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Effects of late winter pruning at different phenological stages on vine yield components and berry composition in La Rioja, north-central Spain

Wei Zheng*, Jesús García, Pedro Balda and Fernando Martínez de Toda

Instituto de ciencias de la vid y del vino, ICVV (Universidad de La Rioja, CSIC, Gobierno de La Rioja), Finca La Grajera, Autovía del Camino de Santiago LO-20 Salida 13, 26007 Logroño, La Rioja, Spain

Abstract

Aims: Under global warming, the desynchrony between technological maturity and phenolic maturity of wine grapes is a worthy concern. Late winter pruning (LWP) has proven to be an effective way to delay grape phenology. The aim of this study was to evaluate the effects of LWP at different phenological stages (based on the Baillod & Baggiolini system) on grape ripening delay, vine yield components, berry composition, and anthocyanin to sugar ratio.

Methods and results: The two-year (2015 and 2016) trial was conducted in the Rioja wine region (north of Spain) on Maturana vines and in each year, four pruning treatments were carried out taking apical buds/shoots as reference: (1) winter pruning at stage A (WPA; dormant bud); (2) LWP at stage C (LWPC; green shoot tip) in 2015 and at stage F (LWPF; inflorescence clearly visible) in 2016; (3) LWP at stage G (LWPG; inflorescences separated); and (4) LWP at stage H (LWPH; flowers separated). LWPC failed to delay the late phenological stages and did not exert an important influence on vine yield and berry composition. LWPG and LWPH succeeded to delay all the phenological stages of grapes, shifting development to a considerably cooler and longer ripening period compared to WPA. Vine yield was not affected by LWPF and was significantly reduced (by 41% on average) by LWPG. LWPH led to greater losses in yield (by 67% on average), especially in 2015. LWPG did not change the fruit composition, while LWPH increased the anthocyanin to sugar ratio and helped to maintain a relatively high level of acidity in berries.

Conclusions: The primary cause of the decline in yield seems to be the reduced berry number per cluster, most likely associated with a loss of flowers and/or a reduction in fruit set percentage in the current season, than losses in inflorescences within buds during the previous season. For Maturana grapes, LWP after stage F could reduce vine yield and be applied as an alternative to the time consuming cluster thinning to meet the needs of yield control. Delaying winter pruning to stage H could improve fruit quality, though the risk of *botrytis* and yield loss would be greater.

Significance and impact of the study: The outcomes of this research open a door for winegrowers to perform yield control in an alternative way. For those who aim for top quality wines (regardless of yield), a very late winter pruning might provide grapes with more desirable attributes. Moreover, winegrowers could postpone the budburst date to some extent, thus reducing the risk of spring frost injury to zero, though this point is not the focus of our study.

Key words: global warming, viticulture, pruning, grape ripening, vine yield, anthocyanins

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*Corresponding author: zheng1987wei@126.com

Introduction

The steady trend of climate warming has had a profound impact on European viticulture (Schultz, 2000). In the Rioja wine region (north of Spain), the average growing season temperature for red wine was 16.3 ºC between 1950-1989 and 18.1 ºC between 1990-1999 while the estimated optimum average value is 17.5 ºC (Jones *et al.*, 2005). Moreover, according to model prediction, this value is expected to increase by 1.33 ºC between 2000-2049 (Jones *et al.*, 2005). That is to say, climate warming is affecting the wine industry of Rioja. One of the greatest problems of high temperatures is the accelerated sugar accumulation, which could result in a high alcohol level accompanied by low acidity, high pH and typical over-ripe aromas in wine (Keller, 2010; Palliotti *et al.*, 2014). Another worrying problem is the temperature-driven decoupling of anthocyanins and sugars in berries of red varieties (Sadras and Moran, 2012), that is, the optimal temperature range for phenol accumulation in berries is lower than that for sugar accumulation (Iland and Gago, 2002). When temperatures are too high, whether during the day or night, anthocyanin synthesis is repressed (Mori *et al.*, 2005; Mori *et al.*, 2007) and berries are less likely to reach the maximum anthocyanin concentration at a regular total soluble solids (TSS) level for harvest (Palliotti *et al.*, 2014). In order to mitigate these effects, a number of cultural attempts have been made in different wine regions around the world to delay grape sugar accumulation so that crops may mature under cooler climatic conditions (Gu *et al.*, 2012; Palliotti *et al.*, 2013; Martínez de Toda *et al.*, 2014; Palliotti *et al.*, 2014; Frioni *et al.*, 2016; Zheng *et al.*, 2017).

Among the cultural approaches, late winter pruning (LWP) has been well known and widely applied since it could delay budburst by a few days, thus reducing the risk of spring frost injury (Trought *et al.*, 1999; Reynier, 2002). Moreover, the timing of budburst exerts a great influence on the subsequent vegetative and reproductive growth (May, 2000). Accordingly, delaying the budburst is a possible way to postpone the following phenological stages, including fruit ripening (Martin and Dunn, 2000; Friend and Trought, 2007). Though temperature is the decisive factor that determines the timing of budburst (May, 2000), there is a general agreement that a delayed budburst can be achieved by LWP (Parkin and Turkington, 1980; Frioni *et al.*, 2016; Gatti *et al.*, 2016). The mechanism of this phenomenon is the imposition of apical dominance, whereby grapevine shoot growth starts in the distal buds of a cane and the development of the basal buds is often inhibited by the budburst of distal buds (Friend and Trought, 2007; Keller, 2015). And then, after a late spur-pruning, basal buds/shoots are forced to break/grow (Howell and Wolpert, 1978).

Though LWP is a promising tool to delay ripening, its effects depend largely on the extent to which the winter pruning is delayed (Palliotti *et al.*, 2014). Before the apical buds open, LWP could merely delay budburst and shoot growth by a few days with limited influence on the subsequent phenological stages (Antcliff *et al.*, 1957; Martin and Dunn, 2000). Nonetheless, Friend and Trought (2007) reported that LWP, shortly before budburst, could alter some yield components depending on the year. On the contrary, alteration neither in yield components nor in grape composition was found for vines pruned before budburst in the study of Frioni *et al.* (2016). After the budburst of apical buds, LWP at stage E (leaves unfolded) and F (inflorescence clearly visible) based on the Baillod and Baggiolini system (Baillod and Baggiolini, 1993; Coombe, 1995) could delay the budburst date by 17 and 31 days, respectively. However, yield losses were significant and LWP at both stages failed to postpone late-season phenological stages under the warm conditions (Gatti *et al.*, 2016). Similarly, Parkin and Turkington (1980) reported that LWP in late October (in the southern hemisphere) delayed the onset of fruit ripening by about 20 days but the fruits matured at about the same time as those from normal-pruned vines; when carried out later (late November), LWP could delay fruit ripening to a larger degree and improve fruit quality at the cost of a great reduction in yield (Parkin and Turkington, 1980). Also, a recent research (Frioni *et al.*, 2016) in central Italy showed that LWP at stage G (inflorescences separated) slowed fruit ripening and reduced yield as well as the number of inflorescences in winter buds, but the LWP berries were lower in TSS while higher in titratable acidity (TA) and in total anthocyanin concentration. In the same study, no yield was obtained by LWP at stage H-I (40% to 50% of flower caps fallen).

Taken together, LWP is a viable approach to delay grape berry ripening as long as it is carried out late enough. However, extremely late winter pruning may lead to an unacceptable low yield. Therefore, it is vital to find an appropriate period to apply LWP with the purpose of significantly delaying fruit sugar accumulation without affecting the yield. To our knowledge, few studies have focused on this point and there is no general agreement. The objectives of this study were to: (1) assess the effects of LWP at different growth stages on yield components and fruit composition; (2) determine a phenological stage from which LWP would reduce vine yield; and (3) verify whether a delayed ripening period due to LWP could improve the anthocyanin to sugar ratio.

Materials and Methods

1. Study site

The two-year (2015 and 2016) study was conducted in the experimental vineyard of the University of La Rioja (42º27′N, 2º25′W, 370 m.a.s.l.), Logroño, north of Spain. Vines of *Vitis vinifera* "Maturana Tinta de Navarrete" (abbreviated to Maturana in this article) grafted on R110 were planted in 2010. The vineyard was north-south oriented, spacing was 1.2 m (within row) × 2.4 m (between rows), and each row had 28 vines. Vertical cordon was trained 1.6 m high from the ground and six spurs were left after winter pruning with the lowest one located 0.7 m aboveground.

2. Winter pruning treatments

Two adjacent vine rows were selected for the trial, and each of them was equally divided into four blocks; four winter pruning treatments were randomly assigned to the four blocks so there were 14 vines for each treatment. Every treatment was applied to the exact same vines in both years. The Baillod & Baggiolini system (Baillod and Baggiolini, 1993) was applied to identify the growth stages of apical buds/shoots. The four treatments were: (1) winter pruning at stage A (WPA; dormant bud); (2) LWP at stage C (LWPC; green shoot tip) in 2015 and at stage F (LWPF; inflorescence clearly visible) in 2016; (3) LWP at stage G (LWPG; inflorescences separated); and (4) LWP at stage H (LWPH; flowers separated). It is worth mentioning that, in 2015, the second pruning treatment was conducted at stage C; however, LWP exerted very few effects compared to WPA (as will be described in detail below). Since the goal of the study was to delay fruit ripening, we decided to shift the time of the second pruning to stage F in 2016. Two buds (excluding crown buds) per spur were left through winter pruning. Moderate trimming was performed when the shoots hindered the passing through the inter-rows. In 2015, drip irrigation was applied to all the treatments with an average amount of 4.5 L/vine/day from the beginning of July until the end of August. In 2016, the same irrigation pattern lasted from the middle of July until the end of August.

3. Climatic parameters

Climatic data were obtained from the nearest meteorological station located in Logroño. In both years, for each treatment, dates of budburst, full bloom, veraison and harvest were recorded, and the number of days (No. days), growing degree days (GDD), cumulative precipitation (CP) and radiation (R) were calculated between every two adjacent phenological dates. Mean temperatures (mean T) during flowering (full bloom date ± 7 days) were also calculated.

4. Bud fertility and yield analysis

Considering the risk of cluster abscission due to possible environmental stress (Keller, 2015), bud fertility was assessed by calculating the average number of inflorescences per shoot right after the full fruit set of the latest pruning treatment. Leaf area (LA) per vine was calculated at harvest by multiplying LA per shoot by shoot number per vine, and the method based on leaf disc sampling (Smart and Robinson, 1991) was used to estimate the LA per shoot. Specifically, at harvest, 15 shoots per treatment were collected randomly. All the leaves on each shoot were removed and weighed. On the other hand, 100 3.80-cm^2 discs from randomly selected leaves were weighed as well. Finally, LA per shoot was estimated by multiplying the quotient of the two weights by 380.

5. Berry analysis

In each year, fruits from all the treatments were harvested and analyzed at the same TSS level (22-23 ºBrix, which is a common range for commercial Maturana grapes in the region). In the case of LWPH in 2016, the berries had not reached the desired ripening level by the time the first symptoms of bunch rot were observed so they were picked at a lower TSS. For each treatment, cluster number per vine was recorded to assess bud fertility, and cluster weight was measured on 40 randomly cut clusters at harvest. Finally, yield per vine was estimated by multiplying cluster number by cluster weight. Average berry weight was determined on 100 randomly sampled berries from all the 14 vines of each treatment, and these berries were subsequently crushed manually for juice analysis. pH and TA were analyzed by standard methods (OIV, 2014). The concentration of total anthocyanins was measured based on Iland *et al.* (2004). For every parameter, measurements were repeated three times per treatment on the same vines.

6. Data analysis and statistics

Statistical package SPSS 16.0 (SPSS Inc., Chicago, US) for Windows was used for the statistical analysis. Data of yield components and berry

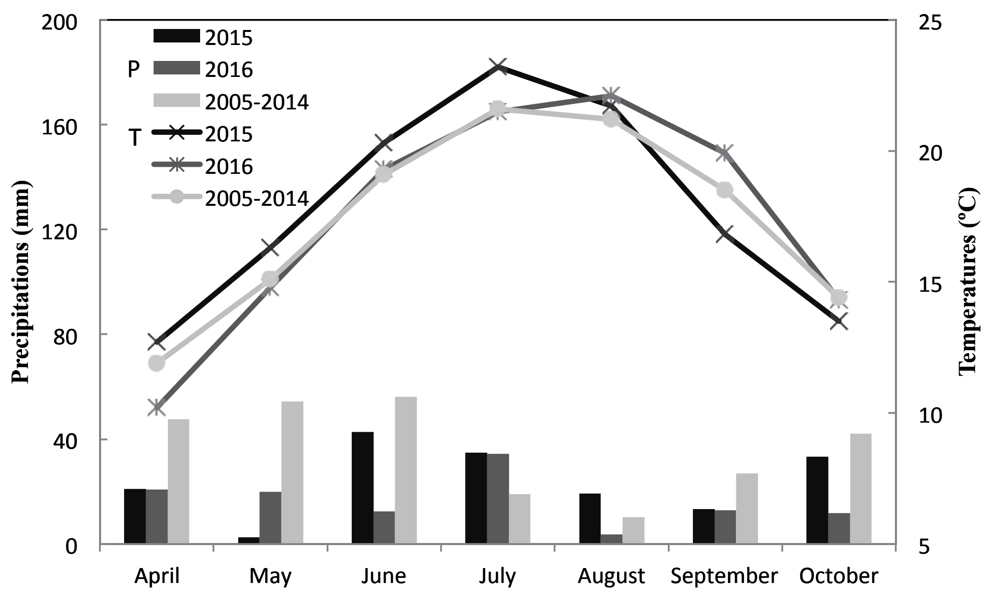

Figure 1. Mean monthly temperature (T) and monthly cumulative precipitation (P) during growing seasons in Logroño.

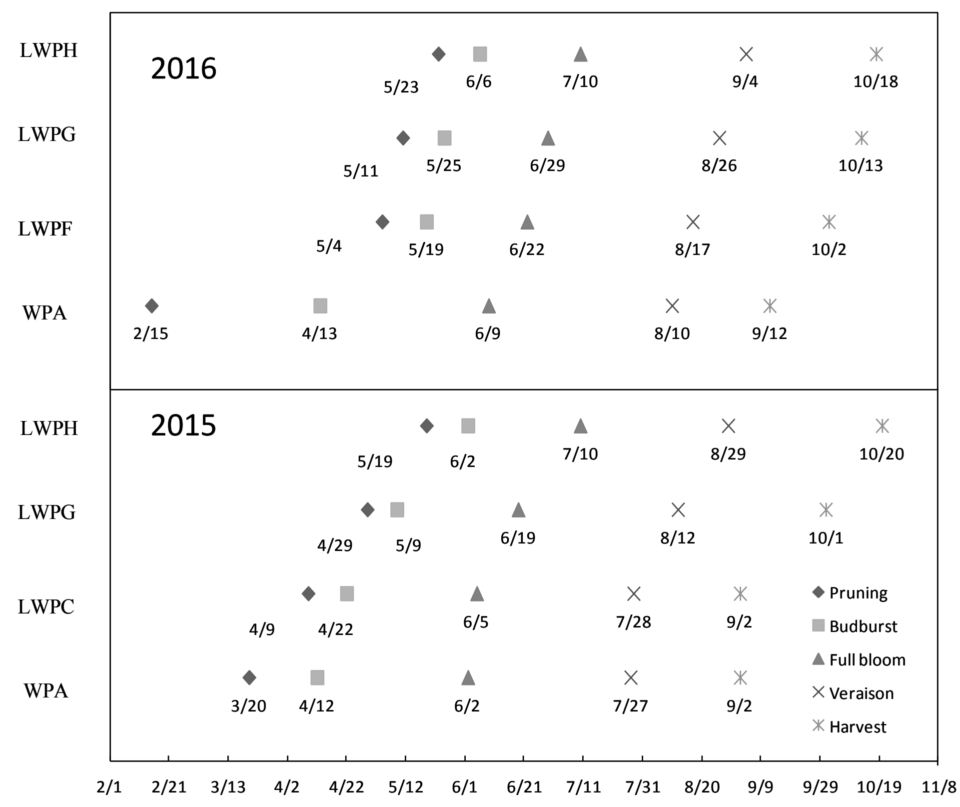

Figure 2. Dates of pruning, budburst, full bloom, veraison and harvest for control (WPA) and late pruning at stage C (LWPC, in 2015) or F (LWPF, in 2016), at stage G (LPWG) and at stage H (LWPH) in 2015 and 2016.

Table 1 - Climatic parameters during different phenological stages of Maturana grapes for control (WPA) and late pruning at stage C (LWPC) or F (LWPF), at stage G (LPWG) and at stage H (LWPH) in 2015 and 2016.

Year	Phenological stages	Parameters	WPA	LWPC (2015) LWPF (2016)	LWPG	LWPH
2015	Flowering time[a]	Mean T[b] (ºC)	20.1	20.1	19.2	24.7
	Budburst to full bloom	No. days	51	44	41	38
		GDD (ºC)	288.3	294.0	316.0	457.9
		CP (mm)	20.8	4.8	69.7	74.2
		R (10^6 MJ/m^2)	1.127	1.028	0.989	0.985
	Full bloom to veraison	No. days	55	53	54	50
		GDD (ºC)	692.6	663.0	732.4	626.2
		CP (mm)	114.8	114.8	56.7	55.2
		R (10^6 MJ/m^2)	1.425	1.371	1.410	1.172
	Veraison to harvest	No. days	37	36	50	52
		GDD (ºC)	434.9	421.4	438.1	310.3
		CP (mm)	47.2	47.2	43.2	66.9
		R (10^6 MJ/m^2)	0.789	0.760	0.930	0.764
2016	Flowering time	Mean T (ºC)	20.2	19.6	22.1	22.3
	Budburst to full bloom	No. days	57	34	35	34
		GDD (ºC)	300.2	304.4	336.8	387.5
		CP (mm)	58.9	25.6	19.7	22.3
		R (10^6 MJ/m^2)	1.279	0.856	0.880	0.871
	Full bloom to veraison	No. days	62	56	58	56
		GDD (ºC)	728.9	696.4	742.9	717.2
		CP (mm)	26.5	30.5	28.7	13.7
		R (10^6 MJ/m^2)	1.568	1.424	1.458	1.341
	Veraison to harvest	No. days	33	46	48	44
		GDD (ºC)	441.9	507.9	442.6	343.0
		CP (mm)	9.5	1.0	8.6	11.0
		R (10^6 MJ/m^2)	0.712	0.836	0.781	0.651

[a] Flowering period is considered as the period between seven days before full bloom and seven days after full bloom.
[b] T, CP, GDD and R stand for temperature, cumulative precipitation, growing degree days and radiation, respectively.

composition were tested for homogeneity of variance using Levene's test and then one-way analysis of variance was run. S-N-K (equal variance assumed) or Dunnett's T3 (equal variance not assumed) method was used for the post-hoc multiple comparisons of means.

Results

1. Weather conditions and phenological stages

In 2015, spring and summer were hot but autumn was cooler than usual (Figure 1). It needs to be pointed out that there was a prolonged period of hot weather in the two weeks at the end of June and the beginning of July. In contrast, the year 2016 had a cold spring and a warm ripening period. Except July and August, rainfall in both years was low compared to the average in the last decade. Each LWP treatment could effectively delay the budburst date and shorten the time interval between budburst and full bloom (Figure 2, Table 1). The effects of LWPC on delaying the phenological stages were only maintained until veraison. By contrast, LWPF (as a replacement of LWPC in 2016) successfully delayed the technological maturity of grapes by 20 days. In both years, LWPG and LWPH succeeded to postpone each

Table 2 - Effects of different pruning time on yield components in 2015 and 2016 (Logroño, La Rioja, Spain).

	WPA[a]	LWPC	LWPG	LWPH	Sig[b]	WPA	LWPF	LWPG	LWPH	Sig
No. berries/cluster	212 a	223 a	132 b	110 b	**	194 a	173 a	113 b	88 b	***
Cluster weight (g)	270 a	317 a	180 b	158 b	*	256 a	228 a	151 b	122 b	***
Berry weight (g)	1.27 b	1.42 a	1.37 ab	1.44 a	*	1.32	1.32	1.34	1.39	ns
No. clusters/vine	14 a	15 a	10 ab	6 b	*	15	16	18	13	ns
No. shoots/vine	13	14	11	14	ns	13	17	16	17	ns
No. clusters/shoot	1.1 a	1.1 a	0.9 a	0.4 b	**	1.2	0.9	1.1	0.8	ns
Yield (Y)/vine (kg)	3.78 a	4.76 a	1.80 b	0.95 c	**	3.84 a	3.65 a	2.72 ab	1.59 b	*
Leaf area (LA)/vine (m^2)	4.70	5.29	5.02	6.50	ns	5.10	5.31	4.47	3.86	ns
LA/Y (m^2/kg)	1.31	1.11	2.79	6.84		1.29	1.49	1.64	2.31	

[a] WPA, LWPC, LWPF, LWPG and LWPH stand for winter pruning at stage A and late winter pruning at stage C, F, G and H, respectively.
[b] Significance level; data were analyzed with one-way Anova; *,**, ***,
ns: significant at p≤ 0.05, p≤ 0.01, p≤ 0.001 or not significant, respectively.
When differences among treatments were significant,
the S-N-K method was used to separate the means; different letters (a, b, c) represent different means at p≤ 0.05.

phenological stage to a great extent and prolong the ripening period by 10-15 days (Figure 2, Table 1).

2. Weather conditions between different phenological stages

In both years, the number of days, growing degree days (GDD, ºC), cumulative precipitation (CP, mm) and cumulative radiation (R, 10^6 MJ/m^2) between different phenological stages were calculated and recorded, as well as the mean temperature during flowering time (Table 1). Only LWPH led to an observably high mean temperature (2-4 ºC higher than WPA) during flowering time. From budburst to full bloom, LWP vines experienced greater heat accumulation than those of WPA, especially LWPG and LWPH. Between full bloom and veraison, in both years, LWPG generated the hottest weather conditions, while LWPH brought about the least rainfall. From veraison to harvest, grapes of LWPF, LWPG and LWPH received higher, similar and lower heat energy than WPA, respectively. Besides, vines of LWPF and LWPG received more illumination relative to WPA, while those of LWPH received the highest precipitation amount.

3. Yield components

LWPC and LWPF did not alter the berry numbers per cluster, while LWPG and LWPH reduced the value of this parameter significantly (Table 2). Similarly, the same trend was observed for cluster weight. In 2015, the LWP treatments significantly increased berry weight except LWPG; however, such difference was not found in 2016. In 2015, LWPH drastically reduced the number of clusters per shoot compared to the other treatments. In 2016, a relatively lower number was also observed for LWPH vines, though the difference was not significant. LWPH reduced the yield per vine to a large extent in both years, while LWPG only reduced it significantly in 2015 (by 52%). Regarding LA to fruit ratio, as a consequence of the lower yield, LWPG had a higher ratio compared to WPA and in the case of LWPH, this value was even higher, especially in 2015.

4. Must composition

Must composition was compared at the same TSS level with the exception of LWPH in 2016. In spite of this, 21.4 ºBrix was obtained by the LWPH berries, only 1.6 ºBrix lower than WPA (Table 3). In 2015, both LWPC and LWPG slightly increased must pH without affecting TA. In 2016, on the contrary, LWPF and LWPG reduced TA by 0.76 and 1.07 g/L, respectively, without significantly increasing must pH. As to LWPH, in both years, the must significantly had a higher TA and a lower pH than all the other treatments. With respect to anthocyanins, only the latest LWP treatment exerted influences: in 2015, must of LWPH was significantly higher in both concentration (mg/g) and content (mg/berry) of total anthocyanins. In 2016, such difference was not found.

Discussion

One of the biggest concerns about delayed winter pruning is the fluctuation of vine yield (Friend and Trought, 2007). Vine yield is a function of the number of buds per vine, bud fertility, the number of berries per cluster, and berry weight (Keller, 2015). Within a winter bud, the formation of grape inflorescences begins around the flowering time of the current season. However, flower initials are not formed before the buds enter in dormancy (May,

Table 3 - Effects of different pruning time on must composition in 2015 and 2016 (Logroño, La Rioja, Spain).

	2015					2016				
	WPA[a]	LWPC	LWPG	LWPH	Sig[b]	WPA	LWPF	LWPG	LWPH	Sig
Total soluble solids at harvest (ºBrix)	23.0	22.6	22.5	22.4	ns	23.0 a	22.8 a	22.8 a	21.4 b	*
Titratable acidity (g/L)[c]	7.15 b	6.35 b	6.56 b	8.89 a	***	6.85 b	6.09 c	5.78 c	7.95 a	**
pH	3.40 b	3.49 a	3.53 a	3.30 c	***	3.46 a	3.52 a	3.58 a	3.31 b	**
Anthocyanin concentration (mg/g)	2.60 b	2.40 b	2.72 b	3.58 a	***	3.14	3.37	3.21	3.39	ns
Anthocyanin content (mg/berry)	3.44 b	3.42 b	3.73 b	5.17 a	***	4.14	4.44	4.27	4.72	ns

[a] WPA, LWPC, LWPF, LWPG and LWPH stand for winter pruning at stage A and late winter pruning at stage C, F, G and H, respectively.
[b] Significance level; data were analyzed with one-way Anova; *,**, ***, ns: significant at $p\leq 0.05$, $p\leq 0.01$, $p\leq 0.001$ or not significant, respectively.
When differences among treatments were significant, the S-N-K method was used to separate the means; different letters (a, b, c) represent different means at $p\leq 0.05$.
[c] Titratable acidity is expressed as g/L tartaric acid.

2000). During the dormancy phase, morphological development cannot be observed and around budburst of the next season, inflorescence growth recommences together with flower formation (Lavee and May, 1997; May, 2000; Vasconcelos *et al.*, 2009). Bud fertility is a gene-controlled trait; however, it is also affected by the environmental conditions prior to inflorescence differentiation. On the other hand, in the following season, the climatic conditions from budburst to fruit set are also a determining factor of the final number of inflorescences and flowers (Keller, 2015). Therefore, delayed winter pruning could have a great influence on the vine yield, not only of the current year but also of the next season.

Friend and Trought (2007) found that LWP at stage E for Merlot vines significantly increased berry weight, due to favorable climatic conditions during flowering. However, the finding of Gatti *et al.* (2016) was precisely the opposite: LWP at stage E and F maintained and reduced berry weight, respectively. In our trial, the influence of LWP on berry weight was inconsistent between the two years, so further study is necessary regarding this aspect. By contrast, the effect of LWP on berry number per cluster was consistent: extremely late winter pruning (at stage G and H) led to fewer berries per cluster, which indicated that vines of LWPG and LWPH had either fewer flowers and/or a poorer fruit set compared to other treatments. Since the availability of carbohydrates is the determining factor of flower induction and fruit set (Friend and Trought, 2007; Vasconcelos *et al.*, 2009; Keller, 2015), it could be speculated that there was a shortage of carbohydrate supply during the process of bloom and/or fruit set. The lack of carbohydrate supply might be attributed to four possible reasons: (1) normally, vines have the least nutritional reserves in their perennial woods around flowering (Bennett *et al.*, 2005; Weyand and Schultz, 2006); moreover, in the case of a very late winter pruning, sources of carbohydrates and nitrogen compounds were removed by pruning otherwise they would contribute to the development of basal shoots (Frioni *et al.*, 2016; Gatti *et al.*, 2016); (2) as seen in Table 1, vines of LWP treatments had a shorter interval between budburst and full bloom compared to WPA (34~44 days vs 51~57 days). As a consequence, at flowering time, LWP vine leaves were not as mature as those of WPA and had a limited capacity of photosynthesis [vine leaves usually attain maximum photosynthetic capacity at 30-35 days of age (Poni *et al.*, 1994; Gatti *et al.*, 2016), though Gatti *et al.* (2016) also stated that LWP could reduce the time required for foliage to reach maximum efficiency]; (3) before flowering, under warm conditions, a growing shoot is a strong sink creating intensified competition with flower differentiation for nutrition, resulting in the occurrence of flower abortion, which is well known as the phenomenon of "filage" (Champagnol, 1984); and (4) extremely high temperatures (>35 ºC) could reduce fruit set percentage (Keller, 2015). However, this case only happened to LWPH vines in 2015, during the long lasting heat wave.

Another important yield component is the number of inflorescences per shoot, which could be considered as an indirect estimation of bud fertility (Vasconcelos *et al.*, 2009). Interestingly, for this parameter, we only observed a significantly lower value on LWPH in 2015. Since it was the first year of the experiment, inflorescence differentiation was assumed to be the same for all treatments in the previous season. The poorer fertility of LWPH buds should be attributed to the loss of pre-developed inflorescences in the current season. In 2015, for LWPH vines, the mean temperature during flowering was 24.7 ºC and the GDD from budburst to full bloom was 457.9 ºC (Table 1), which might be high enough to cause filage. On the contrary, in 2016, under more normal weather conditions (Table 1), no significant difference in bud fertility was found among treatments. In sum, it seems that LWP does not inhibit inflorescence differentiation in the season when buds occur and that a very late winter pruning, especially when combined with unfavorable weather conditions, could give rise to a loss of inflorescences in the following season. Nonetheless, this contention should be supported by long-term data, especially after LWP vines are subjected again to a standard winter pruning during bud dormancy. As reported by Frioni *et al.* (2016), after the pruning was switched back to normal, LWPG vines could obtain a normal bud fertility again while vines previously subjected to LWP at stage H-I still had fewer inflorescences per shoot than control.

Based on the two years' experimental results, setting aside every single yield component, the global effects of LWP on vine yield can be summarized as follows: LWP before or at stage F is unlikely to reduce vine yield. LWPG could significantly reduce vine yield but to a moderate level (about 30% to 50% less than WPA, Table 2). However, LWPH might lead to a severe yield reduction. If converted into yield per hectare, LWPH vines produced about 3200 kg/ha and 5400 kg/ha of grapes in 2015 and 2016, respectively. The former value is too low relative to the average yield in the region as the local wine regulation permits the viticulturists to produce up to 6500 kg/ha of red grape. In fact, in many commercial vineyards within the Rioja wine region, cluster thinning is a cultural practice that is performed every year due to the yield limit, though it is quite time consuming (about 40 hours/hectare). Fortunately, we may dispense with this costly work by simply postponing the pruning date, with variable results depending on the extent of the delay.

We tried to harvest the grapes of all the treatments at the same TSS level because only in this way the rest of the must parameters (TA, pH and anthocyanins) are comparable, since the perception of acidity in the wine is greatly affected by the alcohol content (Jackson, 2009) and the anthocyanin concentration is closely related to the TSS level (Pirie and Mullins, 1977). However, for LWPH, sugar accumulation in the ripening period was so slow that the berries did not reach the desired TSS level before bunch rot began to appear in 2016. The reason for the slow rate of sugar accumulation might be the considerably lower GDD during ripening (Table 1). Though LWPH vines had a much higher LA/Y value than the other treatments (Table 2), the low temperatures might repress the photosynthetic activity greatly (Keller, 2015). Besides, according to Kliewer and Dokoozlian (2005), high LA/Y (>1.2-1.5 m^2/kg) does not help to achieve a higher maximum level of TSS. On the other hand, the lower temperatures during the ripening phase of LWPH grapes were closer to the desirable levels for anthocyanin accumulation (He *et al.*, 2010). Our results on LWPH also support this standpoint. In 2015, the anthocyanin concentration of LWPH must was 38% higher than WPA. In 2016, though the anthocyanin concentration is similar among treatments, it should not be ignored that the LWPH grapes had a significantly lower sugar content. In other words, LWPH grapes had a higher anthocyanin to sugar ratio in both years. It is true that a high LA/Y is also beneficial to anthocyanin accumulation; however, like the case of TSS, as long as the LA/Y is above 1.2 m^2/kg, excessive LA seems ineffective to anthocyanin accumulation (Kliewer and Dokoozlian, 2005). Therefore, the best explanation for the improvement of the anthocyanin to sugar ratio might be that LWPH created cooler ripening conditions by delaying and prolonging the ripening phase. The mean temperatures from veraison to harvest for WPA, LWPC (2015)/LWPF (2016), LWPG and LWPH were 21.8 ºC, 21.8 ºC, 18.9 ºC and 16.1 ºC, respectively, in 2015, and 23.3 ºC, 21.1 ºC, 19.3 ºC and 18.0 ºC, respectively, in 2016. In fact, LWPF and LWPG succeeded to reduce the mean temperature of the ripening phase as well, but the anthocyanin accumulation of grapes was not enhanced, indicating that a sharp decline in mean temperature during ripening (>5 ºC) might be beneficial for anthocyanin accumulation of Maturana grapes. Actually, Maturana is a minor variety cultivated in the Rioja wine region and it is characterized by very high color content (Balda *et al.*, 2013). In the same region, it was reported that a decrease of 2.3 ºC of daily mean temperatures during ripening enhanced the coloration of Garnacha grapes (Martínez de Toda *et al.*, 2014), which usually have much less color than Maturana. So it is conceivable that the sensitivity of anthocyanin accumulation to temperatures might be variety-dependent. Based on the two years of data, delayed winter pruning at stage C, F and G did not have much impact on the acid traits of berries. However, LWPH grapes always kept more acidity and lower pH, the possible reason being that low temperatures repressed the respiratory malate degradation (Keller, 2015).

Conclusions

LWP at stage G and H could effectively delay all the phenological stages of Maturana grapes to a great extent and create considerably cooler ripening conditions than standard winter pruning. Vine yield is unlikely to be affected by LWP before or during stage F; however, LWPG and LWPH could reduce vine yield by reducing berry number per cluster, possibly by inhibiting flower formation and/or fruit set. With an acceptable yield level, LWPG can be applied as a better alternative to cluster thinning. LWPH can improve anthocyanin accumulation and help to maintain a relatively high level of acidity in berries. Delaying the winter pruning to stage H is a promising way to restore the anthocyanin to sugar ratio decoupled by the warming climate, despite the risk of *botrytis* and severe decline in production. Further study should be carried out to evaluate the long-term effects of LWP on grapevines, especially on bud fertility and nutritional reserves of perennial parts. Also, it will be interesting to apply this technique on other varieties to clarify to which degree a delayed winter pruning could improve grape coloration.

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References

Antcliff A.J., Webster W.J. and May P., 1957. Studies on the Sultana vine. V. Further studies on the course of bud burst with reference to time of pruning. *Crop and Pasture Science* 8: 15-23. doi:/10.1071/AR9570015

Baillod M. and Baggiolini M., 1993. Les stades repères de la vigne. *Revue Suisse de Viticulture, Arboric, Horticulture* 25: 7-9.

Balda P., Palacios A., Carrillo D., Zaldivar E., Borinaga I. and Martínez de Toda F., 2013. Expresión de pirazinas en Maturana Tinta de Navarrete: efectos de la radiación solar. *La Semana Vitivinícola* 3412: 1926-1933.

Bennett J., Jarvis P., Creasy G.L. and Trought M.C.T., 2005. Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. *American Journal of Enology and Viticulture* 56: 386-393.

Champagnol F., 1984. *Eléments de physiologie de la vigne et de viticulture générale*. Dehan, Montpellier.

Coombe B.G., 1995. Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research* 1: 104-110. doi:/10.1111/j.1755-0238.1995.tb00086.x

Friend A.P. and Trought M.C.T., 2007. Delayed winter spur-pruning in New Zealand can alter yield components of Merlot grapevines. *Australian Journal of Grape and Wine Research* 13: 157-164. doi:/10.1111/j.1755-0238.2007.tb00246.x

Frioni T., Tombesi S., Silvestroni O., Lanari V., Bellincontro A., Sabbatini P., Gatti M., Poni S. and Palliotti A., 2016. Postbudburst spur pruning reduces yield and delays fruit sugar accumulation in Sangiovese in central Italy. *American Journal of Enology and Viticulture* 67: 419-425. doi:/10.5344/ajev.2016.15120

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. doi:/10.3389/fpls.2016.00659

Gu S., Jacobs S., McCarthy B. and Gohil H., 2012. Forcing vine regrowth and shifting fruit ripening in a warm region to enhance fruit quality in 'Cabernet-Sauvignon' grapevine (*Vitis vinifera* L.). *The Journal of Horticultural Science and Biotechnology* 87: 287-292. doi:/10.1080/14620316.2012.11512866

He F., Mu L., Yan G.-L., Liang N.-N., Pan Q.-H., Wang J., Reeves M.J. and Duan C.-Q., 2010. Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules* 15: 9057-9091. doi:/10.3390/molecules15129057

Howell G.S. and Wolpert J.A., 1978. Nodes per cane, primary bud phenology, and spring freeze damage to Concord grapevines. A preliminary note. *American Journal of Enology and Viticulture* 29: 229-232.

Iland P. and Gago P., 2002. *Australian wine: styles and tastes*. Patrick Iland Wine Promotions, Campbelltown, SA, Australia.

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.

Jackson R.S., 2009. *Wine tasting: a professional handbook*. 2nd edition, Elsevier Academic Press.

Jones G.V., White M.A., Cooper O.R. and Storchmann K., 2005. Climate change and global wine quality. *Climatic Change* 73: 319-343. doi:/10.1007/s10584-005-4704-2

Keller M., 2010. Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. *Australian Journal of Grape and Wine Research* 16: 56-69. doi:/10.1111/j.1755-0238.2009.00077.x

Keller M., 2015. *The science of grapevines: anatomy and physiology*. 2nd edition, Elsevier Academic Press, London.

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.

Lavee S. and May P., 1997. Dormancy of grapevine buds - facts and speculation. *Australian Journal of Grape and Wine Research* 3: 31-46. doi:/10.1111/j.1755-0238.1997.tb00114.x

Martin S.R. and Dunn G.M., 2000. Effect of pruning time and hydrogen cyanamide on budburst and subsequent phenology of *Vitis vinifera* L. variety Cabernet-Sauvignon in central Victoria. *Australian Journal of Grape and Wine Research* 6: 31-39. doi:/10.1111/j.1755-0238.2000.tb00159.x

Martínez de Toda F., Sancha J., Zheng W. and Balda P., 2014. Leaf area reduction by trimming, a growing technique to restore the anthocyanins: sugars ratio decoupled by the warming climate. *VITIS* 53: 189-192.

May P., 2000. From bud to berry, with special reference to inflorescence and bunch morphology in *Vitis vinifera* L. *Australian Journal of Grape and Wine Research* 6: 82-98. doi:/10.1111/j.1755-0238.2000.tb00166.x

Mori K., Sugaya S. and Gemma H., 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae* 105: 319-330. doi:/10.1016/j.scienta.2005.01.032

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. doi:/10.1093/jxb/erm055

OIV, 2014. *Compendium of international methods of analysis of wines and musts*. International Organisation of Vine and Wine (OIV), Paris.

Palliotti A., Panara F., Silvestroni O., Lanari V., Sabbatini P., Howell G., 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. doi:/10.1111/ajgw.12033

Palliotti A., Tombesi S., Silvestroni O., Lanari V., Gatti M. and Poni S., 2014. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. *Scientia Horticulturae* 178: 43-54. doi:/10.1016/j.scienta.2014.07.039

Parkin R. and Turkington C., 1980. Late pruning improves wine quality. *The Agricultural Gazette of New South Wales* 91: 36-37.

Pirie A. and Mullins M.G., 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. *American Journal of Enology and Viticulture* 28: 204-209.

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.

Reynier A., 2002. *Manual de viticultura*. Mundi Prensa, Madrid.

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. doi:/10.1111/j.1755-0238.2012.00180.x

Schultz H., 2000. Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. *Australian Journal of Grape and Wine Research* 6: 2-12. doi:/10.1111/j.1755-0238.2000.tb00156.x

Smart R. and Robinson M., 1991. *Sunlight into wine: a handbook for winegrape canopy management*. Winetitles, Australia.

Trought M.C.T., Howell G.S. and Cherry N., 1999. *Practical considerations for reducing frost damage in vineyards*. Report to New Zealand Winegrowers.

Vasconcelos M.C., Greven M., Winefield C.S., Trought M.C.T. and Raw V., 2009. The flowering process of *Vitis vinifera*: a review. *American Journal of Enology and Viticulture* 60: 411-434.

Weyand K.M. and Schultz H.R., 2006. Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in a cool climate. *American Journal of Enology and Viticulture* 57: 172-182.

Zheng W., del Galdo V., García J., Balda P. and Martínez de Toda F., 2017. Use of minimal pruning to delay fruit maturity and improve berry composition under climate change. *American Journal of Enology and Viticulture* 68: 136-140. doi:/10.5344/ajev.2016.16038