

# Effects of delayed winter pruning on vine performance and grape composition in cv. Merlot

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**Abstract.** Delaying winter pruning until after budburst is a technique that can retard vine phenological phases and reduce grape sugar concentration at harvest. Given these characteristics, many studies have recently been conducted to verify the ability of pruning after budburst to contrast the negative effects of climate change. In our trial, vines of the cv. Merlot, trained to a VSP spur pruned cordon, were pre-pruned leaving 8 nodes per shoot and hand finished when the shoots sprouted by the apical nodes were at BBCH13 (treatment LP) and BBCH18 stage (treatment VLP). Vines refinished during winter were used as control (WP). Anthocyanins and tannins of skin and seeds were analysed after both exhaustive extraction (total content) and extraction conducted with a hydroalcoholic solution (extractable portion). Vines refinished after budburst showed reduced leaf area, yield, cluster and berry weights; technological maturity of these vines was delayed as lower sugar concentration and pH were observed at harvest. Treatment VLP had a stronger effect than LP on these parameters. Considering phenolic compounds, the skin and seed tannin concentration increased only in VLP, while no effect was found on anthocyanins. In conclusion, delaying pruning until after budburst revealed interesting prospects for contrasting the negative effects of climate change.

## 1 Introduction

In the last few decades, accelerated sugar accumulation in *Vitis vinifera* L. berries has been observed in many cultivation areas and different factors have contributed to this phenomenon. For instance, yield limits adopted in the production of appellation of origin wines to preserve their quality obliged grape-growers to reduce crop load that, together with the enhancement of canopy management, resulted in a higher sugar accumulation.

Another factor that contributed strongly to the acceleration of sugar accumulation is global warming. The significant temperature increase of the last decades caused earlier onset of all the phenological phases, shorter phenological intervals, and increased soluble solids concentrations at harvest, and, therefore, higher potential alcohol [1; 2]. It was also demonstrated that the temperature increase caused earlier ripening in Australian vineyards [3] and decoupled sugar and anthocyanin ripening, with acceleration of sugar accumulation and lower anthocyanin concentrations at harvest [4].

In these conditions, grape-growers need to choose one of the following options: i) harvest at technological maturity with the right balance of alcohol content and acidity, but with the risk of poor colour and unpleasant astringency; ii) harvest when phenolic compounds have reached the desired traits for a good anthocyanin

extraction avoiding the risk of astringent tannins, but with too high alcohol and too low acidity levels.

Several approaches were studied to counteract the negative effects of climate change and, among these, limitation of the source of photosynthates at the beginning of ripening by defoliation, trimming or spraying the apical part of the shoots with antitranspirants showed interesting results [5; 6; 7; 8; 9].

In the last years, innovative studies on the delay of winter spur pruning showed good prospects for mitigating the negative effects of global warming. Early work proved that this technique could reduce spring frost damage, since in unpruned shoots, apical buds development inhibits the basal bud burst, whose shoots grow after spur-pruning [10]. Recent studies reported that late winter pruning of Cabernet Sauvignon vines determined a delay of 4-5 days in the main phenological events and lowered soluble solids concentration by about 1 °Brix at harvest [11]. Moreover, [12] described that the later winter pruning was performed, the greater was the sugar ripening delay in Merlot berries. More recently, post-budburst spur pruning on Sangiovese vines caused a reduction in sugar concentration and increase in phenolic compounds, but different responses were reported in relation to the phenological stage at which vines were pruned [13; 14; 15;16].

In this experiment, winter spur pruning was delayed until after budburst to delay the phenological phases, reduce sugar concentration at harvest, and achieve a

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good balance between technological and phenolic ripening in Merlot grapes.

## 2 Materials and methods

The study was conducted in the 2014, 2015 and 2016 seasons in a 12-year-old irrigated vineyard of *Vitis vinifera* L. cv. Merlot (clone R3 grafted onto SO4 rootstock), located in Valsamoggia, Bologna, Italy (latitude 44°28'N; longitude 11°07'E). Vines were spaced 1 m within rows and 3 m between rows and were trained to a vertically shoot positioned (VSP) spur pruned cordon. Each vine was mechanically pre-pruned during dormancy and manually finished leaving 5 nodes of two buds (10 buds per vine). During the growing season, 10 shoots per vine were left by shoot thinning. Shoots were trimmed twice, in June and July, and pest management was conducted according to Emilia-Romagna Region standard practices.

Four randomized blocks were created in two adjacent rows and five vines per treatment were assigned in each block (20 vines per treatment). Spur pruning treatments were based on phenological phase: manual refinishing was performed at BBCH 0 - dormancy (Winter Pruning - WP); refinishing when shoots developed on the apical part of unpruned canes were at stage BBCH13 – three leaves unfolded (Late Pruning - LP); refinishing when control vines and shoots developed on the apical part of unpruned canes were at stage BBCH18 – eight leaves unfolded (Very Late Pruning – VLP). In 2014, 2015 and 2016 manual refinishing was performed on 17, 29 and 21 April for LP, 30 April, 11 and 13 May for VLP respectively.

At the end of vegetative growth, 20 representative shoots per treatment were removed from plants within the blocks and the area of main and lateral leaves were measured with a LI-3100 A (Li-cor, Lincoln, Nebraska USA). The leaf area of each vine was calculated by multiplying the average leaf area of the 20 shoots by the number of shoots per vine.

At harvest (7 October 2014, 16 September 2015 and 14 September 2016), the yield of tagged plants was weighed and number of clusters counted. The incidence of cluster rot was assessed by estimating the surface with symptoms and cluster compactness was estimated using the International Organization of Vine and Wine (OIV) code 204 [17].

Total soluble solids, pH and titratable acidity were analyzed every ten days from veraison, by sampling 50 berries from the five vines of each block (200 berries per treatment), while at harvest, 25 berries were sampled from each tagged plant (500 berries per treatment). On the same date, samples of 80 berries were taken from the five vines of each block (320 berries per treatment) and then divided into three subsamples that were used to determine: a) total anthocyanins (20 berries); b) total tannins (20 berries); c) extractable anthocyanins and tannins (40 berries). The berries for the determination of must biochemical parameters were immediately processed, while the other samples were frozen and stored at –20 °C.

Must samples were analyzed to determine the soluble solids concentration using a temperature-compensating Maselli R50 refractometer (Maselli Misure, Parma, Italy). Must pH and titratable acidity were measured using a Crison Titrator (Crison Instruments, Barcelona, Spain).

Total anthocyanins were extracted from the skins of 20 berries by soaking the peeled skins in 100 mL methanol for 24 h, then storing the extracts at –20 °C [18]. Total tannins were extracted from the skins and seeds of 20 berries ground separately to a fine powder, before extracting 1 mg of the sample in 1 mL 70% (v/v) acetone in water, for 24 h in a dark room [19].

Extractable anthocyanins and tannins were extracted from 40 berries: skins and seeds were soaked separately in tubes containing 80 mL of a hydroalcoholic solution for 15 days at 28 °C [20]. The duration and temperature imposed at the extractions were chosen to simulate the winemaking conditions and thus determine the concentration of extractable anthocyanins and tannins.

Total and extractable anthocyanins were separated by HPLC as described by [18], using a Waters 1525 instrument equipped with a diode array detector (LPD) and a reversed-phase column (RP18 250 x 4 mm, 5 µm) with a pre-column (Phenomenex, Castel Maggiore, BO, Italy).

Total and extractable skin and seed tannins were measured by HPLC with the equipment described above. The tannin content was determined by acid-catalysed cleavage in the presence of excess phloroglucinol [21]. Separation of monomer subunits and cleaved proanthocyanidins was conducted following the two different HPLC methods proposed by [19]. The mean degree of polymerization (mDP) was calculated by summing terminal and extension subunits and dividing by terminal subunits [19].

### 2.1 Statistical analysis

A combined analysis of variance over years was performed using the mixed procedure available in SAS v9.0 (SAS Institute, Inc., Cary, NC, USA). Treatment comparisons were analyzed using the Tukey test with a cut off of  $P \leq 0.05$ .

## 3 Results and discussion

### 3.1. Environmental conditions and phenology

Climatic conditions in the three growing seasons appeared similar from April to June, whereas they strongly differed afterwards: summer in 2014 was colder and wetter than in 2015 and 2016. Indeed, the average temperature of the period July-September was 21.2 °C in 2014, while it was 2.5 °C higher in 2015 and 2016. Total rainfall in the same period reached 216 mm in 2014, but it was only 71 and 69 mm in 2015 and 2016 respectively.

**Table 1.** Delay in occurrence of the main phenological phases compared to control vines.

Treatment	Delay of budburst compared to WP (n° of days)	Delay of bloom compared to WP (n° of days)	Delay of veraison compared to WP (n° of days)
LP	30	12	8
VLP	45	24	17

The delay of LP and VLP phenological phases was long at budburst but decreased at bloom, caused by a reduction of the budburst-bloom interval in the delayed pruning vines. On the contrary, the number of days between bloom and veraison was almost constant in all treatments and, as a consequence, the delay of LP and VLP veraison was still noticeable, as also found by [11]. Bloom-veraison interval appears more stable than budburst-bloom and our results are in agreement with those of a long-term study conducted on the phenology of many *Vitis vinifera* L. varieties [22], in which it is reported that over a period of 46 years, the bloom-veraison interval showed the lowest variation among all the phenological intervals.

### 3.2. Vegetative behaviour, yield components and grape composition

Delaying pruning decreased leaf area, with vines pruned later in the season (VLP) showing the lowest values (Table 2). The reduction in main leaf area was due to the smaller surface of the single leaf, while lateral leaf area was negatively affected by both fewer leaves and smaller surface of the single lateral leaf in 2015 (data not reported).

Yield was reduced by about 40% and 70% in LP and VLP vines respectively, mainly due to the lower cluster weight and, only for VLP, also their number. Likewise, the weights of LP and VLP berries were lower than that of WP, as reported in a similar study conducted on cv. Sangiovese [13].

In the second year of the trial, a decreased cluster number in VLP vines was noted (data not reported), which suggested a carry-over effect on bud fertility linked to the loss of storage reserves in the previous year [23]. We can assume that, in our study, the removal of developing shoots with late pruning may have impoverished the carbohydrate and nitrogen reserves, leaving less available for flower induction in shoots growing from the basal buds. Similarly, [13] found a

drop of bud fertility in the second year of trial, on vines pruned at the beginning of May (BBCH18).

Technological ripening was retarded by delayed pruning, as also found in previous studies [11; 12; 13; 14; 15; 16]. The concentrations of soluble solids in LP and VLP grapes were 1 and 2 °Brix lower than WP at the same date, and pH followed the same trend as sugars. On the contrary, titratable acidity of LP and VLP grapes was about 1 and 2 g/L higher than that of WP, respectively (Table 2). In the three years of study, ripening was affected by the different climatic conditions, as evidenced by an overall increase of sugar concentration and pH were observed in the hot and dry summers of 2015 and 2016 (data not shown).

Leaf-to-fruit ratio (Table 2) increased in the delayed pruning vines but, since the value of WP was also definitely above the optimal for adequate ripening (~ 1.5 m<sup>2</sup> /kg of grape), no incremental effect on sugar concentration of LP and VLP berries should have been induced [24].

It may be hypothesized that three main factors could have contributed to the lower sugar concentration in delayed pruning grapes: 1) the delay in the onset of veraison shortened the veraison-harvest interval, reducing the time for sugar accumulation; 2) the prolonged vegetative competition due to late shoot development could have interfered with the accumulation of soluble solids [13]; 3) the loss of storage reserves, due to the removal of developing shoots with late pruning, could have limited the contribution of carbohydrates from the permanent organs of the plants to berry ripening.

### 3.3. Phenolic maturity

The total anthocyanin concentrations, expressed as mg/kg of berries, did not show any difference between treatments (Table 3). Despite the delay in the onset of anthocyanin accumulation being reported as a cause of the reduction of their content at harvest [42], in our LP and VLP berries, the delay of veraison had no negative effect on anthocyanin concentration at harvest. Higher leaf-to-fruit ratio is reported to stimulate the accumulation of both sugars and anthocyanins [25], but in the delayed pruning vines, lower sugar levels were associated with similar concentrations of anthocyanins, as also found by [15]. These particular results could be partially due to reaching—in all treatments (WP, LP and VLP)—very high values of leaf-to-fruit ratio, which correspond to the plateau phase of the negative exponential curve describing the correlation between sugar and anthocyanin accumulation and leaf-to-fruit ratio [24]. So in our conditions, factors other than leaf-to-fruit ratio are probably involved in sugar and anthocyanin accumulations.

**Table 2.** Leaf area, yield components and compositional traits of Merlot vines pruned in different phenological phases. Data averaged over 2014-2016.

Treatment	Leaf area (m <sup>2</sup> /vine)	Yield (kg/vine)	Clusters / vine (n)	Cluster weight (g)	Berry weight (g)	Soluble solids (°Brix)	pH	Titrateable acidity (g/L)	Leaf-to-fruit ratio
WP	6.10 a	2.68 a	13.9 a	185 a	2.13 a	24.5 a	3.62 a	5.78 c	2.66 c
LP	5.26 ab	1.60 b	13.3 a	116 b	1.89 b	23.5 b	3.53 b	6.76 b	4.05 b
VLP	3.44 b	0.78 c	11.5 b	65 c	1.54 c	22.5 c	3.47 b	7.84 a	6.05 a
Significance	*	**	*	**	**	*	*	*	*
Year	*	**	ns	**	**	**	*	ns	*
T x Y	ns	ns	ns	ns	ns	ns	ns	ns	ns

Different letters within a column indicate significant differences after Tukey test. Asterisks indicate significance at: \* P ≤ 0.05; \*\* P ≤ 0.01 ; ns not significant.

**Table 3.** Total and extractable flavonoids of Merlot vines pruned in different phenological phases. Data averaged over 2014-2016.

Treatment	Total Anthocyanins (mg/kg)	Extractable Anthocyanins (mg/kg)	Total skin tannins (mg/kg)	Extractable skin tannins (mg/kg)	Skin-to-pulp ratio	Total seed tannins (mg/kg)	Extractable seed tannins (mg/kg)	Seed-to-pulp ratio
WP	1488	499	1003 b	480 b	0.179 b	1706 b	932 b	0.054 c
LP	1460	494	1050 b	484 b	0.192 a	1766 b	985 b	0.061 b
VLP	1564	525	1250 a	579 a	0.201 a	2120 a	1305 a	0.069 a
Significance	ns	ns	**	**	*	**	**	*
Year	*	*	**	**	*	**	**	*
T x Y	ns	ns	ns	ns	*	**	**	*

Different letters within a column indicate significant differences after Tukey test. Asterisks indicate significance at: \* P ≤ 0.05; \*\* P ≤ 0.01 ; ns not significant.

Further investigations that take into account the complex factors involved in the accumulation of sugars and in the balance between the synthesis and degradation of anthocyanins during ripening are needed to clarify the underlying mechanisms.

The concentrations of extractable anthocyanins were not influenced by the delayed pruning treatments and this result is important from a practical point of view since this portion of anthocyanins represents what can be obtained in wine. Given that, and above all the considerations about the synthesis and extractability of these phenolic compounds, it appears that delayed pruning did not cause detrimental effects on the colour extractability obtained from the maceration of these grapes.

Considering the results of total skin tannins, it appears that the highest concentration was found when pruning was delayed to BBCH18 (VLP), while LP did not differ from WP. The higher skin-to-pulp ratio certainly contributed to this increase, but the different degree of ripening could also have interfered in the fate of total skin tannins. [19] described a decrease of these compounds from veraison to harvest, indicating that unripened grapes showed higher level of skin tannins. Since the interval between veraison and harvest was 15 days shorter for VLP grapes than for WP, and the sugar level was 2 °Brix lower, it is possible that the concentration of resulting skin tannins was higher in VLP berries also due to the incomplete ripening of these

grapes. The concentrations of extractable skin tannins resembled those of total, with the highest concentration found in VLP treatment.

Total seed tannins were also higher in VLP grapes and appeared well correlated with the modifications of the seed-to-pulp ratio, indicating that the reported increases were mainly due to smaller berry size rather than variations in the synthesis of tannins. The changes in extractable seed tannin concentrations resembled those of total, but appeared to be of greater magnitude, in particular for VLP treatment that showed increases of about 40% (compared to WP). Neither total nor extractable seed tannins showed any difference between WP and LP.

## 4 Conclusion

This study has provided detailed information on the effects of three consecutive years of delayed pruning after budburst, carried out at the stages BBCH13 and BBCH18, on the vegetative and reproductive behaviour of vines and technological and phenolic maturity of the grapes. This pruning technique retarded all the phenological phases, reduced yield and lowered sugar concentration at harvest. The latter result was one of the main goals of our study. The effects were more evident when vines were pruned later (BBCH18), but in this case the reuse of the same technique over the years led to

reducing yield below acceptable levels due to the carry-over effect that negatively influenced bud fertility and cluster weight. The analysis of the extractable portion of the phenolic compounds has important implications from a practical point of view, and two main results can be identified: i) extractable anthocyanins were not reduced by the delay in pruning compared to the control; ii) skin and seed tannins were higher when pruning was delayed to BBCH18 (VLP), while no effect was observed in LP treatments. Considering the results of a previous study conducted on Shiraz [26], in which the quality of wine was positively correlated with higher concentration of anthocyanins and skin tannins but negatively with those of seeds, we cannot exclude the possibility that the increase of extractable seed tannins found in VLP berries could cause negative effects on the sensory attributes.

The results of the study suggest that in our conditions it is not advisable to delay pruning until BBCH18: the yield drop is too drastic and doubts are caused by the increase in extractable seed tannins. Instead, delaying pruning until BBCH13 determined a yield loss of about 40%, which can be acceptable for the production of high quality wines, associated with a decrease in potential alcohol and no change in phenolic maturity. These results support the applicability of this technique performed at BBCH13 if viticulturists wish to control yield level and slow down sugar accumulation without detrimental effects on phenolic maturity.

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