

## Influence of pruning timing on the agronomic and qualitative potential of red wine grape cv. Merlot in Southern of Brazil

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**Abstract:** The study aimed to evaluate the influence of pruning timing on the phenology, productivity, and quality of grapes and wine of red wine grape cv. Merlot cultivar during the 2022/2023 growing cycle. The treatments involved four pruning seasons: late May (autumn), June, July and August in winter, using a block design with three replications. Were evaluated phenology (beginning and end of budbreak, flowering, and ripening), budburst percentage, productivity, grape and wine quality. The number of clusters per plant, cluster weight and size, productivity per plant and per hectare were assessed. The important analyses such as pH, total acidity, and soluble solids (°Brix) were also included. Wine analyses evaluated pH, alcohol, total acidity, color, and sensory attributes. Early pruning did not affect plant phenology. Late pruning decreased and reduced budburst, as well as shortened the duration of phenological cycles, without advancing harvest time. Early pruning increased productivity without influencing grape and wine composition. Plants pruned in May and July were more productive, producing 9300 and 9900 kg of grapes per hectare. Late pruning in August reduced productivity (7143 kg). Winter pruning can be brought forward or extended without significantly altering the quality of the grape and wine, considering treatments with early pruning in May and late pruning in August.

**Keywords:** Phenology, production, wine, harvest, *Vitis vinifera* L.

**Abbreviations:** A\_Absorbance; BO\_Bud burst onset; CH\_Color Hue; CI\_Color intensity; CV\_Coefficient of variation; EB\_End of budburst; EF\_End of flowering; ER\_End of ripening; FO\_Flowering onset; FTIR\_Fourier-transform infrared spectroscopy; NM\_Nanometers; OR\_Onset of ripening; pH\_potential of Hydrogen; SO<sub>2</sub>\_Sulfor dioxide; SS\_Soluble solids; T\_Treatment.

### Introduction

Grapevine (*Vitis vinifera* L.), although originally from the Caucasus Mountains in Asia Minor (Ruiz-García, 2018), is now cultivated worldwide (Vanderweide et al., 2021). It is one of the most important fruit species, known for its demanding cultivation practices and edaphoclimatic requirements, which affect the duration of phenological stages, ripening, quality, and yield of the grapes (Pessenti et al., 2021).

In Brazil, viticulture is an important source of income for producing regions (Miranda, 2020), with Rio Grande do Sul standing out, accounting for more than 62% of the country's vineyard area. The Campanha Gaúcha region is the second-largest producer of fine wines in Brazil, representing over 30% of the country's production (Manfio, 2019). Its soil and climate conditions have proven favorable for quality wine production, due to sandy, well-drained soils, high solar radiation levels, and low rainfall during ripening (Costa et al., 2019).

Various practices are employed to monitor vine growth, fruit development, and ripening under many environmental conditions, particularly in temperate climates with harsh winters and hot summers. Among the key vineyard management practices, pruning stands out as an activity that maintains consistent productivity and standardizes both the size and quality of grape clusters (Villa et al., 2018).

Pruning allows growers to manipulate vine balance by altering yield and the leaf area-to-fruit ratio, which also affects berry composition and ripening (Allebrandt et al., 2017; Leão et al., 2018). This practice determines the number of buds left on

the vine and aims to ensure a balance between vegetative and reproductive vigor by distributing photoassimilates between the source (leaf area) and the sink (clusters) (Mendonça et al., 2016).

The grapevine undergoes a period of vegetative rest during autumn and winter, known as dormancy, when growth is temporarily suspended (Lamela et al., 2020). Dormant pruning (winter pruning) is performed on canes and arms of the plants during this dormancy period (Maciel et al., 2017). It is usually carried out at the end of winter, a time when vines begin to release water from cane cuts (Maciel et al., 2020). This phenomenon signals the onset of bud break, in response to rising temperatures (Maciel et al., 2020).

Pruning requires skilled labor (Würz et al., 2017). Since it is a time-consuming task that must be performed within a short window (Buesa et al., 2021), growers often have to carry it out outside the recommended period or hire outsourced labor, increasing production costs (Tesser and Pauletti, 2020). Thus, an alternative would be to extend the dormant pruning period, staggering labor needs (Souza and Bender, 2022). This involves carrying out early or late pruning, in May (autumn) or August (winter), respectively, leading to a better distribution of labor throughout the cycle (Souza and Bender, 2022).

Based on the context outlined above, the objective of this study was to evaluate the effect of different dormant pruning timings on the development of Merlot grapevines, and their influence on grape and wine production and quality.

## Results and Discussion

The plants pruned in May remained dormant for a longer period; however, budburst occurred at the same time as in plants pruned in June and July (Table 1). In early pruning (autumn or early winter), the roots remain dormant (as does the aerial part), and even after the canes are cut, budburst does not occur due to lower soil temperatures (Santos and Silva, 2016, cited by Maciel et al., 2020). Pruning performed in August (T4) delayed both the onset and completion of budburst by eight days.

The vine cycle ranged from 195 days (plants pruned in August) to 202 days (plants pruned in June and July). In this study, the total cycle length was longer when compared to results reported by Radünz et al. (2015), who found an average of 161 days for Merlot. Studies conducted by Perin et al. (2023) with Syrah and Malbec concluded that the seasonal patterns of phenological development, between late and conventional pruning treatments, resumed phenological processes at a similar rate, with only about a one-week difference between late pruning treatments and others. All treatments, after the onset of ripening, followed similar phenological stages, a behavior consistent with that observed in the cultivar evaluated in this study.

The number of chilling hours that plants are exposed during dormancy determines budburst and flowering quality, and this requirement varies widely among species and cultivars (Monteiro et al., 2013, cited by Fogaça et al., 2022). The same number of chilling hours was required for budburst when plants were pruned in May, June and July (282 hours). However, for plants pruned in August, the accumulation hours was higher (328 hours), which may be related to the longer dormancy due to late pruning. The accumulated chilling hours were sufficient to ensure uniform and satisfactory budburst across all pruning periods.

During endodormancy, bud inhibition results from a series of biochemical and physiological processes that vary in duration and intensity, and it is overcome with the accumulation of chilling hours at  $\leq 7.2^{\circ}\text{C}$  (Anzanello, 2019). Longer exposure to cold during dormancy promotes earlier budburst (Anzanello, 2019). A total of approximately 300 chilling hours is required to overcome endodormancy in Merlot buds under a constant thermal regime of  $7.2^{\circ}\text{C}$  (Monteiro et al., 2013).

The results of production and yield evaluations are presented in Table 2. Cluster weight and length showed no differences among treatments. However, the number of clusters was higher when plants were pruned in May and August, whereas plants pruned in June produced fewer clusters. Clusters from plants pruned in August showed shorter length, differing significantly from those from plants pruned in June and July, and resembling those originated from plants pruned in May.

Regarding yield per plant, plants pruned in May and July were the most productive, producing 3.7 and 3.9 kg of grapes per plant, respectively, equivalent to 9,300 kg and 9,900 kg of grapes per hectare. Plants pruned in August showed the lowest yield. Buesa et al. (2021) observed that late pruning in Bobal and Tempranillo resulted in lower yields, attributing this outcome to a reduction in cluster mass, without affecting the number of bunches per plant. In previous work with Chardonnay, Brighenti et al. (2017) observed that performing pruning later than one week after the control date, may lead to yield losses up to 58%.

Table 2 also presents grape must analysis results. For reducing sugars and soluble solids, grapes originating from plants pruned in July differed from the other treatments. Regarding total acidity, the highest value was observed when plants were pruned in August. Must pH was highest when plants were pruned in June and lowest when plants were pruned in August. The genetic characteristics of each cultivar, the length of phenological stages, and especially climatic factors during ripening, may have influenced grape composition. In this case, the higher values of sugars and pH, combined with lower acidity levels, may have been influenced by climatic factors favorable to grape ripening.

Temperature plays a critical role in sugar accumulation in berries, with the optimal range for grapevine photosynthesis being between  $25^{\circ}\text{C}$  and  $35^{\circ}\text{C}$  (Gutiérrez-Gamboa et al., 2021). The 2022/2023 growing season was characterized by the La Niña phenomenon, which minimized rainfall and resulted in higher temperatures in the region of Campanha Gaúcha. This phenomenon may have influenced grape composition by altering vine physiology, especially during ripening.

Grapes originating from plants pruned in August exhibited higher total acidity levels and lower soluble solids and reduced sugars. These results were influenced by the shorter duration of phenological cycles, defined by late pruning. According to Netzer et al. (2022), late pruning was not effective in delaying harvest but did retard sugar accumulation and the degradation of total acidity in Malbec grapes.

**Table 1.** Number of days required for the occurrence of phenological periods and subperiods, days to the solstice, chilling hours, and budbreak for different pruning times in the *Vitis vinifera* cv. Merlot during the 2022/2023 cycle.

Pruning time	Days to solstice	07	16	60	68	81	89	Chilling gHours	Budbreak
		BBCH (07-16)	BBCH (16-60)	BBCH (60-68)	BBCH (68-81)	BBCH (81-89)	BBCH (total)		
May	-33	55 a (8b)	63 a (67 b)	130 a (8a)	138 a (57a)	195 a (61b)	256 a (201b)	282	93%
June*	-3	55 a (8b)	63 a (67 b)	130 a (8a)	138 a (57a)	195 a (62c)	257 b (202c)	282	93%
July	33	55 a (8b)	63 a (68 b)	131 b (9b)	140 b (57a)	197 b (60a)	257 b (202c)	282	93%
August	63	55 a (7a)	70 b (61a)	130 a (8a)	138 a (57a)	197 b (60a)	258 c (195a)	328	91%
	CV (%)	0.53 (1.85)	0.45 (0.73)	0.22 (1.74)	0.29 (0.25)	0.21 (0.34)	0.19 (0.2)		

Beginning of Budburst (07 BBCH) and end of budburst (16 BBCH), beginning of flowering (60 BBCH) and end of flowering (68 BBCH); onset of variation (81 BBCH) and end of maturation (89 BBCH); budbreak percentage (%); chilling hours (below 7.2°C). CV: Coefficient of variation; a'b'c': different letters in the columns indicate statistically significant differences. Statistical parameters from ANOVA with t-test at 0.05 (5%) error probability. May: vine pruning 33 days before the winter solstice; June: vine pruning two days after the winter solstice; July: vine pruning 33 days after the solstice; August: 63 days after the winter solstice. (\*): Control, usual pruning time of the grower.

**Table 2.** Average evaluations of productivity and physicochemical analysis of the must from the *Vitis vinifera* Merlot cultivar pruned at different times.

Pruning time	Clust Weight (g)	Cluste Number	Cluste Length (cm)	Yield (kg plant <sup>-1</sup> )	Yield (kg ha <sup>-1</sup> )	Reducin g Sugars	Soluble Solids	Total Acidity	pH
May	175.7 a	21.1 b	16.4ab	3.7 bc	9739 bc	232.4 a	22.8 a	69.3 b	3.59 b
June*	186.4 a	15.5 a	17.0 b	2.9 ab	7278 ab	232.7 a	22.8 a	68.0 a	3.67 d
July	207.8 a	18.9 ab	17.0 b	3.9 c	9937 c	234.9 b	23.1 b	67.1 a	3.63 c
August	149.6 a	19.7 b	14.0 a	2.8 a	7143 a	231.9 a	22.7 a	71.1 c	3.58 a
CV (%)	41.24	28.27	20.33	15.53	15.6	0.24	0.18	0.79	0.08

CV = Coefficient of Variation; C = Length; P = Production; RS = Reducing Sugars; SS = Soluble Solids; TA = Total Acidity; a'b'c' = different letters in the columns indicate statistically significant differences. Statistical parameters from ANOVA, with t-test at 0.05 (5%) probability of error. (\*): Control, usual pruning time of the producer.

Buesa et al. (2021), evaluating Bobal and Tempranillo, reported that berry ripening was significantly affected by pruning time, increasing the total acidity compared to early pruning. Brighenti et al. (2017) also found that delay of pruning in 'Chardonnay' delays the grape ripening. Gatti et al. (2016) found that winter pruning was unable to delay berry ripening and harvest in Sangiovese vines. Nevertheless, they observed that late pruning showed potential to limit yield and improve fruit quality. Petrie et al. (2017) reported that pH and soluble solids values increased with later pruning times, which differs from the results of the present study.

Table 3 presents wine physicochemical analysis results. Reducing sugars confirmed the classification of the wines as dry (residual sugar below 4 g L<sup>-1</sup>), according to Brazilian legislation (Law 7.678/1988). For pH, wines originated from grapes of plants pruned in June had the highest value.

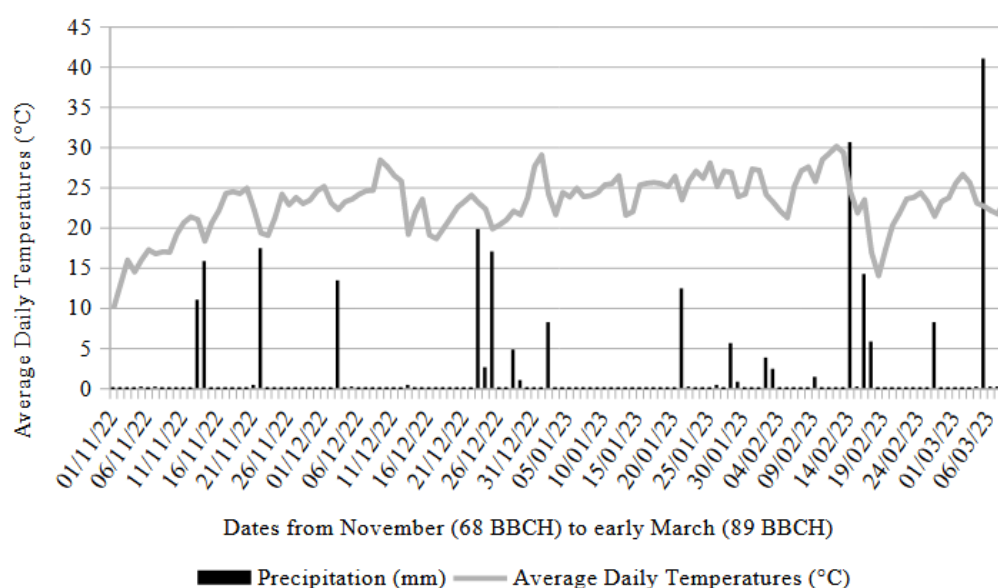
Wine produced from grapes of plants pruned in August showed the lowest alcohol concentrations, following the same trend observed for soluble solids and sugars in the must. In this treatment, the shorter phenological cycle duration, due to late pruning, influenced the lower sugar accumulation in the grapes, reflected in the alcohol content. Regarding acidity, values were higher when pruning was performed in May and August. However, must acidity was lower than wine acidity. This could be related to fruit composition concerning organic acids and berry size, which affects the skin-to-pulp ratio, as well as the acidity of the berry skin. An increase in total acidity after fermentation was also observed in Isabel grapes, due to the release of organic acids from the skin—a characteristic of vinification in this grape (Rizzon and Miele, 2006).

Color parameters in red wines showed differences among treatments. A420 was higher in wines produced from grapes of plants pruned in May and August (0.689 and 0.687), with a similar trend observed for A520. A620 was highest when plants were pruned in August (0.220). In optical density measurements, A520 and A420 nm represent red and yellow colors, respectively, while A620 nm reflects the blue/violet color of young wines (Ribéreau-Gayon et al., 2006). Color hue differed among treatments. Color hue corresponds to the progression towards orange hues in young wines, typically ranging between 0.5 and 0.7 (Ribéreau-Gayon et al., 2006).

**Table 3.** Physicochemical analyses of wine from *Vitis vinifera* cv. Merlot of the 2023 vintage, pruned at different times.

Pruning time	Alcohol	Total acidity	pH	Reducing Sugars	A420	A520	A620	Color Hue	Color Intensity
May	13.0b	117.3d	3.47a	2.36a	0.689d	1.171d	0.192c	0.588 a	2.051d
June*	13.1c	94.7a	3.72c	2.86c	0.575a	0.824a	0.162a	0.697 d	1.561a
July	13.0bc	105.3b	3.58b	2.56b	0.618b	0.958b	0.178b	0.645 c	1.754b
August	12.6a	114.6c	3.47a	2.46ab	0.687c	1.131c	0.220d	0.605 b	2.038c
CV (%)	0.59	0.11	0.11	2.25	0.16	30.78	0.38	0.25	0.26

CV = Coefficient of variation; a'b'c'd': different letters in the columns express statistically significant differences. Anova statistical parameters, with t-test at 0.05 (5%) probability of error. Alcohol (%v/v); Total acidity (meq.L<sup>-1</sup>); Reducing sugars (g.L<sup>-1</sup>). (\*): Control, usual pruning time of the producer.



**Fig. 1.** Climatological variables of daily precipitation and daily average temperature from November (68 BBCH) to March (89 BBCH), covering the 2022/2023 cycle of the Merlot variety. Source: INMET, 2023. Max. temp.: Maximum temperature; Min. temp.: Minimum temperature; mm: millimeters; 68 BBCH: end of flowering; 89 BBCH: end of maturation.

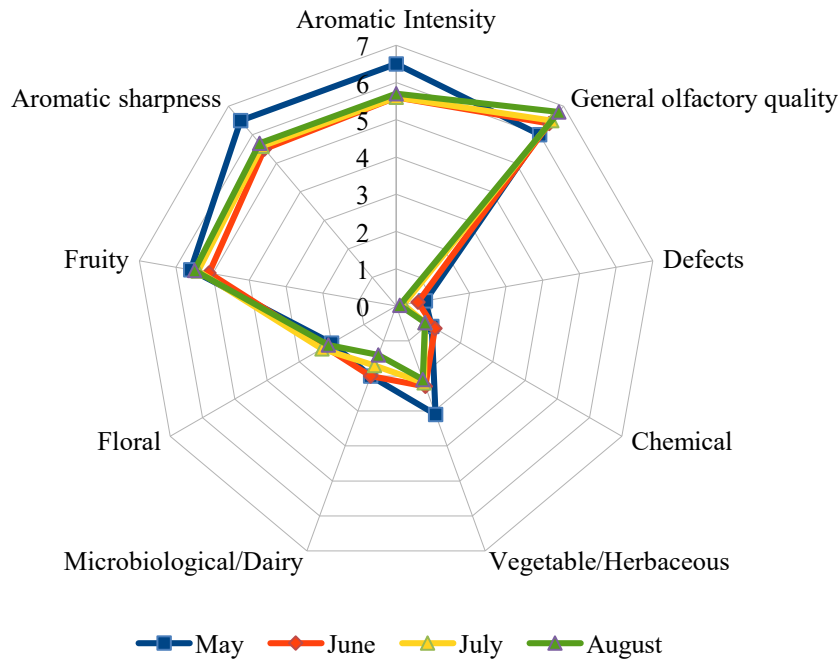
Color intensity also differed among treatments, typically varying between 0.3 and 1.8 depending on cultivar and wine type (Ribéreau-Gayon et al., 2006). Moran et al. (2018) reported that late pruning in Shiraz did not affect wine color hue but did increase color intensity in wines from late pruning. Buesa et al. (2021) also observed that late pruning increased color intensity compared to conventional and early pruning times in Tempranillo wines.

In the sensory analysis of the wines, visual assessment revealed that the predominant color recognized by the evaluators was ruby red, similar across all treatments. In the olfactory analysis, the most prominent descriptor was red fruits, particularly strawberry and cherry, as well as spices (clove and pepper). Additionally, oak, menthol, tobacco, and fermentative (lactic) aromas were identified.

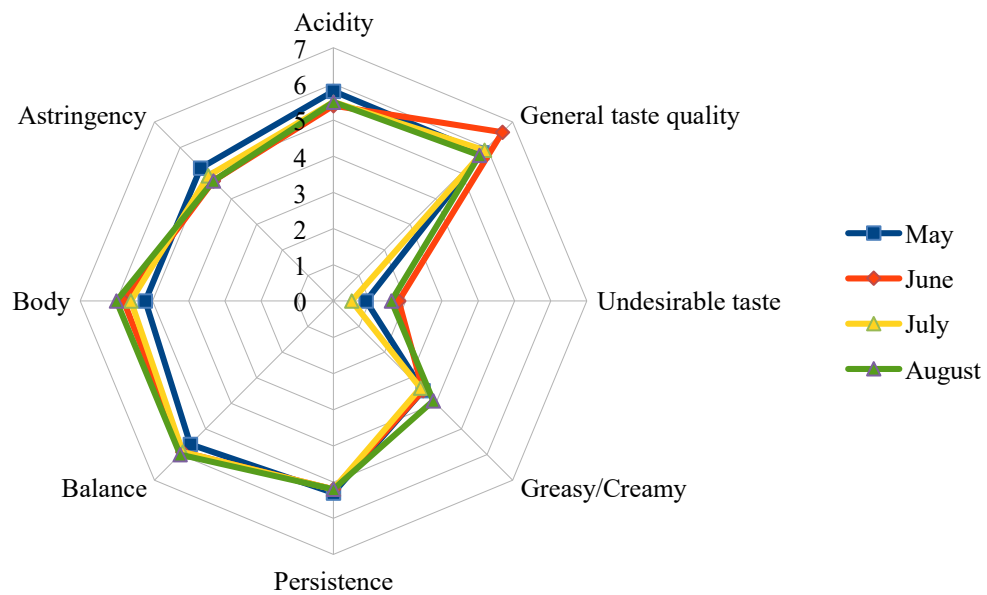
Figure 1 illustrates the results obtained for the treatments, considering quality, aromatic intensity and clarity, defects, and the aroma groups identified. The earliest pruning (May) showed higher aromatic clarity and intensity, with fruity and vegetal/herbaceous aromas. However, the latest pruning (August) demonstrated better overall olfactory quality.

Figure 2 presents the gustatory analysis results. The earliest pruning (May) showed more pronounced acidity and astringency on the palate. However, for balance, body, and persistence, the latest pruning (August) exhibited higher expression. Notably, the control (pruning performed in June) obtained the best overall gustatory quality.

According to Guerra et al. (2005), flavonoids are mainly responsible for taste sensations, especially tannins, which also contribute to wine body, together with alcohol, influencing astringency and bitterness sensations. Figure 3 shows the global wine evaluation as described by the evaluators, considering all previously described parameters. The highest overall evaluation was for wines originating from grapes produced in latest pruning (August), which presented the best olfactory quality, body, balance, and mouthfeel/creaminess. The wine with the lowest overall score was originating from grapes produced in earliest pruning (May)



**Fig. 2.** Olfactory aspects of wines produced from grapes of Merlot vines pruned at different times. T1: vine pruning 33 days before the winter solstice (May); T2: vine pruning two days after the winter solstice (June); T3: vine pruning 33 days after the solstice (July); T4: 63 days after the winter solstice (August).



**Fig. 3.** Gustatory aspects of wines produced from Merlot grapevines pruned at different times. T1: vine pruning 33 days before the winter solstice (May); T2: vine pruning two days after the winter solstice (June); T3: vine pruning 33 days after the solstice (July); T4: 63 days after the winter solstice (August).

## Materials and Methods

### Experimental design

The experiment was conducted in commercial vineyards owned by the company Rigo Vinhedos e Olivais, located in Dom Pedrito, during the 2022/2023 growing cycle. The vineyard covers an area of 24.8 hectares, situated at the geographical coordinates of 31°08'46.7" S and 54°11'53.8" W, at an altitude of 378 m above sea level. The local soil is classified as Argisol, with medium to high depth (Streck, 2018).

The regional climate is classified as humid subtropical (Cfa type) according to Köppen's classification (Moreno, 1961). Based on INMET (National Institute of Meteorology) climatological normals, the average annual precipitation is approximately 1.400 mm, and the mean annual and minimum temperatures are 18°C and 14°C, respectively. The vineyard has a northeast-southwest sun exposure, with plant spacing of 3.30 m between rows and 1.20 m between plants (2,525 plants ha<sup>-1</sup>), trained on a vertical shoot positioning trellis system.

### **Plant materials**

The grapevine variety evaluated in this study was Merlot, clone 346, grafted onto Gravesac rootstock. The experimental design was a randomized block design with four treatments (different dormant pruning timings) and three replicates (five plants each), totaling 60 plants. Dormant pruning was conducted following vineyard-standard criteria, using spur pruning with two buds.

### **Treatments**

To determine the duration (in days) of each phenological stage, the number of days required for each stage was counted from the date of the winter solstice (June 21, 2022). The first dormant pruning was performed in May, 33 days before the winter solstice (T1); the second pruning in June, two days after the solstice (T2); the third in July, 33 days after the solstice (T3); and the fourth in August, 63 days after the solstice (T4), all within the 2022 growing season. June, the standard pruning month for the vineyard, was considered the control.

### **Agronomic performance**

Phenological stages were evaluated according to the following parameters: budburst onset (BO) and end of budburst (EB), considering the green tip and 5/6 unfolded leaves stages, respectively; flowering onset (FO) marked by the first open flowers, and end of flowering (EF) when 80% of flowers were open; onset of ripening (phenological stage 35; OR) and end of ripening (ER), based on the phenological scale proposed by Eichorn and Lorenz (1984). Budburst percentage (%) was assessed as the ratio between the number of shoots and the total number of buds per plant. The duration of the phenological cycle (days) was measured from budburst date to the end of ripening (harvest date).

Chilling Hours accumulation (below 7.2°C) was calculated following the methodology proposed by Weinberger (1950), as an index of chilling requirement for bud dormancy release in fruit crops. The calculation was based on data from the INMET meteorological station (Bagé/RS – the nearest station to the vineyard), considering temperatures from the end of May (first pruning date and start of leaf fall) until budburst.

Yield data were evaluated based on the average number of clusters per plant, yield per plant (kg), cluster length (cm), average cluster weight (g), and estimated yield per hectare, calculated by multiplying the yield per plant by planting density (plants ha<sup>-1</sup>). The average number of clusters per plant was determined by counting clusters on five plants (per replicate) for each treatment. Average cluster weight (g) was calculated by weighing 10 clusters per treatment after harvest. Cluster length was measured using a ruler (width and length) on 10 randomly selected clusters.

Grapes were harvested at enological maturity, the point when the main grape compounds reach the most favorable concentration for wine production. In this case, grapes were intended to produce young dry red wine (standards established by the company). The harvest was performed manually and randomly. Clusters were placed in boxes (20 kg capacity), separated by treatment. After harvest, grapes were transported to the Federal University of Pampa (Dom Pedrito). They were then stored in a cold chamber (4°C) for 24 hours.

### **Microvinifications**

Microvinifications were conducted at the experimental winery of the Federal University of Pampa. After weighing, the grapes underwent mechanical destemming and crushing, and samples were collected for must analysis by Fourier-transform infrared spectroscopy (FTIR) using a Wine Scan device (Wine Scan™ SO<sub>2</sub>, Foss®, Denmark) and Foss Integrator software version 1.6.0. The following parameters were evaluated: soluble solids content expressed in °Brix (SS), pH (potential of Hydrogen), reducing sugars (g L<sup>-1</sup>), and total titratable acidity (meq L<sup>-1</sup>).

During winemaking, SO<sub>2</sub> (potassium metabisulfite) was added at a dose of 100 mg L<sup>-1</sup>, along with *Saccharomyces cerevisiae* AWRI 796® yeast (25 g hL<sup>-1</sup>), and Gesferm® nutrient (25 g hL<sup>-1</sup>). Alcoholic fermentation was monitored daily by measuring density and temperature (maintained between 21°C and 24°C) for seven days. After malolactic fermentation, the wines were subjected to cold stabilization in a cold chamber at a constant temperature of 4°C, followed by SO<sub>2</sub> adjustment for subsequent bottling.

### **Quality analysis**

Wines were analyzed by FTIR spectroscopy using the Wine Scan device (Wine Scan™ SO<sub>2</sub>, Foss®, Denmark) and Foss Integrator software version 1.6.0 for the following parameters: pH (potential of Hydrogen), total titratable acidity (meq L<sup>-1</sup>), alcohol content (% v/v), reducing sugars (g L<sup>-1</sup>), and color absorbance at 420 nm, 520 nm, and 620 nm. Additionally, color intensity (CI = 420 nm + 520 nm + 620 nm) and color hue (CH = 420 nm / 520 nm) were determined.

The wines underwent sensory analysis by a panel of 10 previously trained judges. Quantitative descriptive profile forms were used, specifically developed for the study. The panelists scored the attributes on a structured scale ranging from excellent to insufficient. The visual component included evaluation of color intensity, clarity, and overall visual quality. The olfactory component included intensity, clarity, absence of defects, and overall olfactory quality. Taste characteristics assessed included acidity, astringency, body, balance, persistence, smoothness/creaminess, off-flavors, and overall gustatory quality. Finally, a global score was assigned to each sample.

### **Statistical analysis**

The data obtained for the evaluated variables were subjected to ANOVA statistical analysis, using the t-test with a 5% probability of error, performed with the Sisvar® statistical software.

## Conclusions

Early pruning did not alter the duration of the phenological subperiods, nor did it bring forward the harvest, in relation to the control. Late pruning decreased and reduced bud burst, in addition to reducing the duration of the phenological cycles, without bringing forward the harvest. Early pruning increased productivity. However, it did not influence the composition of grapes and wines. Late pruning reduces productivity. Winter pruning can be brought forward or extended, without significantly altering the quality of grapes and wines.

## Ethical statement

Ethical guidelines were followed, respecting all participants, who were aware of the importance of the research and the need to ingest the product to fully define its attributes.

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