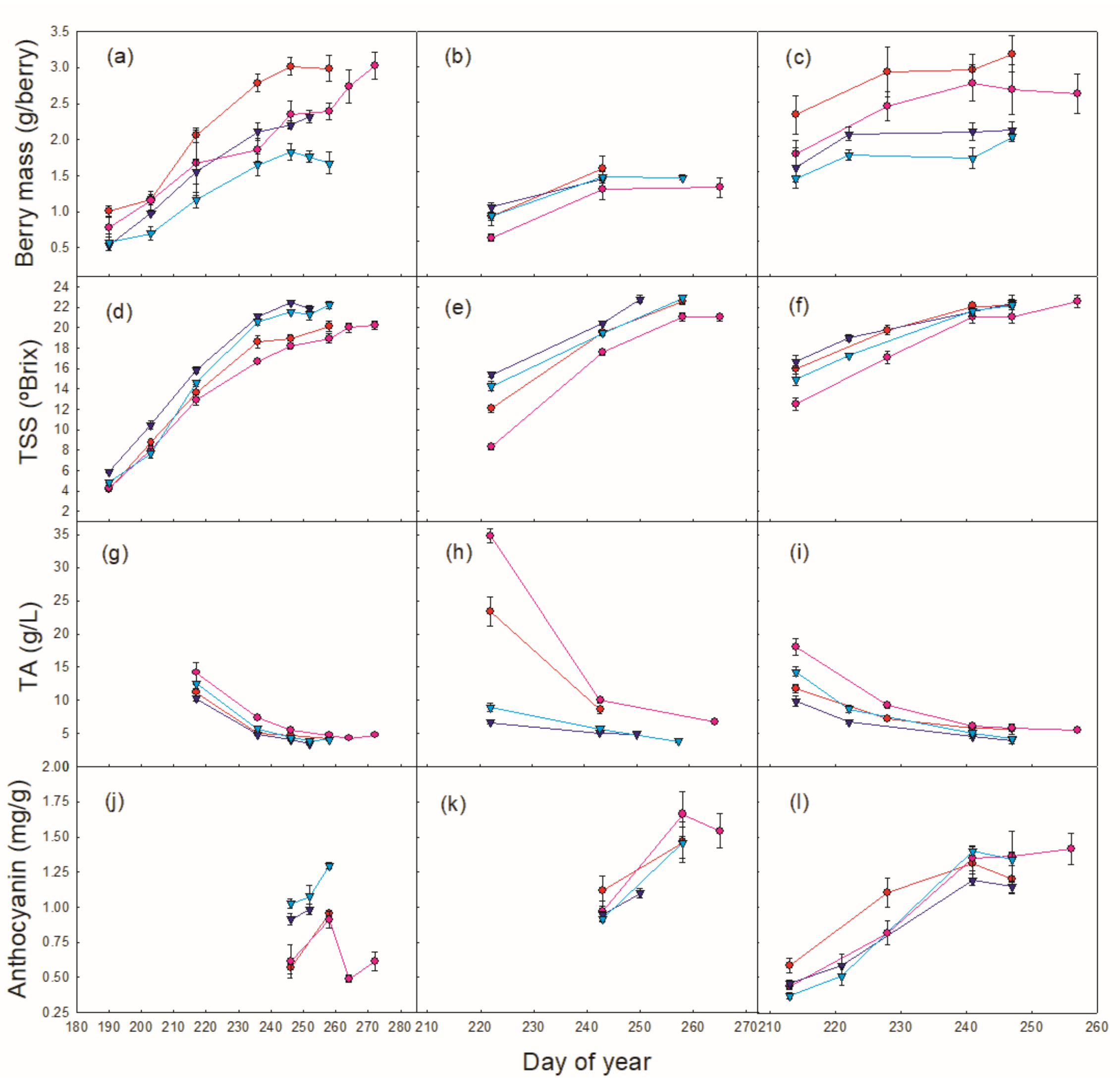
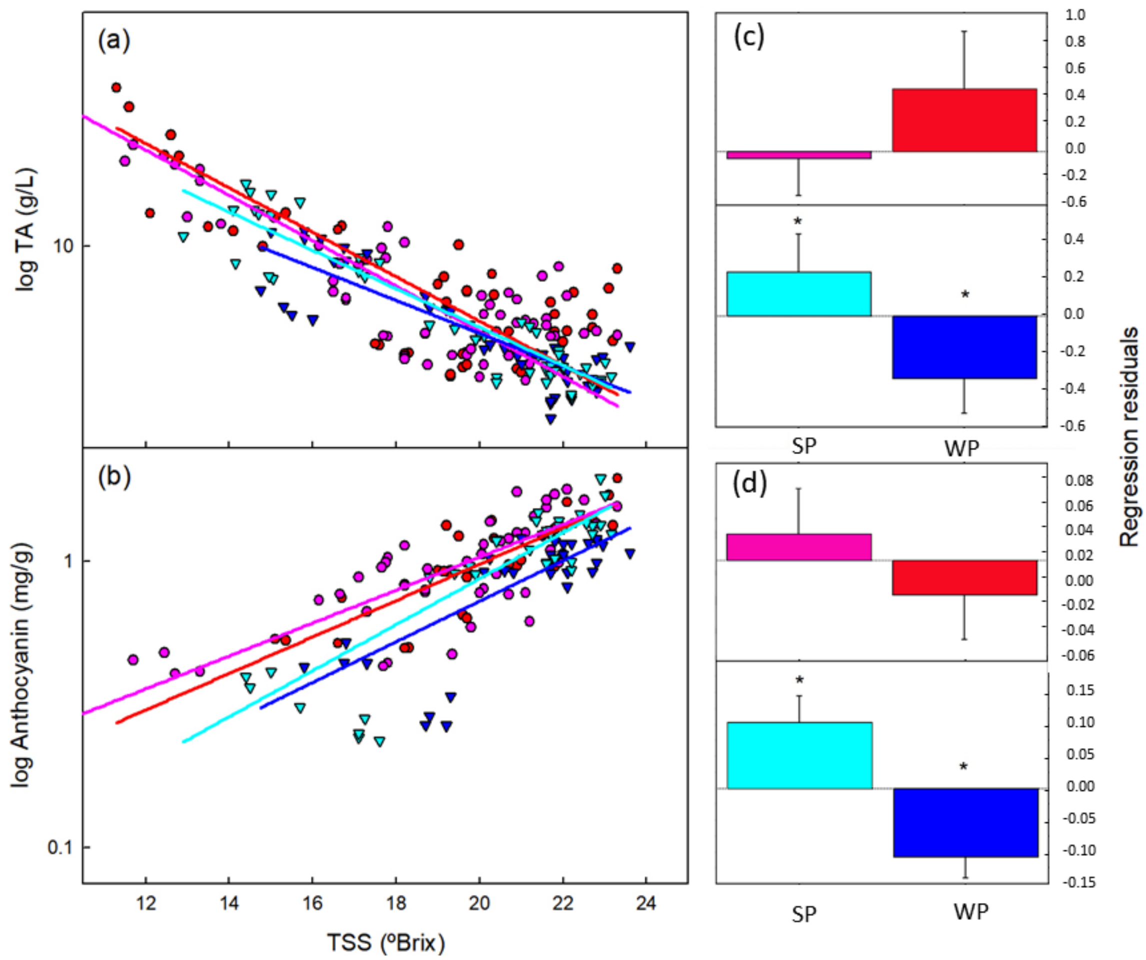


Figure 2. Effect of winter pruning (●,▲) and late pruning (●,▲) of the cultivars Bobal (●,●) and Tempranillo (▲,▲) on the relationship between (a) TSS and TA; and (b) TSS and anthocyanin concentration in berries during 2015–2017. Data are averages of two values per block in each cultivar and each date. Fitted regressions for each treatment and cultivar are shown in their respective colour, as well as the residuals for each cultivar in (c) and (d). Asterisks indicate significant differences in regression residuals between treatments in Bobal and Tempranillo (*P*<0.05). (a) Bobal WP R^2 = 0.74, *P*<0.0001, SE = 0.21; LP R^2 = 0.93, *P*<0.0001, SE = 0.259; Tempranillo WP R^2 = 0.72, *P*<0.0001, SE = 0.189; LP; R^2 = 0.82, *P*<0.0001, SE = 0.213 (b) Bobal WP R^2 = 0.69, *P*<0.0001, SE = 2.815; LP R^2 = 0.56, *P*<0.0001, SE = 2.048; Tempranillo WP R^2 = 0.66, *P*<0.0001, SE = 1.223; LP; R^2 = 0.78, *P*<0.0001, SE = 1.462.



Supplementary information

Figure S1. Detail of grapevine phenology when late pruning was performed in Bobal (a) and in Tempranillo (b) cultivars.

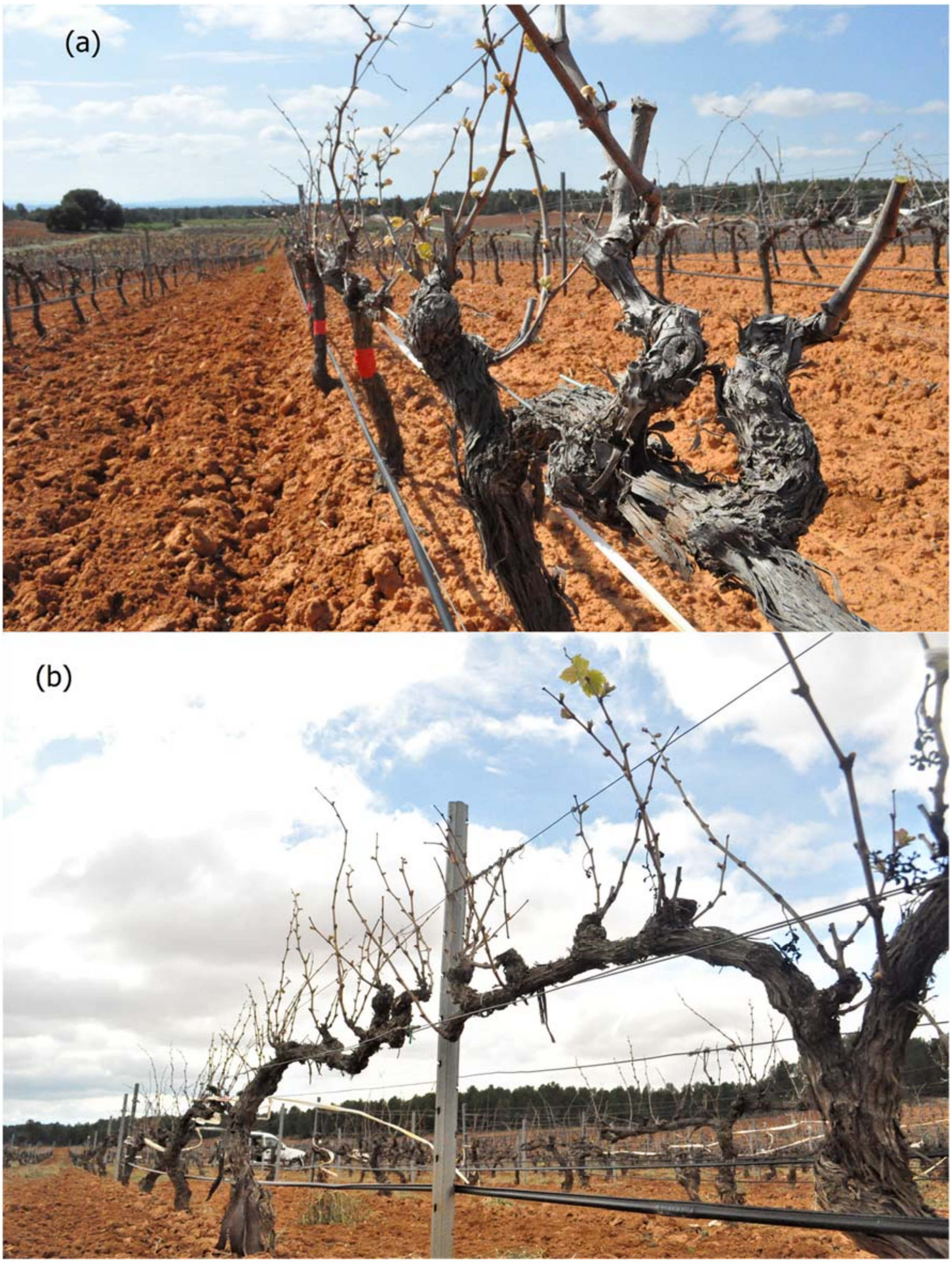


Figure S2. Seasonal patterns of the daily maximum air temperature (●), mean temperature (●), and minimum temperature (□), and rainfall (|) for (a) 2015, (b) 2016 and (c) 2017 in Requena, Valencia, Spain. The day of year follows a continuous time scale starting on 1 January 2015.

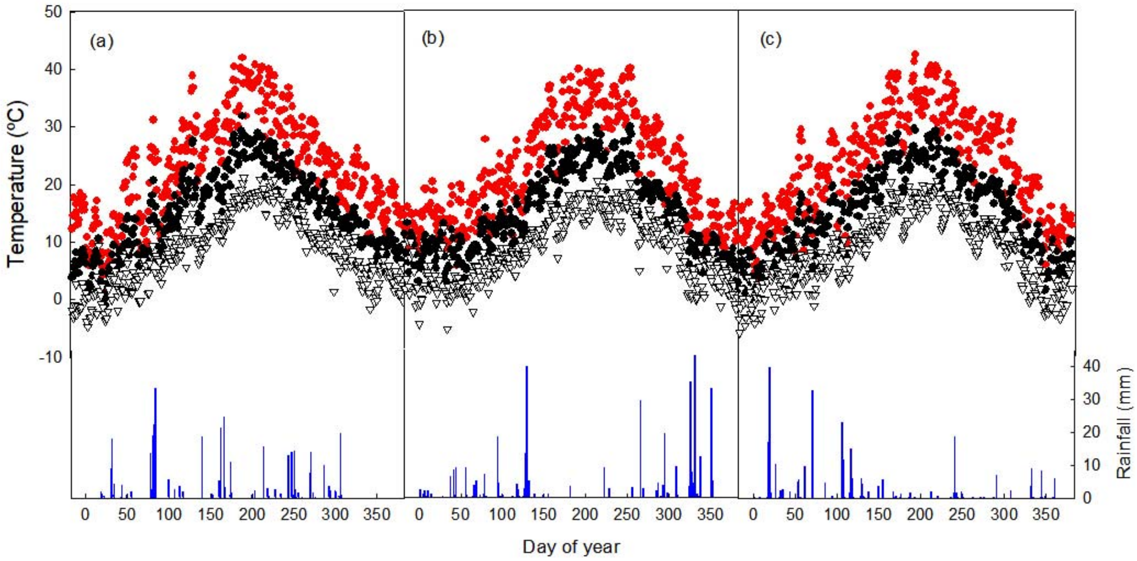


Figure S3. Effect of winter pruning (●, ▲) and late pruning (●, ▲) of the vineyards of the cultivars Bobal (●,●) and Tempranillo (▲, ▲) on the seasonal evolution of midday stem water potential (Ψ_{stem}) in (a,b) 2015, (c,d) 2016 and (e,f) 2017. Data are averages and standard errors of eight leaves per treatment and date.

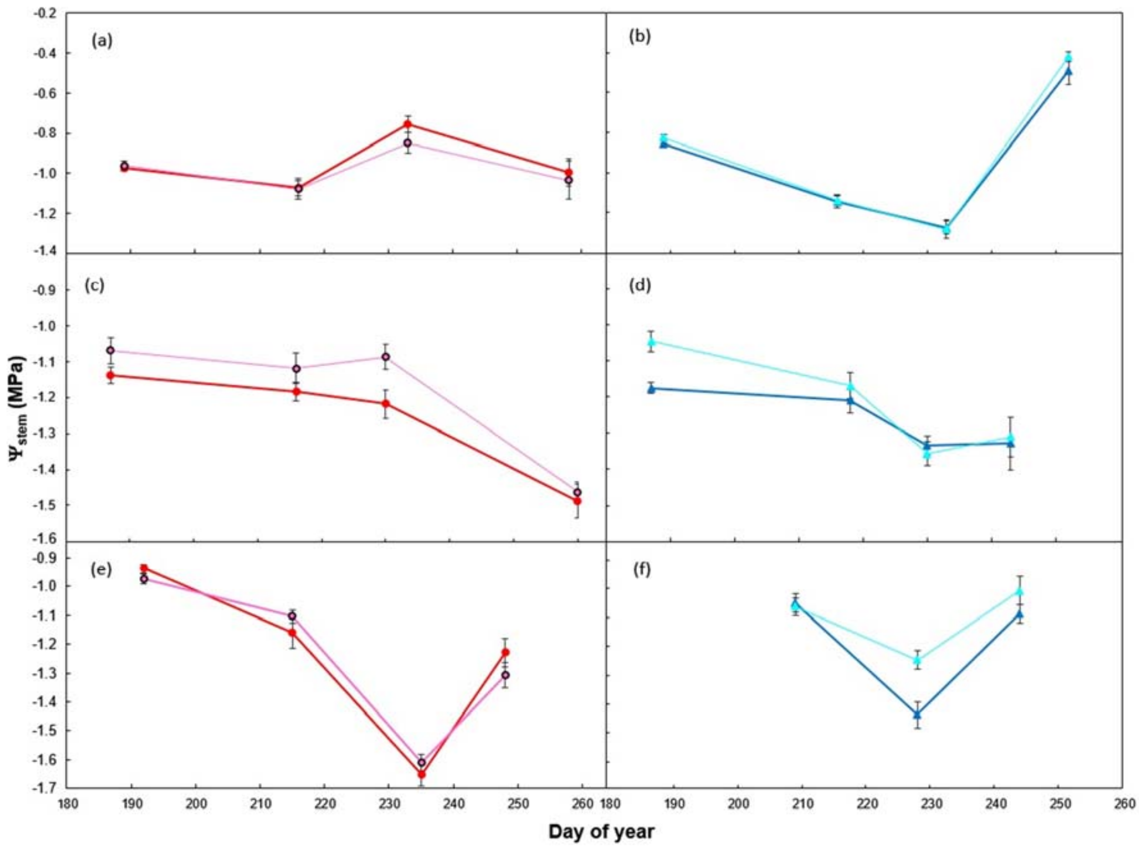


Table S1. Total amounts of water irrigated, rainfall, thermal time and reference evapotranspiration (ET_o) from 1 April to 30 September at the Bobal and Tempranillo experimental vineyards during each of three growing seasons (2015–2017).

Growing season	Irrigation (mm)		Rainfall	Thermal time	ET_o
	Bobal	Tempranillo	(mm)	(ºC day)	(mm)
2015	107.0	30.1	206.6	1934	916.7
2016	61.8	56.0	166.0	1846	923.8
2017	67.7	65.2	118.6	1915	924.5

Data are seasonal averages.

Table S2. Effect of winter pruning nnad late pruning on berry composition attributes at harvest of *Vitis vinifera* L. cultivars Bobal and Tempranillo in the three seasons in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Treatment		Significance of effects		
			Winter pruning	Late pruning	Treatment	Season	T*S
TSS (ºBrix)	Bobal	2015	20.1 a	20.3 b	**0.003**		
		2016	22.6 b	21.0 a	**0.000**		
		2017	22.2 a	22.6 b	**0.000**		
		Average	21.6	21.3	0.189	**0.000**	**0.003**
	Tempranillo	2015	21.7	22.2	0.250		
		2016	22.8	22.9	0.587		
		2017	22.4	22.2	0.228		
		Average	22.3	22.4	0.371	**0.000**	0.198
TA (g/L)	Bobal	2015	3.8 a	4.6 b	**0.000**		
		2016	7.3 b	6.4 a	**0.000**		
		2017	5.3	5.2	0.629		
		Average	5.5	5.4	0.525	**0.000**	**0.001**
	Tempranillo	2015	2.9 a	3.6 b	**0.045**		
		2016	4.6 b	4.1 a	**0.000**		
		2017	3.6 a	3.9 b	**0.021**		
		Average	3.7 a	3.9 b	**0.041**	**0.000**	**0.000**
pH	Bobal	2015	3.70 b	3.61 a	**0.003**		
		2016	3.10 a	3.14 b	**0.017**		

		2017	3.65	b	3.56	a	**0.000**		
		Average	3.48	b	3.44	a	**0.003**	**0.000**	**0.000**
	Tempranillo	2015	3.91	b	3.79	a	**0.044**		
		2016	3.63	b	3.20	a	**0.019**		
		2017	3.77		3.74		0.085		
		Average	3.77	b	3.58	a	**0.008**	**0.000**	**0.034**
Phenolic substances (mg/g)	Bobal	2015	2.3		2.0		0.015		
		2016	3.3	a	4.2	b	**0.004**		
		2017	4.9	a	5.2	b	**0.000**		
		Average	3.5	a	3.8	b	**0.004**	**0.000**	**0.000**
	Tempranillo	2015	2.7		2.7		0.860		
		2016	3.1		3.2		0.644		
		2017	4.7	a	5.1	b	**0.011**		
		Average	3.5	a	3.7	b	**0.048**	**0.000**	**0.083**
Anthocyanin (mg/g)	Bobal	2015	1.0	b	0.6	a	**0.000**		
		2016	1.5		1.5		0.655		
		2017	1.2	a	1.4	b	**0.000**		
		Average	1.2		1.2		0.820	**0.000**	**0.007**
	Tempranillo	2015	1.0	a	1.3	b	**0.000**		
		2016	1.1	a	1.5	b	**0.002**		
		2017	1.1	a	1.3	b	**0.002**		
		Average	1.1	a	1.4	b	**0.000**	**0.038**	0.172
Anthocyanin (g/vine)	Bobal	2015	0.19		0.13		0.105		
		2016	0.52		0.75		0.168		
		2017	0.44		0.63		0.282		
		Average	0.38		0.50		0.122	**0.000**	0.237
	Tempranillo	2015	0.13	a	0.20	b	**0.005**		
		2016	0.27		0.36		0.117		
		2017	0.19	a	0.23	b	**0.045**		
		Average	0.19	a	0.26	b	**0.002**	**0.000**	0.570

Data are the average values for 2015, 2016 and 2017 (n=4). Within each row, mean values followed by a different letter are significantly different at $P<0.05$. For data analysis, the statistical significance effect of the treatment, season and their interaction are also indicated. Bold values indicate significant effects of a given factor. Letters on a given parameter and season indicate significant differences between treatments.

Table S3. Effect of winter pruning and late pruning on the average values of wine composition parameters made from Bobal and Tempranillo winegrapes in Requena, Valencia, Spain.

Parameter	Cultivar	Season	Treatment		Significance of effects		
			Winter pruning	Late pruning	Treatment	Season	T*S
Alcohol (% v/v)	Bobal	2015	11.5	12.4	0.061		
		2016	12.8 b	11.8 a	**0.028**		
		2017	13.1	-			
		Average	-	-	-	-	-
	Tempranillo	2015	12.0	12.7	0.261		
		2016	12.8	13.4	0.211		
		2017	12.6	12.5	0.534		
		Average	12.5	12.9	0.108	**0.040**	0.368
TA (g/L)	Bobal	2015	4.7	4.7	0.557		
		2016	7.5	7.4	0.871		
		2017	6.1	-			
		Average	-	-	-	-	-
	Tempranillo	2015	4.0 a	4.9 b	**0.040**		
		2016	4.6	4.7	0.636		
		2017	5.6 a	5.8 b	**0.042**		
		Average	4.7 a	5.1 b	**0.028**	**0.000**	0.117
pH	Bobal	2015	3.70	3.66	0.287		
		2016	3.19	3.18	0.972		
		2017	3.65	-			
		Average	-	-	-	-	-
	Tempranillo	2015	3.85	3.79	**0.244**		
		2016	3.90	3.90	0.920		
		2017	3.81	3.81	1.000		
		Average	3.85	3.83	0.439	**0.027**	0.618
Glycerol (g/L)	Bobal	2015	9.1 a	10.2 b	**0.004**		
		2016	9.4	8.8	0.180		
		2017	10.0	-			
		Average	-	-	-	-	-
	Tempranillo	2015	9.7	10.8	0.288		
		2016	10.2 a	11.1 b	**0.019**		
		2017	9.7 a	9.9 b	**0.037**		
		Average	9.9 a	10.6 b	**0.035**	0.116	0.561

Data are the average values for 2015, 2016 and 2017 (n=4). Within each row, mean values followed by a different letter are significantly different at $P<0.05$. For data analysis, the statistical significance effect of the treatment, season and their interaction are also indicated. Bold values indicate significant effects of a given factor. Letters on a given parameter and season indicate significant differences between treatments.