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**Post-budburst hand-finishing of winter spur pruning can delay technological ripening without altering phenolic maturity of Merlot berries**

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**RUNNING TITLE**

Effect of post-budburst pruning on Merlot berries

## Abstract

**Background and Aims:** Global warming can lead to technological ripening occurring in advance of phenolic maturity for red wine cultivars. This study evaluated the effect of post-budburst winter pruning on the phenology, yield components, berry composition and phenolic maturity in *Vitis vinifera* L. cv. Merlot.

**Methods and Results:** Mechanically pre-pruned vines followed up with hand pruning during winter (Control), were compared to vines that were mechanically pre-pruned and followed up with hand pruning after budburst when distal buds developed shoots with either three unfolded leaves (DF3) or eight unfolded leaves (DF8). Late pruning delayed budburst, flowering and, to a lesser extent, veraison. The delays were greater for DF8 than DF3 treatments. Yield decreased by about 40 and 71% in DF3 and DF8 vines, respectively, while sugar accumulation and reduction of titratable acidity were delayed by both pruning treatments. The concentration of anthocyanin and tannin and of extractable anthocyanin and tannin (of skin and seeds analysed separately) were not influenced by DF3 treatment while tannin concentration increased in DF8 berries.

**Conclusions:** Delaying hand pruning of mechanically pre-pruned vines until after budburst of distal nodes can delay technological ripening without affecting the concentration of anthocyanin and tannin of berries. Yield, however, is substantially reduced.

**Significance of the Study:** We verified the feasibility of a cost-effective technique that can be adopted to counteract the hastening of sugar accumulation and organic acid decline caused by global warming on valuable black grapes.

**Keywords:** *anthocyanins, global warming, late pruning, phenology, tannins*

## **Introduction**

In the last few decades, accelerated sugar accumulation and faster declines of organic acids have been observed in grapes grown in many viticultural areas. Different factors have contributed to this phenomenon. For instance, yield limits adopted in the production of appellation of origin wines forced grapegrowers to reduce crop load and this, together with improved canopy management (Palliotti et al. 2014), resulted in faster technological ripening, i.e. TSS, pH and TA (Palliotti et al. 2013a, Poni et al. 2013), that leads to higher sugar and lower acid concentration in berries at harvest.

Another factor that has been attributed to the acceleration of technological ripening is global warming. Temperature increase in the last few decades have led to advancing phenology, shorter phenological intervals and a higher concentration of TSS at harvest (Jones and Davis 2000, Duchêne and Schneider 2005). It has also been demonstrated that higher temperature delays the onset of anthocyanin synthesis and accelerates sugar accumulation in many wine areas. Therefore, the consequent decoupling between sugar and anthocyanin accumulation makes it difficult to determine appropriate harvest dates (Petrie and Sadras 2008, Sadras and Moran 2012).

Indeed, in the current context of climate change, producers of grapes for red wine often have to choose one of the following options approaching harvest: (i) harvest performed at optimal technological ripening to obtain wines with the right balance between alcohol concentration and acidity, but with the risk of poor colour and unpleasant astringency; and (ii) delayed harvest until the concentration and extractability of anthocyanin are satisfactory, and tannin is not too astringent, in other words when phenolic maturity is completed. The latter choice, however, may lead to wines with high alcohol concentration and low acidity.

In the last decade, many studies have aimed to slow down sugar accumulation by limiting photosynthesis at the beginning of ripening. Leaf removal of the apical part of the shoots, trimming or spraying the same part of the canopy with antitranspirants reduced sugar accumulation without detrimental effects on phenolic substances (Palliotti et al. 2013a,b, Poni et al. 2013, Filippetti et al. 2015, Gatti et al. 2016).

Studies on delaying winter pruning showed the potential to mitigate the negative effects of global warming. Early work proved that this technique could reduce spring frost damage, since in unpruned canes, the growth of apical shoots inhibits the development of basal buds, which is delayed until vines are pruned (Howell and Wolpert 1978). Martin and Dunn (2000) reported that late winter pruning on Cabernet Sauvignon vines delayed phenology by 4–5 days and lowered TSS at harvest by about 1°Brix. Moreover, Friend and Trought (2007) reported that the later the vines are pruned the greater is the delay in sugar accumulation in Merlot berries.

In the last few years, many studies have showed the effects of post-budburst spur pruning on red wine cultivars and it was reported that the phenological stage at which vines were pruned had a substantial effect on yield and final TSS concentration (Frioni et al. 2016, 2019, Gatti et al. 2016, 2018, Moran et al. 2017, 2019, Palliotti et al. 2017, Petrie et al. 2017, Silvestroni et al. 2018). It was also demonstrated that this technique, if applied appropriately, is able to delay sugar accumulation and increased the concentration of phenolic substances of berries whilst not affecting anthocyanin (Palliotti et al. 2017, Silvestroni et al. 2018).

As is well known, the concentration of berry phenolic substances is an important parameter of grape composition but information about the extractability and composition of anthocyanin and tannin could improve our understanding of the effect of this approach

on phenolic maturity, which is a key factor for predicting important characteristics of red wine (Río Segade et al. 2008). We report the results of a 3-year experiment on the effect of delayed winter pruning after budburst on phenology, yield components, technological ripeness and phenolic maturity of Merlot grapes.

## **Materials and methods**

### *Plant material and experimental design*

The study was conducted over three consecutive seasons, 2014, 2015 and 2016, in a 12-year-old irrigated vineyard situated on a north-facing slope of  $\approx 7\%$  located in Valsamoggia, Bologna, Italy (latitude  $44^{\circ}28'N$ ; longitude  $11^{\circ}07'E$ , about 200 m asl). Vines were *Vitis vinifera* L. cv. Merlot (clone R3 grafted onto SO4 rootstock). The vineyard was established in a silty-clay soil (18% sand, 49% loam, 33% clay) with low content of organic matter (0.9%). The average growing degree days from 1 April to 31 October of the period 2004–2013 was 1962, corresponding to Region IV after Winkler et al. (1974). The vines were spaced at 1 m within the row (oriented north to south) and 3 m between rows, comprising 3333 vines/ha, and were trained to a vertical shoot positioned (VSP) spur-pruned cordon.

The experiment was conducted on 60 vines along two adjacent rows of 150 vines each and three pruning treatments were laid out in a randomised block design with four blocks of 15 vines each. In each block, five vines per treatment were used as experimental units ( $n=20$ ).

Vines were mechanically pre-pruned during dormancy leaving shoots of seven–eight buds using a rotary disc pre-pruner (Tanesini Technology model Girasole, Faenza, Italy) and manually finished leaving five two-bud spurs at three different times: (i) hand-

pruning a few days after mechanical pre-pruning (Control); (ii) hand pruning when the distal buds of pre-pruned canes had burst and were at stage BBCH13 (Lorenz et al. 1995) with three unfolded leaves (DF3); or (iii) hand-pruning when distal buds of pre-pruned canes had burst and were at stage BBCH18 with eight unfolded leaves (DF8). In 2014, 2015 and 2016 the vines were finished manually on day of the year (DOY) 107, 119 and 112 (17, 29 and 21 April) for DF3, and on DOY 150, 131 and 134 (30 April, 11 and 13 May) for DF8, respectively. Pruning treatments were repeated on the same vines during the 3 years of the experiment.

During the growing season, vines were shoot thinned to leave ten shoots per vine. Shoots were trimmed twice at 1.2 m above the cordon and plants were sprayed to control downy mildew, powdery mildew and insects (i.e. *Eupoecilia ambiguella*, *Lobesia botrana* and *Scaphoideus titanus*) according to Emilia-Romagna Region standard practices.

#### *Climate data*

Daily average temperature and rainfall data were kindly provided by the meteorological service of the Emilia-Romagna Region (ARPAE), from a weather station 5 km away from the vineyard.

#### *Phenology*

During the 2014, 2015 and 2016 seasons, phenological stages were identified according to the BBCH scale (Lorenz et al. 1995) twice weekly from budburst to veraison. Budburst stage was assessed as the average of all the nodes per each tagged vine. The dates of flowering and veraison were determined by visual assessment of the proportion of open bottom-flowers and of coloured berries, on all inflorescences and bunches per each tagged vine.

*Berry sampling, ripening kinetics and yield components at harvest*

Two batches of samples were collected, one to assess TSS, pH and TA and the other to assess the concentration of phenolic substances. Berry sampling for technological ripening was conducted every 10 days from veraison to harvest, while that for phenolic maturity only at harvest. An extra sample was collected from Control vines at the last sampling date before harvest to compare phenolic parameters of Control and DF3 berries at similar TSS. Berries were sampled from the bottom, middle and top part of the bunch, on both sides of the canopy. From each five-vine plot 50 berries were collected (200 berries per treatment) to assess TSS, pH and TA. For the analysis of phenolic substances, one sample of 80 berries per five-vine plot was collected (320 berries per treatment) and then stored at – 80°C. Before analysis, each sample was divided into three subsamples to determine: (i) anthocyanin (20 berries); (ii) tannin (20 berries); and (iii) extractable anthocyanin and tannin (40 berries).

At harvest, on DOY 280 in 2014 (7 October), 259 in 2015 (16 September) and 258 in 2016 (14 September), a sample of 25 berries per tagged plant (500 berries per treatment) was collected to assess technological ripeness. The yield of each vine was then weighed and the number of bunches counted. The incidence of bunch rot was assessed by estimating the proportion of berries with visual symptoms. Bunch compactness was estimated according to the Organisation Internationale de la Vigne et du Vin (OIV) code 204 (Organisation Internationale de la Vigne et du Vin, 1983). These assessments were made for all harvested bunches.



### *Chemical analysis of must*

Total soluble solid concentration was measured with a temperature-compensating Maselli R50 refractometer (Maselli Misure, Parma, Italy), while pH and TA were measured with a Crison Titrator (Crison Instruments, Barcelona, Spain).

### *Leaf area and pruning mass*

Immediately after harvest, in the two rows in which the experiment was set, 20 shoots per treatment were randomly removed from other vines that were subjected to the three pruning treatments (Control, DF3 and DF8). The areas of main and lateral leaves were measured with a LI-3100 A (LI-COR Biosciences, Lincoln, NE, USA) and leaf area of each tagged vine, for every treatment, was calculated per vine by multiplying the average leaf area of the 20 shoots by the number of shoots per vine. In January 2017, at the end of the experiment, all the tagged plants were spur pruned by hand to two buds and the wood was weighed.

### *Exhaustive extraction of berry phenolic substances*

Anthocyanin was extracted from the skins of 20 berries by soaking the peeled skins in 100 mL methanol for 24 h and then storing the extracts at  $-20^{\circ}\text{C}$  (Mattivi et al. 2006). Tannin was 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 (Downey et al. 2003). Skin and seed extracts were then centrifuged (15 min, 18530 g) and two 400  $\mu\text{L}$  aliquots of the supernatant were dried under vacuum at  $20^{\circ}\text{C}$ . Pellets were stored at  $-20^{\circ}\text{C}$ .

### *Extraction of berry phenolic substances using a model hydroalcoholic solution*

Whole, unground skins and seeds from 40 berries were soaked separately and shaken daily, in different tubes containing 80 mL of a hydroalcoholic solution for 15 days at  $28^{\circ}\text{C}$

(Allegro et al. 2016). The duration and temperature imposed were chosen to simulate winemaking conditions and thus determine a realistic concentration of extractable anthocyanin and tannin. The hydroalcoholic solution comprised 6 g/L tartaric acid, 40 mL/L 1 N NaOH, 100 mg/L potassium metabisulfite and a proportion of ethanol that was raised from 0 to 13% in the first 12 days of extraction. This concentration was reached by adding 2 mL of ethanol absolute (12 mL total) every 2 days to simulate alcoholic fermentation. The extracts were centrifuged (15 min, 18530 g) and aliquots of the supernatant (400  $\mu$ L) were dried under vacuum at 20°C. Pellets were stored at -20°C.

#### *Anthocyanin determination*

Anthocyanin and extractable anthocyanin were separated by HPLC as described by Mattivi et al. (2006) using a Waters 1525 instrument equipped with a diode array detector (DAD) and a reversed-phase column (RP18 250 x 4 mm, 5  $\mu$ mol) with a pre-column (Phenomenex, Castel Maggiore, BO, Italy). The concentration, expressed in mg per kg of grape, was determined by measuring absorbance at 520 nm. A calibration curve was established using a malvidin-3-glucoside standard (Sigma-Aldrich, St Louis, MO, USA).

#### *Analysis of berry tannin*

Tannin and extractable tannin were separated by the HPLC equipped as for anthocyanin determination. For the analysis of free monomers, one pellet was re-suspended in 100  $\mu$ L methanol acidified with 1% HCl and then neutralised with 100  $\mu$ L sodium acetate (200 mmol, pH 7.5). The other pellet was used for the analysis of terminal and extension subunits and underwent acid-catalysed cleavage of the proanthocyanidins in the presence of excess phloroglucinol, following Kennedy and Jones (2001). Determination of the cleaved and uncleaved samples was performed following two different procedures proposed by Downey et al. (2003). For the uncleaved samples, solvent A, 0.2% phosphoric

acid, solvent B, 4:1 acetonitrile: 0.2% phosphoric acid (gradient of solvent B: zero min, 0%; 5 min, 10%; 40 min, 10%; 55 min, 17%; 65 min, 19%; 75 min, 19%; 80 min, 100%; 85 min, 100%; 86 min, 0%). For the cleaved samples, solvent A, 0.2% acetic acid, solvent B, methanol (gradient of solvent B: zero min, 1%; 40 min, 1%; 120 min 30%; 120.1 min, 100%; 125 min, 100%; 126 min, 1%). For both methods, 25  $\mu$ L of sample was injected at 25°C with a flow rate of 1 mL/min.

The concentration of free monomers and hydrolysed terminal subunits were determined from standard curves prepared with commercial standards of catechin, epicatechin, epicatechin-gallate and epigallocatechin (Extrasynthese, Genay, France) by measuring absorbance at 280 nm (Downey et al. 2003). The concentration of extension subunit-phloroglucinol adducts was calculated from published molar extinction coefficients (Kennedy and Jones 2001). The mean degree of polymerisation (mDP) was calculated by summing terminal and extension subunits and dividing by terminal subunits (Downey et al. 2003).

#### *Statistical analysis*

All data were subjected to ANOVA over years using the mixed procedure available in SAS v9.0 (SAS Institute, Cary, NC, USA). Treatment comparisons were analysed using the Tukey's HSD (Honestly Significant Difference) with mean separation at  $P \leq 0.05$ .

## **Results**

### *Environmental conditions*

Rainfall from April to October in 2014 was high compared to the average for the previous decade (2004–2013) and unusually in July (Table 1). In contrast, drought occurred in the

period July–September of 2015 and 2016. In this 3-month period for both years, only 70 mm of rainfall was recorded.

The summer drought of 2015 and 2016 was associated with temperature that was about 2.5°C higher than the same period in 2014 and about 1°C higher than the average for the decade 2004–2013 (Table 1, Figure S1).

Growing degree-days (GDD, base 10 °C) from April to October in 2014 were 60 lower than in the decade 2004–2013, while in 2015 and 2016 were more than 100 higher.

#### *Vine phenology*

Over the 3 years, the budburst of basal buds was delayed from 24 to 37 days for DF3 and by 37 to 56 days in DF8 vines (Table 2). The basal buds of hand-finished unpruned canes were still dormant (BBCH00) or beginning to expand inside the scales (BBCH01). This delay of late-pruned vines decreased at anthesis, being 10 to 13 days in DF3 vines and 22 to 26 days for DF 8. Veraison was delayed 5 to 11 days for DF3 vines and by 12 to 21 days in DF8 vines.

#### *Leaf area*

Late pruning treatments had a differing impact on leaf area. The main leaf area of DF3 vines was lower than that of the Control vines but there was no effect on lateral leaf area. The DF8-treated vines had lower main shoot and lateral shoot leaf area, amounting to a 44% of total leaf area (Table 3).

#### *Fruit composition*

From 2014 to 2016, sugar accumulation and TA decline were delayed by late pruning. Lower TSS and higher TA level was recorded in DF3 and DF8 berries throughout ripening (Figure 1). The effect of late pruning on TSS and TA, averaged over 3 years of the experiment, was more pronounced for DF8 vines than for DF3 vines. At harvest, TSS of

DF8 and DF3 fruit was 2°Brix and 1°Brix lower than that of Control fruit, respectively, while TA was 2 g/L and 1 g/L higher, respectively (Table 4). No significant treatment x year (T x Y) interactions were found for any of the fruit composition parameters, but an effect of the year was observed: in the cold and rainy season (2014) TSS ranged between 21.3 and 22.2°Brix, while in the following hot and dry seasons (2015 and 2016) values were higher, ranging between 23 and 25.9°Brix.

*Bunch morphology, rot incidence, yield components and leaf-to-fruit ratio*

Bunch compactness was not affected by the pruning treatments and a negligible incidence of rot was observed only in 2014 (Table 5). Compared to Control vines, the DF3 and DF8 vines showed a yield lower of 40 and 71%, respectively. In DF3 vines, yield reduction was mainly due to the lower bunch mass (-37%) while in DF8 both number (-17%) and mass (-65%) of bunches were lower. The reduction of bunch mass in DF vines was related to lower berry mass and fewer berries per bunch. Leaf-to-fruit ratio increased in the DF vines due to their lower yield.

*Phenolic maturity*

At harvest, the concentration of anthocyanin and extractable anthocyanin was not affected by delayed pruning (Table 6). Consequently, anthocyanin extractability, expressed as the proportion of the extractable portion on the total amount, was not different between treatments.

Skin tannin increased by 25% and extractable skin tannin by 21% in DF8 compared to Control berries, while no difference between DF3 and Control berries was observed. As for anthocyanin, skin tannin extractability was not affected by delayed pruning. The skin-to-pulp ratio of DF berries was higher than that of Control but a significant T x Y

interaction was observed. While in 2014 and 2015 DF berries had a higher value than the Control berries, in 2016 the skin-to-pulp ratio of DF3 berries was the lowest (Figure 2).

Similar to skin tannin, seed tannin and extractable seed tannin of DF8 berries were also higher than that of Control berries, while no difference was observed between DF3 and Control berries (Table 7). For both seed tannin and extractable seed tannin, however, significant T x Y interactions were observed. In 2015, the increase in DF8 seed tannin was much higher than that observed in 2014 and 2016 (Figures 3, 4). Seed-to-pulp ratio increased with the delay of pruning, primarily due to a decrease in berry mass relative to seed mass. Delayed pruning did not affect the composition of skin and seed tannin (Tables S1, S2).

#### *Pruning mass*

In the winter following the last harvest of the experiment, pruning mass of DF8 vines was about 45% less than that of the Control vines, while pruning mass of DF3 and Control vines did not differ (Table 8).

#### *Main oenological and phenolic parameters of DF3 and Control berries at similar TSS (around 25°Brix)*

Delayed winter pruning was imposed in an effort to avoid excessive TSS while attaining satisfactory phenolic maturity in seasons with relatively high temperature. To test this approach, we compared the main oenological and phenolic parameters of DF3 berries at harvest (16 September 2015 and 14 September 2016) with those of Control berries when TSS was 25°Brix, threshold level that could indicate excessive TSS for premium Merlot wine. Control berries reached this TSS 9 days before harvest date (7 September 2015 and 5 September 2016). We excluded the data of the DF8 treatment, because of the unacceptable low yield obtained, and the results for 2014, since in this season TSS at harvest was not

excessive, probably in response to the cold and rainy summer. In other words, we compared the Control and DF3 berries, both sampled at proper technological ripeness. For DF3 treated vines this stage was delayed 9 days. As expected, pH and TA did not differ between Control and DF3, while DF3 berries extractable anthocyanin and the anthocyanin-to-sugar ratio were higher than that of the Control berries (Table 9).

## **Discussion**

Late hand pruning during winter following mechanically pre-pruning for Merlot vines, delayed all the phenological stages and harvest. Budburst of the basal buds was inhibited by the development of shoots from the apical buds of pre-pruned canes, according to the phenomenon known as acrotony (Lauri 2007) or primigenic dominance (Bangerth 1989). The broad length of the delay at budburst was reduced at anthesis, probably because DF3 and DF8 shoots grew in a period of warmer temperature and more hours of light compared to Control shoots, and this probably accelerated their development (Palliotti et al. 2017). After anthesis the delay remained relatively constant. This result is in accord with the findings of Tomasi et al. (2011) who reported that over a period of 46 years, the interval from anthesis to veraison of several cultivars showed the lowest variation among all of the phenological intervals.

At budburst, reserves of carbohydrates and nitrogen are mobilised from the permanent organs of vines to support shoot growth (Zapata et al. 2004). In delayed pruning vines the removal of shoots originating from apical buds of pre-pruned canes, may have caused considerable loss of these reserves (Moran et al. 2017). The subsequent development of basal buds may have been constrained by the lower storage reserves that,

in turn, may have limited the development of leaf area observed in this study, particularly in the later pruning treatment (DF8).

Late pruning delayed the onset of TSS accumulation, from 1 to 3 weeks, across three seasons that were characterised by different environmental conditions. The later the vines were pruned, the lower was TSS at harvest. Similar results were found for Merlot grown in New Zealand (Friend and Trought 2007) and for Shiraz and Cabernet Sauvignon grown in Australia (Petrie et al. 2017). In those studies, however, the yield increase of delayed pruning vines and significant rain events before harvest may have contributed to the reduction in TSS.

Coincident with lower TSS, berries of late-pruned vines had lower pH and higher TA, indicating that pruning after budburst delayed all the parameters of technological ripening, as reported by Frioni et al. (2019).

Ripening rates were similar over the 3 years of the study, with the exception of the cold and rainy summer of 2014, where TSS was not excessive (22.2°Brix for Control berries). In the following years, with hotter and drier summers, TSS of Control berries was above 25.5°Brix, a TSS that would lead to an excessive alcohol level in wine (> 15% v/v). Moreover, pH was near 3.70, a level that may not, on its own, ensure microbiological stability of wine. Conversely, DF3 berries showed optimal technological parameters for red wine production: TSS and pH at harvest being 24.5°Brix and 3.60 respectively, and TA between 6 and 7 g/L. In the same seasons, DF8 berries had lower TSS and similar pH. However, TA, around 8 g/L, may be considered too high for a red wine.

The yield of DF3 vines in comparison to Control vines was lowered by 40% and this was due to reduction of bunch mass, which in turn was due to fewer berries per bunch and lower berry mass. The large yield drop of 71% for DF8 vines, when compared with



Control vines, was due to both lower bunch mass and fewer bunches per vine. Similar results were found for Sangiovese by Silvestroni et al. (2018) where the reduction of storage reserves due to removal of the developing shoot may have caused a reduction of inflorescence number per vine and flower number per inflorescence, in the following season (Bennett et al. 2005). Considering that the reduction of yield components of our Merlot vines occurred from the first year of the trial and remained almost constant over the following seasons, it appears that fewer berries per bunch and the fewer bunches per vine were not affected by inflorescence induction and differentiation in the previous year. Therefore, our results suggest that only the last stages of flower differentiation, around budburst in the current season, was negatively influenced by the potential depletion of storage reserves due to late pruning, which may have reduced the number of flowers as well as causing a reversion of developing inflorescence to tendrils (Gatti et al. 2016, Petrie et al. 2017). Delaying pruning until BBCH18, however, reduced yield to an unacceptable level ( $< 1$  kg/vine in each year) and therefore, this treatment is not recommended commercially (Petrie et al. 2017).

The reduction of yield caused by late pruning was relatively greater than the reduction of leaf area, thus leaf to fruit ratio increased with delayed pruning. Even if all these parameters were outside the optimal range for an adequate ripening (Kliewer and Dokoozlian 2005), a higher source to sink ratio of DF vines may have influenced ripening kinetics, and hastened sugar accumulation after veraison (Figure 1). Therefore, in addition to the effect on TSS accumulation, late pruning also increased the rate of TSS accumulation. In this respect, faster sugar accumulation could counterbalance the delay to the onset of ripening, leading to equal or even higher TSS at harvest as reported by other researchers (Moran et al. 2017, Silvestroni et al. 2018).

Pruning mass measured at the end of the experiment (January 2017) reflected the leaf area results, confirming the overall reduction in vegetative growth of DF8 vines.

Delayed winter pruning did not affect anthocyanin concentration at harvest, as found by other authors (Palliotti et al. 2017, Silvestroni et al. 2018, Moran et al. 2019). The decrease of DF berry mass contributed to the increase of the skin-to-pulp ratio that, together with lower yield, may have counteracted the effect of the delay in the onset of anthocyanin accumulation (from 5 to 21 days later), as was suggested by Sadras and Moran (2012). Similarly, the concentration of extractable anthocyanin was not influenced by late pruning, indicating that this technique did not alter their extractability. This has important implications, since the extractable portion of anthocyanin depends on their concentration in skin and the ability of skin tissues to release these compounds (Allegro et al. 2016). It therefore appears that delayed pruning is able to reduce TSS without any detrimental effects on colour obtained from the maceration of DF skins.

The lack of difference in the concentration and composition of skin and seed tannin and extractable tannin between DF3 and Control, suggests that delaying pruning to BBCH13 may not have any negative influence on the sensory attributes due to the presence of these compounds in wine (i.e. astringency and bitterness). Instead, the DF8 treatment, however, increased both skin and seed tannin. Skin-to-pulp and seed-to pulp ratios contributed to these increases. In addition, the overall increase of extractable seed tannin (+40%) may intensify undesired sensations in wine as seeds contain galloylated tannins (Allegro et al. 2018) and these are responsible for rough sensations, such as coarseness, drying and chalkiness (Vidal et al. 2003).

Comparison between the main oenological and phenolic parameters of Control and DF3 berries at similar TSS (25°Brix), which was achieved with 9 more days of ripening for

DF3 berries, showed an increase in extractable anthocyanin, partially due to higher proportion of skin-to-pulp, and of anthocyanin-to-sugar ratio of DF3 berries. These results suggest that delayed pruning may improve wine composition by bringing technological ripeness and phenolic maturity closer together (Petrie et al. 2017).

## **Conclusion**

Winter spur pruning performed after budburst on mechanically pre-pruned vines delayed phenology and technological ripeness and reduced yield substantially. The magnitude of the yield loss increased with the delay in pruning and has to be taken into consideration. Delaying winter pruning to stage BBCH13 reduced yield by 40%, while delaying pruning to stage BBCH18 reduced yield by 71%. A similar concentration of anthocyanin and extractable anthocyanin and tannin in Control and DF3 berries suggest that delaying pruning to BBCH13 should not have a negative influence on the sensory attributes due to the presence of these compounds in wine (i.e. astringency and bitterness).

In conclusion, our study suggests that delaying winter pruning to stage BBCH13 is a technique that can counteract the negative effect of global warming, allowing grapes to be obtained with proper technological ripening and optimal phenolic maturity, also in hot and dry seasons.

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## TABLES

**Table 1.** Environmental conditions on a monthly basis in the experimental vineyard from April to October of the 3 years of the experiment (2014–2016) and of the average in the decade 2004–2013.

	April	May	June	July	August	September	October	April- October†
<b>Precipitation (mm)‡</b>								
2014	98	54	47	131	38	94	29	491
2015	83	79	48	7	56	9	51	333
2016	50	84	58	9	18	42	75	336
2004–2013	65	67	68	17	27	55	110	409
<b>Average temperature (°C)</b>								
2014	13.9	17.3	22.4	22.7	22.4	18.5	15.0	18.9
2015	13.5	18.1	22.4	27.3	24.4	19.5	13.1	19.8
2016	14.2	16.7	21.4	25.6	23.9	21.2	14.9	19.7
2004–2013	13.1	17.6	21.9	24.8	23.8	19.1	13.8	19.2
<b>Growing degree-days§</b>								
2014	117	223	368	392	386	250	156	1892
2015	105	249	368	541	441	291	96	2091
2016	125	201	335	482	435	340	152	2070
2004–2013	92	237	356	459	427	273	118	1962

† Interval from 1 April to 31 October. ‡Precipitation and temperature data were provided by the meteorological service of the Emilia-Romagna Region (ARPAE). §Growing degree-days (daily temperature base 10°C).

**Table 2.** Phenology of Merlot vines following different pruning treatments.

	2014			2015			2016		
	Control	DF3	DF8	Control	DF3	DF8	Control	DF3	DF8
Budburst	88 <sup>†</sup> (29 March)	+29	+42	104 (14 April)	+24	+37	86 (26 March)	+37	+56
Anthesis	150 (30 May)	+13	+23	152 (1 June)	+10	+22	154 (2 June)	+13	+26
Veraison	214 (2 August)	+11	+18	213 (1 August)	+5	+12	214 (1 August)	+10	+21

<sup>†</sup>Day of the year. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 3.** Effect of delayed pruning on the leaf area of Merlot vines at the end of the vegetative cycle.

	<b>Main leaf area (m<sup>2</sup>/vine)</b>	<b>Lateral leaf area (m<sup>2</sup>/vine)</b>	<b>Total leaf area (m<sup>2</sup>/vine)</b>
<b>Treatment (T)</b>			
Control	3.81 a	2.29 a	6.10 a
DF3	3.22 b	2.04 a	5.26 ab
DF8	2.39 c	1.05 b	3.44 b
Significance	**	**	*
<b>Year (Y)</b>			
2014	2.64 b	1.78	4.42 b
2015	3.42 a	1.85	5.27 a
2016	3.36 a	1.75	5.11 a
Significance	*	ns	*
T x Y	ns	ns	ns

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. T x Y, treatment x year interaction. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 4.** Effect of delayed pruning on grape composition of Merlot berries at harvest.

	TSS (°Brix)	pH	TA (g/L)
<b>Treatment</b>			
Control	24.5 a	3.62 a	5.78 c
DF3	23.5 b	3.53 b	6.76 b
DF8	22.5 c	3.47 b	7.84 a
Significance	*	*	*
<b>Year</b>			
2014	21.7 b	3.43 b	6.79
2015	24.4 a	3.69 a	6.45
2016	24.4 a	3.51 b	7.15
Significance	**	*	ns
T x Y	ns	ns	ns

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. T x Y, treatment x year interaction. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 5.** Effect of delayed pruning on bunch morphology, rot incidence, yield components and leaf-to-fruit ratio of Merlot vines.

	<b>Bunch compactness (1-9)</b>	<b>Rot incidence (%)</b>	<b>Yield (kg/vine)</b>	<b>Bunches / vine (No.)</b>	<b>Bunch mass (g)</b>	<b>Berry mass (g)</b>	<b>Berries/bunch (No.)</b>	<b>Leaf-to-fruit ratio (m<sup>2</sup>/kg)</b>
<b>Treatment</b>								
Control	4.82	0.08	2.68 a	13.9 a	185 a	2.13 a	87.4 a	2.66 c
DF3	5.25	0.05	1.60 b	13.3 a	116 b	1.89 b	60.3 b	4.05 b
DF8	4.85	0.07	0.78 c	11.5 b	65 c	1.54 c	41.6 c	6.05 a
Significance	ns	ns	**	*	**	**	**	*
<b>Year</b>								
2014	5.02	0.82 a	2.09 a	13.4	140 a	2.17 a	62.7 ab	2.85 b
2015	4.85	0 b	1.24 b	12.6	94 b	1.58 b	55.8 b	6.17 a
2016	5.05	0 b	1.73 ab	12.6	133 a	1.81 ab	70.8 a	3.75 b
Significance	ns	**	**	ns	**	**	**	*
T x Y	ns	ns	ns	ns	ns	ns	ns	ns

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. T x Y, treatment x year interaction. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 6.** Effect of delayed pruning on anthocyanin and skin tannin traits of Merlot berries.

	<b>Anthocyanin (mg/kg)</b>	<b>Extractable anthocyanin (mg/kg)</b>	<b>Anthocyanin extractability (%)</b>	<b>Skin tannin (mg/kg)</b>	<b>Extractable skin tannin (mg/kg)</b>	<b>Skin tannin extractability (%)</b>	<b>Skin to pulp ratio</b>
<b>Treatment</b>							
Control	1488	499	32.9	1003 b	480 b	49.2	0.179 b
DF3	1460	494	33.3	1050 b	484 b	46.6	0.192 a
DF8	1564	525	32.4	1250 a	579 a	47.7	0.201 a
Significance	ns	ns	ns	**	**	ns	*
<b>Year</b>							
2014	1289 b	473 b	36.9 a	912 b	469 b	52.3 a	0.138 c
2015	1679 a	570 a	30.7 b	1210 a	518 ab	43.8 ab	0.244 a
2016	1544 ab	476 b	31.0 b	1181 a	557 a	47.5 ab	0.191 b
Significance	*	*	*	**	**	*	*
T x Y	ns	ns	ns	ns	ns	ns	*

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. T x Y, treatment x year interaction. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 7.** Effect of delayed pruning on the concentration of seed tannin and extractable seed tannin of Merlot berries.

	<b>Seed tannin (mg/kg)</b>	<b>Extractable seed tannin (mg/kg)</b>	<b>Seed tannin extractability (%)</b>	<b>Seed to pulp ratio</b>
<b>Treatment</b>				
Control	1706 b	932 b	54.9	0.054 c
DF3	1766 b	985 b	56.1	0.061 b
DF8	2120 a	1305 a	61.5	0.069 a
Significance	**	**	ns	*
<b>Year</b>				
2014	1766 b	927 b	52.3 b	0.051 b
2015	2143 a	1317 a	61.7 a	0.076 a
2016	1674 b	978 b	58.6 ab	0.057 b
Significance	**	**	*	*
T x Y	**	**	ns	ns

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. T x Y, treatment x year interaction. Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.



**Table 8.** Effect of delayed pruning on pruning mass of Merlot vines, recorded at the end of the trial (January 2017).

<b>Treatment (T)</b>	<b>Pruning mass (kg/vine)</b>
Control	0.75 a
DF3	0.67 a
DF8	0.41 b
Significance	*

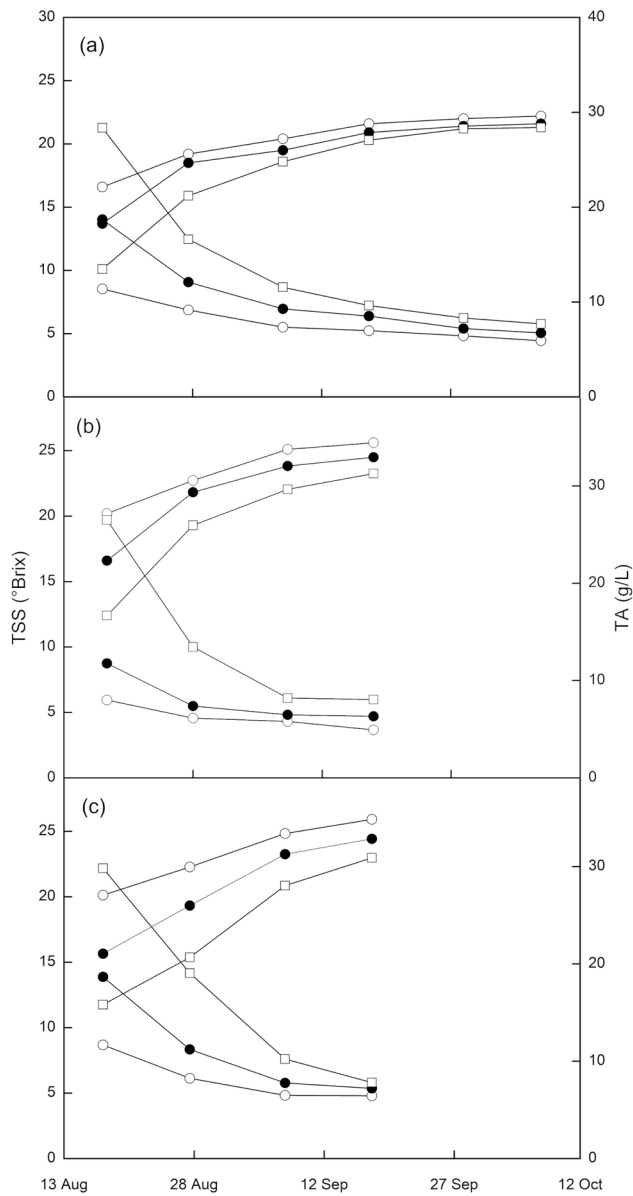
Different letters within the column indicate a significant difference after Tukey's HSD test. Asterisk indicates significance at \*,  $P < 0.05$ . Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves; DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves.

**Table 9.** Main oenological and phenolic parameters of DF3 and Control berries at TSS around 25°Brix.

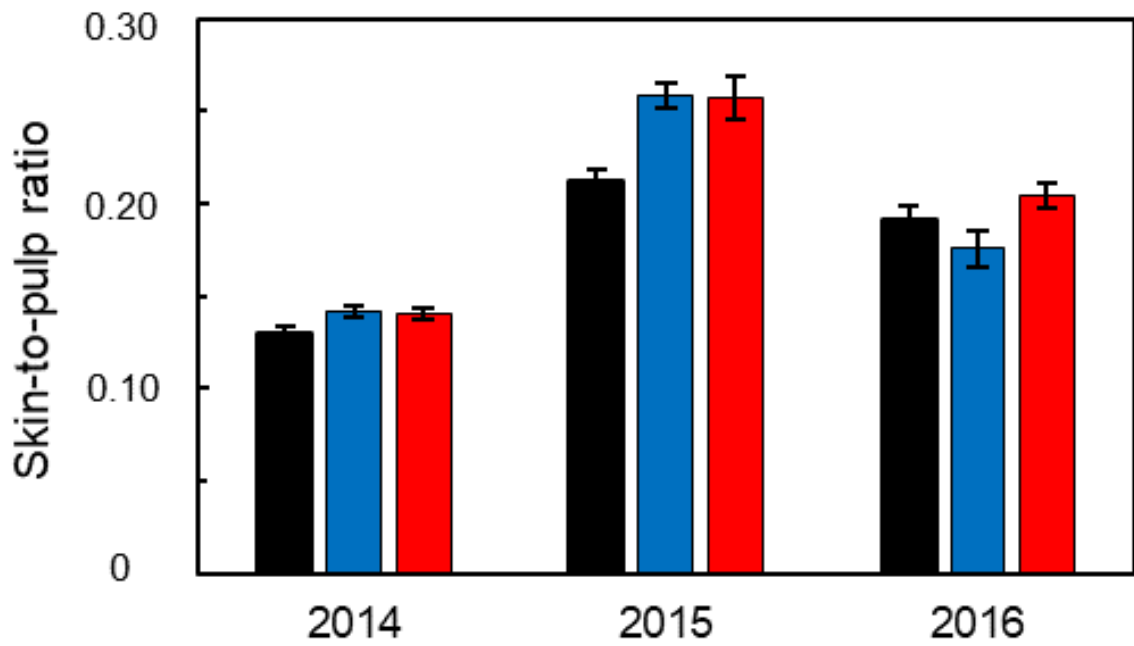
Year	Treatment	TSS (°Brix)	pH	TA (g/l)	Extractable anthocyanin (mg/kg)	Anthocyanin to sugar ratio
2015	Control (9 days BH)	25.1	3.73	5.79	519 b	20.7 b
	DF3 (harvest)	24.5	3.70	6.32	552 a	22.5 a
	Significance	ns	ns	ns	*	**
2016	Control (9 days BH)	24.8	3.58	6.49	423 b	17.0 b
	DF3 (harvest)	24.4	3.48	7.21	465 a	19.0 a
	Significance	ns	ns	ns	*	**

Different letters within a column indicate a significant difference after Tukey's HSD test. Asterisks indicate significance at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant. BH, before harvest; Control, hand pruning in winter; DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves..

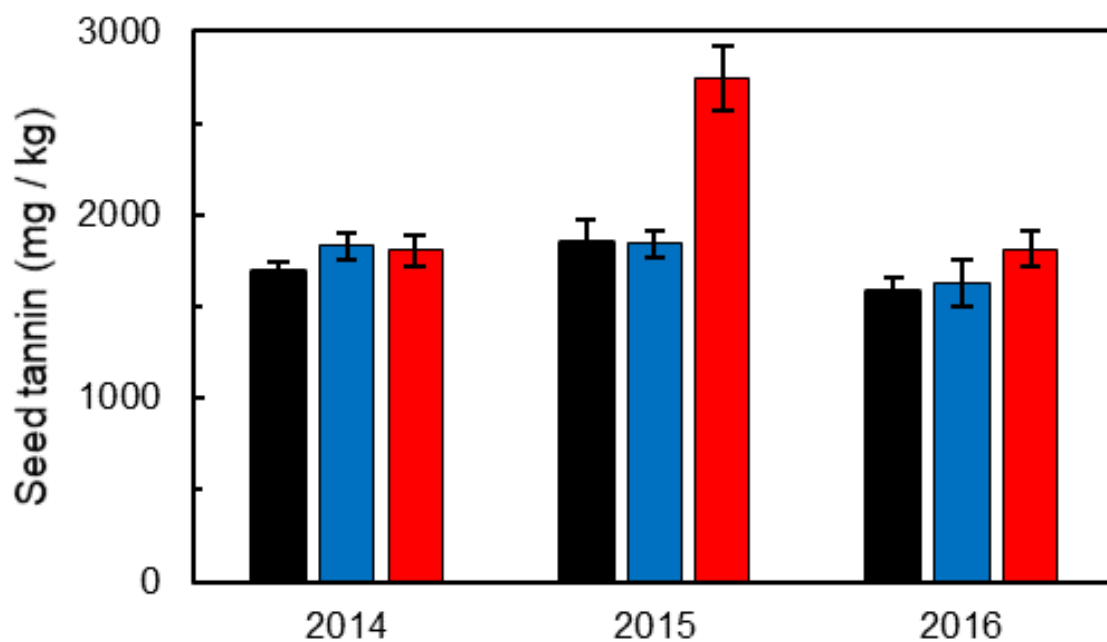
## FIGURE LEGENDS



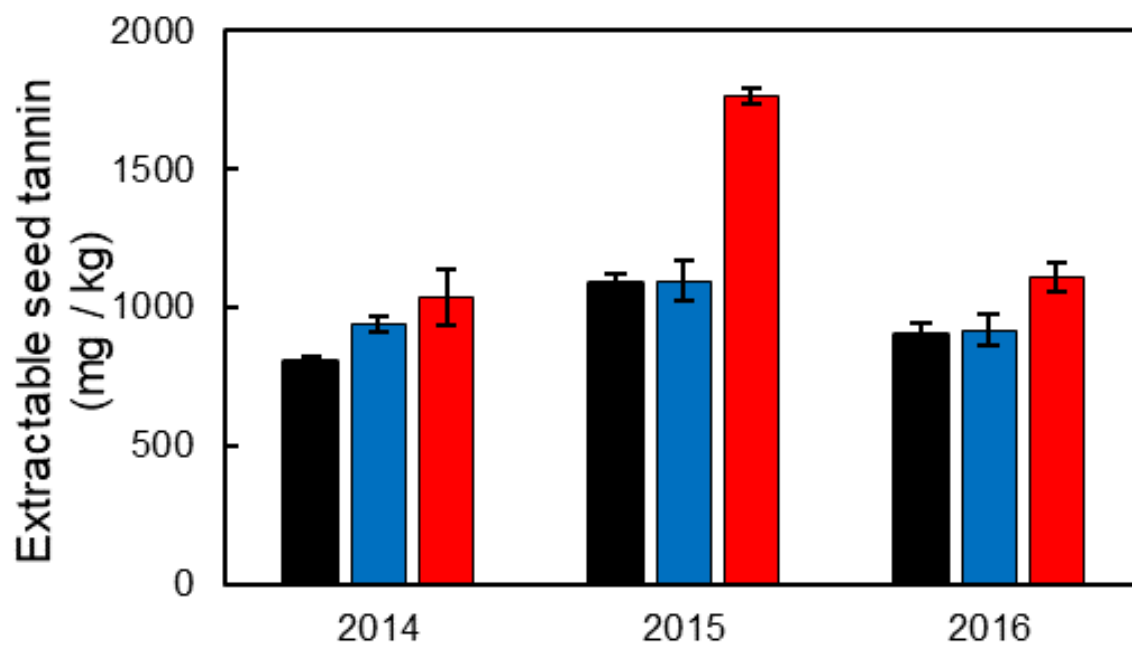
**Figure 1.** Dynamics of TSS and TA in Merlot berries in (a) 2014, (b) 2015 and (c) 2016. Control, hand pruning in winter (○); DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves (●); DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves (□). Vertical bars represent SE.



**Figure 2.** Interactive effect of treatments and year on skin to pulp ratio of Merlot vines subjected to different pruning treatments. Control, hand pruning in winter (■); DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves (■); DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves (■). Vertical bars represent SE.



**Figure 3.** Interactive effect of treatments and year on seed tannin of Merlot vines subjected to different pruning treatments. Control, hand pruning in winter (■); DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves (■); DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves (■). Vertical bars represent SE.



**Figure 4.** Interactive effect of treatments and year on extractable seed tannin of Merlot vines subjected to different pruning treatments. Control, hand pruning in winter (■); DF3, hand pruning when shoots originated from the distal buds had three unfolded leaves (■); DF8, hand pruning when shoots originated from the distal buds had eight unfolded leaves (■). Vertical bars represent SE.

## SUPPORTING INFORMATION

**Figure S1.** Average air temperature in the experimental vineyard from day of the year (DOY) 91 (1 April) to 304 (31 October). Average of the decade 2004–2013 (—); 2014 (—); 2015 (—); 2016 (—).