

Effect of the application of abscisic acid and ethephon on the quality of Merlot grapes grown in Serra Gaúcha, South Brazil

Efeito da aplicação de ácido abscísico e etefom sobre a qualidade de uvas Merlot cultivadas na região da Serra Gaúcha, Sul do Brasil

Efecto de la aplicación de ácido abscísico y etefón en la calidad de uvas Merlot cultivadas en la región de Serra Gaúcha, sur de Brasil

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Abstract

Currently, viticulture is using plant growth regulators as an alternative to increasing grape and must quality for vinification. This work aimed to evaluate the effect of different doses of abscisic acid and ethephon on the quality of Merlot grapes. The plant growth regulators were applied isolated and combined, on the stages of veraison and 15 days before harvest (DBH). The parameters of mass and berry diameter, soluble solids content, titratable acidity, must pH, phenolic compounds, and total anthocyanin contents. The results showed that the application of ethephon at the dose of 200 mg·L⁻¹ on veraison caused an earlier bunch threshing, an increased berry peel sensibility, and rupture ripening grapes, in both productive cycles. The use of abscisic acid at the dose of 600 mg·L⁻¹ helped increase the soluble solids content of the grapes, however, a strong influence of the climatic conditions was observed on the effect of the plant growth regulations on Merlot grapes.

Keywords: *Vitis vinifera*; Plant growth regulator; Ethylene promoter; Viticulture.

Resumo

Atualmente, a viticultura utiliza reguladores vegetais como alternativa para aumentar a qualidade da uva e do mosto para vinificação. Este trabalho teve como objetivo avaliar o efeito de diferentes doses de ácido abscísico e etefom na qualidade de uvas Merlot. Os reguladores vegetais foram aplicados isolados e combinados, nas fases de veraison e 15 dias antes da colheita (DAC). Foram avaliados os parâmetros massa e diâmetro do bago, teor de sólidos solúveis, acidez titulável, pH do mosto, compostos fenólicos e teores de antocianinas totais. Os resultados mostraram que a aplicação de etefom na dose de 200 mg·L⁻¹ no veraison provocou debulha dos cachos mais precoce, aumento da sensibilidade da casca da baga e ruptura da maturação das uvas, em ambos os ciclos produtivos. A utilização de ácido abscísico na dose de 600 mg·L⁻¹ auxiliou no aumento do teor de sólidos solúveis das uvas, no entanto, observou-se uma forte influência das condições climáticas sobre o efeito da aplicação destes reguladores de crescimento vegetal sobre as uvas Merlot.

Palavras-chave: *Vitis vinifera*; Regulador de crescimento vegetal; Promotor de etileno; Vitivinicultura.

Resumen

Actualmente, la viticultura utiliza reguladores vegetales como alternativa para aumentar la calidad de la uva y del mosto para la vinificación. Este trabajo tuvo como objetivo evaluar el efecto de diferentes dosis de ácido abscísico y etefón sobre la calidad de las uvas Merlot. Los fitorreguladores se aplicaron aislados y combinados, en las fases de pinta y 15 días antes de la cosecha (DAC). Los parámetros evaluados fueron masa y diámetro de baya, contenido de sólidos solubles, acidez titulable, pH del mosto, compuestos fenólicos y contenido de antocianinas totales. Los resultados mostraron que la aplicación de etefón a la dosis de 200 mg·L⁻¹ en la fase de pinta provocó un trillado más temprano de los racimos, aumento de la sensibilidad de la piel de la baya e interrupción de la maduración de la uva, en ambos ciclos productivos. El uso de ácido abscísico en dosis de 600 mg·L⁻¹ ayudó a incrementar el contenido de sólidos solubles de

las uvas, sin embargo, hubo una fuerte influencia de las condiciones climáticas en el efecto de la aplicación de estos fitorreguladores en las uvas Merlot.

Palabras clave: *Vitis vinifera*; Regulador de crecimiento vegetal; Promotor de etileno; Viticultura.

1. Introduction

In the last few years, Brazilian viticulture has been standing out due to the quality of its wines, a reflex of the development of management techniques and the quality of wine grapes grown in the country. Worldwide wine grape production in 2020 was 78.03 million tonnes, and the Brazilian production in 2021 was 1.75 million tonnes (FAO, 2022; IBGE, 2022a). In Brazil, the state of Rio Grande do Sul stands out as one of the main wine grape producers. The production volume in 2021 harvest was 951.254 t, both table and wine grapes in this Brazilian state (IBGE, 2022b).

The Merlot grape is one of the four wine grape varieties regarded as a classic, along with the Cabernet Sauvignon, Pinot Noir, and Syrah varieties, these four varieties being the most cultivated grape wines worldwide. The largest Brazilian production of these varieties occurs in the states of Rio Grande do Sul and Santa Catarina because of a better adaptation to these soils, which have a clayey characteristic (Camargo, 2017).

The Serra Gaúcha region, South Brazil, has unique characteristics relative to other viticultural regions of Brazil, enabling the elaboration of fine wines with high enological quality. Among them, the Merlot variety is the second red grape cultivar in acreage in the state of Rio Grande do Sul, with 472 ha, being surpassed only by the Cabernet Sauvignon variety (IBRAVIN, 2018).

In red wine grapes, such as Merlot grapes, the occurrence of high concentrations of phenolic compounds is characteristic, especially in berry skin. The profile of phenolic compounds in grapes and wines varies with the harvest, ripening time, edaphoclimatic conditions, and vineyard management. It is also important to point out that the vinification technique and fermentation time may also influence the presence and concentration of these compounds, resulting in wines with distinct characteristics (Burin et al., 2011).

Phenolic compounds are responsible for some sensory attributes of the grapes such as color, flavor, and aroma, being found mainly in the skin and, in lower contents, in the pulp. These compounds are formed through photosynthesis and are important for plant growth and reproduction, acting as antioxidants under stress conditions. Phenolic compounds also contribute to pigmentation, astringency, aroma, and oxidative stability of the grapes, enhancing the wines' characteristics such as color, flavor, and body (Rusjan et al., 2012).

According to Rizzon and Miele (2003), the Merlot cultivar (*Vitis vinifera* L.) has expressivity in its products, being used in the production of varietal wines, blend wines with the Cabernet Sauvignon or Tanat varieties, or as a base for sparkling wines, rendering the drink tasty due to the soft tannins, mellow and rounded, reminiscent of dark fruits merged with spices when the wine is aged in wood barrels. These wines generally have a violet-red color, with a typical aroma, and have a balanced palate between acidity and softness.

The agricultural versatility of the Merlot variety, such as the adaptation to several soil types, good ripening degrees to precocity, average grape skin thickness, good seed ripening, and lack of bunch compacting justify the success in the harvesting of this cultivar, being possible to use it to produce both young and old wines (Rizzon and Miele, 2009).

The incidence of rainfall during grape ripening, associated with low thermal amplitude in veraison may harm the ripening process, reducing skin coloration and the concentration of phenolic compounds and anthocyanins in red grapes (Buran et al., 2012).

There is a strong influence of climate on the development of vines and the duration of the phenological stages, bunch development, and berry ripening. As a consequence, grape quality and vineyard productivity are affected. The main edaphoclimatic factors that influence the vines are temperature, humidity, rainfall, and incidence of sunlight (Gardin et al., 2012).

Since anthocyanin content is partially regulated by abscisic acid and ethylene, exogenous applications of these plant growth regulators may increase the concentrations of phenolic compounds and anthocyanins in grape skins, influencing the visual and organoleptic properties of the wines. The more intense the color, the more interesting is the wine because a darker color generally indicates higher contents of anthocyanins and phenolic compounds, increasing the nutraceutical properties of the wine (Abe et al., 2007).

Several authors reported improvements in grape quality when using abscisic acid and ethephon at different doses and epochs of application on several European grape varieties, citing an increase in bunch mass, number of berries per bunch, and berry mass and diameter, among others. However, there are studies in which no improvement was observed, such as the reduction of soluble solids content, increase in titratable acidity, and reduction of berry mass and diameter (Vieira et al., 2008).

Abscisic acid is one of the phytohormones responsible for regulating the color in red wines, which gives important characteristics in the elaboration of high-quality fine wines due to the increase in the contents of phenolic compounds (Abe et al., 2007). Ethylene induces chlorophyll degradation in grape skin, promoting fruit ripening. This increases soluble solids content and grape color, enhancing the grape characteristics for wine production (Fracaro, 2000).

Plant growth regulators based on ethylene (or ethylene promoters, such as ethephon) and abscisic acid mimic the effects of the phytohormones naturally produced by the vines, which have a direct influence on plant metabolism (Kondo et al., 2014). In addition to the use of abscisic acid, some studies also used ethephon to promote an increase in grape color, however, the doses required to increase the content of pigments also caused a reduction in grape firmness, anticipating harvest time, with deleterious effects on overall grape quality (Peppi et al., 2007).

Considering that is necessary to identify the application form, epoch, and concentration of each plant growth regulator to maximize the effect on the quality of wine grapes, this work aimed to evaluate the effects of the application of different doses of abscisic acid and ethephon, applied in two distinct phenological stages, on the quality of Merlot grapes grown in Serra Gaúcha region, South Brazil.

2. Materials and Methods

2.1 Experimental conditions of the harvests

The study was conducted in a commercial vineyard, located in the municipality of Campestre da Serra, Serra Gaúcha, South Brazil (geographical coordinates 28°40' S; 51°06' W, and an altitude of 756 m above sea level). The climate of the region is classified as Cfb according to the Köppen classification, with a yearly average rainfall of 1,790 mm and average relative humidity of 83 %. The soil was classified as a nitisol, with more than 55 % clay (class 1), and wavy smooth relief (EMBRAPA, 2006). The soil had adequate fertility in both productive cycles according to the recommendations of the Commission of Soil Chemistry and Fertility (CQFS, 2016) for vineyards.

The vineyard was established in December 1998, with an approximate age of 22 years. The cultivar Merlot (*Vitis vinifera* L.) was grafted on the 'Paulsen 1103' rootstock (*Vitis berlandieri* × *Vitis rupestris*), conducted using an trellis system. Spacing was 1.5 m between plants and 3.5 m between lines, orientated in an east-west direction. Pruning in both productive cycles was carried out using the double Guyot system, on 2018-08-12 and 2019-08-02. Harvests were carried out on 2019-03-03 and 2020-03-01, corresponding to a cycle of 202 days for the 2018/2019 harvest and 213 days for the 2019/2020 harvests, respectively.

2.2. Treatments and application of plant growth regulators

The treatments were constituted of different application stages (veraison and 15 days before harvest) of the plant growth regulators ethephon and S-abscisic acid (S-ABA). The applications in veraison were carried out when the color of the berries was changing from green to light purple, with 50 % of the bunch colored. The second stage of the application was 15 days before

the expected harvest date (15 DBH). The treatments applied are detailed in Table 1.

Table 1 – Detailing of the treatments applied in the present study in both harvests.

Code	Treatment	Ethephon dose (mg·L ⁻¹)	Abscisic acid dose (mg·L ⁻¹)	Application stage (ethephon/S-ABA)
T0	Control	0	0	-/-
T1	ETF (V)	200	0	V/-
T2	ETF (15 DBH)	200	0	15 DBH/-
T3	ETF (V) + S-ABA (15 DBH)	200	600	V/15 DBH
T4	S-ABA (V)	0	600	-/V
T5	S-ABA (15 DBH)	0	600	-/15 DBH
T6	S-ABA (V) + S-ABA (15 DBH)	0	300 + 300	-/V, 15 DBH
T7	S-ABA (V) + ETF (15 DBH)	200	600	15 DBH/V

V: veraison; 15 DBH: 15 days before harvest. Source: Authors (2022).

The commercial product Ethrel[®] (Bayer CropScience, Germany), which contains 24 wt.% ethephon, was used as the ethephon source. The commercial product Protone[®] (Sumitomo Chemical Corporation Ltda, Japan), containing 10 wt.% S-ABA ((S)-abscisic acid), was used as the abscisic acid source. Both products were applied using the non-ionic silicon adhesive spreader Break-Thru[®] (Evonik Goldschmidt Chemical Corp., USA), composed of a polyether-polymethyl siloxane (PE-PDMS) copolymer 1,000 g·L⁻¹ at the concentration of 0.1 vol.%. The treatments were applied in the morning, between 08:30 and 10:00 h, using a backpack sprayer with a capacity of 10 L, applied until runoff, being applied directly on the bunches and leaves.

2.3 Grape harvest and sample preparation

Grape harvest in the 2018/2019 cycle was conducted on 2019-03-03, whereas the harvest in the 2019/2020 cycle was performed on 2020-03-01. Harvest time was determined by the technological ripening when the stalk presented a red-brown color and the soluble solids content increased with time. All bunches of the central plants of each parcel were collected, totaling 12 plants per treatment.

After the harvests, the grapes were stored for 12 h in a cold chamber at 10 °C and 95 % RH. The collected bunches were inspected and weighed, and the berries were extracted from the bunches. Withered, rotten, and unripe berries were manually separated and removed from the sample.

2.4 Evaluation of grape quality parameters

The biometric parameters of berry diameter and mass of a hundred berries were analyzed. To determine berry diameter, four berries from five bunches of each parcel were randomly collected, totaling 80 berries per treatment. Berry diameter was measured using a digital caliper with a resolution of 0.01 mm. The results were expressed in millimeters.

The mass of a hundred berries was determined by selecting randomly 100 berries in each parcel. The berries were weighed jointly using an analytical balance (AL500C, Marte, Brazil), with a resolution of 0.01 g. The results were expressed in grams.

Soluble solids content was determined according to IAL method 315/IV (IAL, 2008) and the results were expressed as Brix degree (°Brix). Titratable acidity was determined following IAL method 312/IV (IAL, 2008), the results were expressed as grams-equivalent of tartaric acid per 100 g of grapes.

The determination of phenolic compounds was carried out following the procedures of Pereira et al. (2018). Total anthocyanin contents were determined by the differential pH method (AOAC method 2005.02), described by Lee et al. (2005).

2.5 Experimental design and statistical analysis

The experiment was conducted in field conditions, with a randomized blocks design, with four parcels (replicates), and each replicate was composed of five plants. The two plants at the ends of the parcels were considered as a border and received no treatment. The three central plants of each parcel received the treatments with growth plant regulators and their grapes were collected.

The obtained results underwent Levene's test (homoscedasticity) and Shapiro-Wilk test (homogeneity of residuals), followed by Analysis of Variance (ANOVA) and Tukey's multiple range test at 5 % error probability ($\alpha = 0.05$). The statistical analyses were carried out using the Statistica 12 software (Statsoft, USA).

3. Results and Discussion

3.1 Edaphoclimatic conditions of the 2018/2019 and 2019/2020 harvests

For 2018, the municipality of Campestre da Serra had total chilling hours of 268 h (temperature below 7.2 °C), an amount inferior to the chilling requirement for Merlot grape, which is about 300 h to achieve adequate dormancy. For 2019, the total chilling hours was 322 h, supplying the necessity of the vines (CPTEC, 2020).

The temperatures in the dormancy period influence the nutritional reserves of the vines for the next productive cycle and remained within the historical series of the last 30 years of the region (CPTEC, 2020). The average monthly temperature was similar in both harvests. In August, when pruning was conducted, the average temperature was 10.7 °C and 9.6 °C in the 2018/2019 and 2019/2020 harvests, respectively. In November, time of bunch development, the average temperatures were 18.4 °C and 19.1 °C. In February, the period immediately before harvest, the thermal amplitude was 11.3 °C and 11.0 °C for the cycles (CPTEC, 2020).

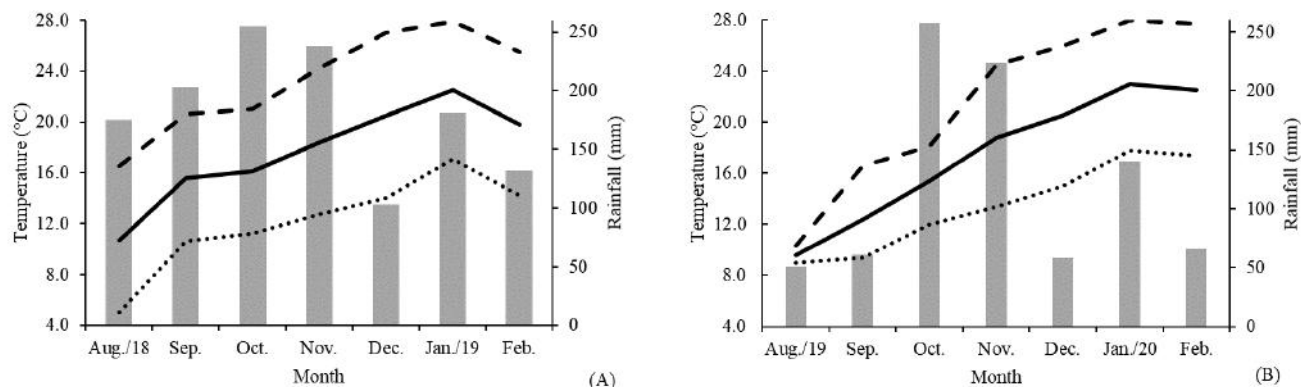
Rainfall, in both harvests, was within the values of the climatological normal of the region. The months of October and November had accumulated rainfall above the water requirements for vines in both cycles, which. This period is considered the most critical because this is the flowering and bunch forming periods. The accumulated rainfall in this period was 512 mm in the 2018/2019 cycle and 459 mm in the 2019/2020 cycle.

For January and February of the 2018/2019 harvest, when berries change color and increase soluble solids content, the accumulated rainfall was 313 mm. For the same period in the 2019/2020 harvest, the accumulated rainfall was 206 mm. Accumulated rainfall in both cycles was well beyond vine requirements, which are 384 mm according to Manica and Pommer (2006).

The data on temperature and rainfall from August to February in both productive cycles are compiled in Figure 1.

According to Jungues et al. (2020), in South Brazil, the main grape-producing regions are characterized by a cycle that initiates in September, when budding starts, ending in early March, with the ripening and harvesting of late varieties. In this period occurred the largest rainfall volumes, in which the ones in January and February, in both harvests, surpassed the water requirements of the vines throughout the cycle.

Figure 1 – Compilation of temperature (maximum, average, and minimum) and rainfall data for the municipality of Campestre da Serra, relative to the productive cycles of 2018/2019 (A) and 2019/2020 (B). Source: adapted from CPTEC (2019, 2020).



Source: Authors (2022).

High rainfall volumes next to harvest, when the grapes are in late ripening stages, associated with high temperatures in summer, tend to decrease soluble solids content, contributing to an increase in the titratable acidity of the berries (Manica & Pommer, 2006; Jungues et al., 2020).

In most years, delays in grape ripening cause a reduction in berry quality due to lower sunlight incidence. This is attributed to drought periods with overcast days. In this situation, when rain occurs, is generally intense, increasing the incidence of fungal diseases because of the impact of water drops on the softened grape skins (Gardin et al., 2012). According to Rocha and Guerra (2008), sunlight is one of the most important edaphoclimatic factors for berry growth, influencing the chemical profiles of the grapes through activation or by reducing the activity of enzymes that are responsible for the synthesis of compounds, such as phenylalanine ammonia-lyase (PAL), a key enzyme for production and accumulation of phenolic compounds in grapes and other plants.

3.2 Effect of the application of abscisic acid and ethephon on the quality parameters of Merlot grapes

Regarding the biometric parameters of the grapes in the 2018/2019 harvest, there were no statistical differences in berry diameter and mass of a hundred berries. The same behavior was observed for berry diameter in the 2019/2020 harvest (Table 2).

For the mass of a hundred berries in the 2019/2020 cycle, T3 - ETF (V) + S-ABA (15DAC) - had greater mass (191 g) relative to the control (T0, 164 g). On the other hand, T6 - S-ABA (V) + S-ABA (15DAC) - had the smallest mass (125 g) among the treatments.

Pessenti (2017), evaluating the application of different S-ABA doses on 'Primitivo' grapes in Água Doce, South Brazil, reported that the use of abscisic acid did not affect berry mass. Similar results were reported by Koyama et al. (2015), who evaluated different application epochs and doses of S-ABA on 'Isabel' grapes grown in Londrina, South Brazil. Both authors explained that the biometric parameters of grapes are strongly influenced by environmental factors, with little effect on plant growth regulators and ripening inducers. Similarly, Fracaro (2000), using the ethephon doses of zero, 720, 960, and 1.200 mg·L⁻¹ on 'Rubi' table grapes in Jales (Southeast Brazil), observed no differences regarding berry diameter and mass.

Table 2 – Berry diameter and mass of a hundred berries of Merlot grapes treated with the plant growth regulators abscisic acid and ethephon in the 2018/2019 and 2019/2020 harvests.

Harvest	2018/2019		2019/2020	
Treatment	Berry diameter (mm)	Mass of 100 berries (g)	Berry diameter (mm)	Mass of 100 berries (g)
T0 - Control	13.8 a	174.6 a	13.53 a	164.0 bc
T1 - ETF (V)	13.8 a	195.5 a	13.14 a	155.0 cd
T2 - ETF (15 DBH)	13.8 a	186.3 a	13.35 a	144.0 cde
T3 - ETF (V) + S-ABA (15 DBH)	13.8 a	185.7 a	12.77 a	191.0 a
T4 - S-ABA (V)	13.5 a	182.8 a	12.90 a	134.5 de
T5 - S-ABA (15 DBH)	13.9 a	190.7 a	12.50 a	181.3 ab
T6 - S-ABA (V) + S-ABA (15 DBH)	13.5 a	188.8 a	12.45 a	125.0 e
T7 - S-ABA (V) + ETF (15 DBH)	13.3 a	187.7 a	12.79 a	139.5 de
Coefficient of variation (%)	3.16	8.07	3.58	6.04

ETF(V): ethephon 200 mg·L⁻¹ in veraison; ETF (15 DBH): ethephon 200 mg·L⁻¹ 15 days before harvest; ETF (V) + S-ABA (15 DBH): ethephon 200 mg·L⁻¹ in veraison + S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V): S-ABA 600 mg·L⁻¹ in veraison; S-ABA (15 DBH): S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V) + S-ABA (15 DBH) S-ABA 300 mg·L⁻¹ in veraison + S-ABA 300 mg·L⁻¹ 15 days before harvest; S-ABA (V) + ETF (15 DBH): S-ABA 600 mg·L⁻¹ in veraison + ethephon 200 mg·L⁻¹ 15 days before harvest. Means in column followed by the same letter do not differ between themselves by Tukey's multiple range test at 5 % error probability ($\alpha = 0.05$). Source: Authors (2022).

Regarding the 2019/2020 harvest, all treatments that used S-ABA in veraison (T4, T6, and T7) had a lower average mass of a hundred berries, whereas the treatments T3 and T5 had the greatest mass of a hundred berries. It was also possible to observe that the application epoch had an important influence on the mass of a hundred berries. The application of the plant growth regulators ethephon in veraison and S-ABA 15 days before harvest (T3) increased berry mass (191.0 g). However, when the same doses were used, but in inverse application epochs (T7), the mass of a hundred berries was one of the lowest values (139.5 g) observed among the treatments.

According to Rizzon and Miele (2003), berry mass is related to the accumulation of sugars, but it can be also influenced by soil moisture and air humidity. Pessenti (2017) observed an increase in berry mass of 'Primitivo' grapes relative to the control with the application of S-ABA 600 mg·L⁻¹. However, this trend was not observed in both harvests in this study, likely due to differences in edaphoclimatic factors between the productive cycles. Carvalho (2000) and Vilas Boas (2000) stated that the mass loss between ABA application and harvest occurs because this phytohormone induces a quick and strong berry ripening, causing early senescence, with berry decay, wilting, and wrinkling. However, this behavior was observed only when ethephon was applied in veraison (T1).

The results of soluble solids content, pH of must, and titratable acidity of the grapes treated with ethephon and S-ABA in both harvests are compiled in Table 3.

For the 2018/2019 harvest, it was possible to observe that the soluble solids content was not influenced by the application order of the plant growth regulators, as T3 and T7 had similar soluble solids contents (21.1 °Brix and 21.0 °Brix, respectively). In this harvest, the two treatments that differed statistically (T3 and T5) were characterized by the application of S-ABA 15 days before harvest. In the 2019/2020 cycle, the treatments which applied only ethephon in veraison had the highest soluble solids content (23.97 °Brix), whereas the treatments using S-ABA in any epoch, or ethephon 15 days before harvest, had lower contents of soluble solids.

Table 3 – Soluble solids content, must pH, and titratable acidity of Merlot grapes treated with the plant growth regulators abscisic acid and ethephon in the 2018/2019 and 2019/2020 harvests.

Harvest	2018/2019			2019/2020			
	Treatment	Soluble solids (°Brix)	pH	Titratable acidity (% m/v)*	Soluble solids (°Brix)	pH	Titratable acidity (% m/v)*
	T0 - Control	20.5 ab	2.98 c	0.57 a	21.88 d	3.39 cd	0.55 a
	T1 - ETF (V)	20.3 ab	2.99 c	0.39 b	23.97 a	3.57 a	0.48 b
	T2 - ETF (15 DBH)	20.5 ab	3.07 b	0.38 b	23.05 b	3.42 bcd	0.48 b
	T3 - ETF (V) + S-ABA (15 DBH)	21.1 a	3.11 ab	0.55 a	21.72 d	3.47 b	0.51 ab
	T4 - S-ABA (V)	21.05 ab	3.10 b	0.54 a	22.92 bc	3.43 bc	0.51 ab
	T5 - S-ABA (15 DBH)	20.2 b	3.11 ab	0.51 a	22.97 b	3.28 e	0.55 a
	T6 - S-ABA (V) + S-ABA (15 DBH)	21.0 ab	3.17 a	0.56 a	22.30 cd	3.28 e	0.56 a
	T7 - S-ABA (V) + ETF (15 DBH)	21.0 ab	3.11 ab	0.54 a	22.87 bc	3.35 de	0.49 b
	Coefficient of variation (%)	1.86	0.90	8.07	1.25	0.88	4.97

ETF(V): ethephon 200 mg·L⁻¹ in veraison; ETF (15 DBH): ethephon 200 mg·L⁻¹ 15 days before harvest; ETF (V) + S-ABA (15 DBH): ethephon 200 mg·L⁻¹ in veraison + S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V): S-ABA 600 mg·L⁻¹ in veraison; S-ABA (15 DBH): S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V) + S-ABA (15 DBH) S-ABA 300 mg·L⁻¹ in veraison + S-ABA 300 mg·L⁻¹ 15 days before harvest; S-ABA (V) + ETF (15 DBH): S-ABA 600 mg·L⁻¹ in veraison + ethephon 200 mg·L⁻¹ 15 days before harvest. Means in column followed by the same letter do not differ between themselves by Tukey's multiple range test at 5 % error probability ($\alpha = 0.05$). * - grams-equivalent of tartaric acid per 100 g of must. Source: Authors (2022).

Pires and Botelho (2001) reported that the application of ethephon did not affect soluble solids content in Merlot grapes, only decreasing the titratable acidity. The same authors also stated that ethephon, when applied in bunches with more than 15 % of color change, accentuates and anticipate color development of red grapes. On the other hand, when applied during berry softening, ethephon anticipates grape ripening. The results of the two harvests presented the same trend in which the application of ethephon increased the soluble solids content of the grapes relative to S-ABA and the control.

Relative to the pH of the must, the observed values for the 2018/2019 harvest were above the ones of the control, except T1. The treatments that used S-ABA had pH values higher than the control (T0), indicating a less acid must than when S-ABA is not applied. Considering the pH results for the 2019/2020 harvest, the highest value (3.57) occurred in T1 (ethephon applied in veraison); in this harvest, there was no specific trend regarding the use of S-ABA, implying that the edaphoclimatic factors of this cycle may have influenced this parameter along with the use of the plant growth regulators. According to Brazilian regulations, there is no pH range for wines, only that the pH must be equal to or above 2.9 (BRASIL, 1998). All treatments in both harvests had pH values following the Brazilian law.

Regarding titratable acidity for the 2018/2019 harvest, only T1 and T2 (both composed of ethephon applications) were statistically smaller than the control (T0), the others have not differed from it. In the 2019/2020 harvest, the titratable acidity of the treatments with ethephon was smaller than those of the control, whereas the presence of S-ABA had no impact on this parameter. Pessenti (2007) commented that the relationship between titratable acidity and the pH of the must is inversely proportional. Lerin (2014) has not observed statistical differences in soluble solids content, pH of must, and titratable acidity in the application of 200, 400, 600, and 800 mg·L⁻¹ of S-ABA on Cabernet Sauvignon grapes in veraison in Serra Gaúcha region, a behavior partially similar to the one observed in this study.

Rizzon and Miele (2003) commented that smaller berries may enhance the release of minerals, influencing the salification of acids, reducing pH values, and increasing the titratable acidity. This was observed in the present work in the 2019/2020 harvest, the treatments T5 and T6 had the smallest berry diameters, the smallest pH values, and the highest titratable acidity among all treatments. Pires and Botelho (2001) also stated that ethephon, by generating ethylene, may induce a reduction in the titratable acidity of the grapes, a phenomenon also observed in this study.

Fregoni (1998) stated that there is a relationship between the increase in pH values and the reduction of titratable acidity

because these parameters are influenced by the same factors. This could be observed in treatments T1 and T6 in the 2019/2020 cycle, in which T1 had higher pH and lower titratable acidity and T6 had lower pH and higher titratable acidity among the treatments.

The results of phenolic compounds and total anthocyanins of Merlot grapes treated with S-ABA and ethephon in the two productive cycles are presented in Table 4.

Table 4 – Phenolic compounds and total anthocyanin contents of Merlot grapes treated with the plant growth regulators abscisic acid and ethephon in the 2018/2019 and 2019/2020 harvests.

Harvest	2018/2019		2019/2020	
	Phenolic compounds (mg·100 g ⁻¹) ¹	Anthocyanins (mg·kg ⁻¹) ²	Phenolic compounds (mg·100 g ⁻¹) ¹	Anthocyanins (mg·kg ⁻¹) ²
T0 - Control	81.4 b	532.1 bc	56.6 a	432.1 abc
T1 - ETF (V)	81.7 b	546.0 bc	56.8 a	478.1 a
T2 - ETF (15DAC)	82.7 b	506.1 bc	41.8 bc	393.3 bc
T3 - ETF (V) + S-ABA (15DAC)	71.5 c	501.3 bc	32.9 c	362.7 c
T4 - S-ABA (V)	74.7 bc	514.7 bc	47.6 ab	458.7 ab
T5 - S-ABA (15DAC)	76.6 bc	470.8 c	38.2 bc	464.1 ab
T6 - S-ABA (V) + S-ABA (15DAC)	80.1 bc	588.5 b	37.4 bc	391.1 bc
T7 - S-ABA (V) + ETF (15DAC)	102.0 a	719.3 a	46.8 ab	420.0 abc
Coefficient of variation (%)	5.13	8.69	10.93	7.59

ETF(V): ethephon 200 mg·L⁻¹ in veraison; ETF (15 DBH): ethephon 200 mg·L⁻¹ 15 days before harvest; ETF (V) + S-ABA (15 DBH): ethephon 200 mg·L⁻¹ in veraison + S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V): S-ABA 600 mg·L⁻¹ in veraison; S-ABA (15 DBH): S-ABA 600 mg·L⁻¹ 15 days before harvest; S-ABA (V) + S-ABA (15 DBH) S-ABA 300 mg·L⁻¹ in veraison + S-ABA 300 mg·L⁻¹ 15 days before harvest; S-ABA (V) + ETF (15 DBH): S-ABA 600 mg·L⁻¹ in veraison + ethephon 200 mg·L⁻¹ 15 days before harvest. Means in column followed by the same letter do not differ between themselves by Tukey's multiple range test at 5 % error probability ($\alpha = 0.05$). ¹ – grams-milliequivalent of gallic acid per 100 g of berries; ² – grams-milliequivalent of cyanidin-3-glycoside per kilogram of berries. Source: Authors (2022).

It can be observed that, in the 2018/2019 harvest, treatment T7 had the highest phenolic compounds and anthocyanin contents relative to the other treatments. This may indicate a possible synergism between S-ABA and ethephon, when they are applied in veraison and 15 days before harvest, respectively. According to Domingues Neto et al. (2017), abscisic acid is responsible for the synthesis and accumulation of anthocyanins in berries. Gardin et al. (2012) reported that when ethephon and abscisic acid are applied together, abscisic acid potentiates the effect of ethephon by increasing ethylene release. This makes it possible to anticipate grape ripening and intensifying berry color.

In the 2019/2020 harvest, however, the observed behavior was different relative to both phenolic compounds and anthocyanins, in which the use of plant growth regulators have not followed a specific trend, probably due to the interaction between these phytohormones and edaphoclimatic factors inherent to the productive cycle. Gardin et al. (2012) commented that ethephon, when associated with abscisic acid, tends to increase the contents of phenolic compounds than when applied isolated. Vaccaro et al. (2019) also observed similar behavior in Cabernet Sauvignon grapes, in which the synergistic action between S-ABA and ethylene (ethephon) enhanced the quality of the grapes compared to the individual use of plant growth regulators.

This synergism is attributed to the effect of abscisic acid on the synthesis and accumulation of anthocyanins and the influence of ethephon on grape ripening through the release of ethylene. Colli and Purgat (2008) stated that abscisic acid may also act as an ethylene-releasing promoter, which helps explain the behavior observed in this study. The results observed for T7 suggest that, when used jointly, these phytohormones may act synergistically, enhancing berry pigmentation and promoting the accumulation of anthocyanins and phenolic compounds; this may anticipate grape ripening.

La Campagne et al. (2010) commented that S-ABA is a coregulator of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), enzymes responsible for the synthesis of (+)-catechins and (-)-epicatechins in grapes. Vaccaro et al. (2019) observed an increase in the content of phenolic compounds in Cabernet Sauvignon grapes treated with ethephon

and ethephon plus S-ABA, with increases of 41 % and 48 %, respectively, relative to the control. Rodrigues et al. (2010) reported that using ethephon enhanced the color of Rubi grapes, even under unfavorable edaphoclimatic conditions to the development of color in the berries.

According to Ruiz-Garcia et al. (2012), who studied the Sauvignon Blanc variety in Spain, the musts of grapes treated with ethephon tend to have higher contents of phenolic compounds than grapes not treated if ethephon is applied shortly before harvest. However, this behavior was not observed in this work, considering the treatments in which ethephon was applied alone in veraison (T1) or 15 days before harvest (T2).

Rufato et al. (2016) observed an increase in phenolic compounds content in the skin of Isabel grapes treated with S-ABA 600 mg·L⁻¹ in veraison, grown in the municipality of Pinto Bandeira (Serra Gaúcha region, South Brazil). Sandhu et al. (2011) evaluated the effect of two applications of S-ABA 300 mg·L⁻¹ on Alachua table grape and Noble wine grape grown in Apopka (USA), in which S-ABA promoted the accumulation of phenolic compounds in the Noble variety whereas there was no influence of this phytohormone on the Alachua grapes. Pessenti (2017), studying the application of plant growth regulators on Malbec grapes, reported that anthocyanin contents in grapes were not influenced by the application of S-ABA. This behavior was observed in this work, in which anthocyanin contents were statistically similar in both harvests with and without the use of S-ABA.

According to Almeida et al. (2016), the incidence of sunlight and temperature is extremely important for anthocyanin biosynthesis. More exposition to sunlight also increases the contents of soluble solids in berry skin, promoting the accumulation of anthocyanins. For the European grapes (*Vitis vinifera* L.), anthocyanins are mostly produced in veraison, characterized by changes in berry color and texture due to the accumulation of anthocyanins in grape skin. Concomitantly, there is an increase in phenolic compounds, which will increase the color intensity of wines. However, it is also important to point out that anthocyanin content in berries may vary widely, as observed by Tardaguila et al. (2010), who studied Grenache grapes in Rioja (Spain).

The results of the present study demonstrate that there is a potential for the use of plant growth regulators to standardize the maturation of grapes and their quality, which may impact the quality of the wines produced. However, due to the climate variability between the assessed crops, it is necessary to carry out a new assessment considering the productive cycles and the climatic variations to adjust the application of these growth regulators on Merlot grapes in Serra Gaúcha region.

4. Conclusion

The application of abscisic acid 600 mg·L⁻¹ in veraison increased the content of soluble solids. The use of S-ABA in the veraison plus ethephon 15 DBH promoted an increase in the content of phenolic compounds and anthocyanins in the 2018/2019 harvest, but with no significant effect on the 2019/2020 cycle. This shows the strong influence of climatic conditions on the effect of plant growth regulators on 'Merlot' grapes.

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