



Agronomic and physicochemical parameters of must and wine as a function of changes in ‘Cabernet Sauvignon’ grapevine canopy

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ABSTRACT. This study aimed to evaluate the influence of vegetative canopy height on the agronomic characteristics and grape must and wine physicochemical properties of a ‘Cabernet Sauvignon’ vineyard in an espalier-trained system. The evaluated parameters comprised agronomic characteristics of ‘Cabernet Sauvignon’ grapevines and physicochemical compositions of ‘Cabernet Sauvignon’ musts and wines, as well as their phenolic compositions (anthocyanins, stilbenes, and flavonoids), and impact on wine contents of methoxypyrazines (volatile compounds that impart vegetal or earthy odors to wine, which are considered undesirable in large intensity). To that end, four heights of the vegetative canopy were tested: 60 cm (T1), 80 cm (T2), 100 cm (T3), and 120 cm (T4). The experiment was carried out in a commercial vineyard in the region of “Campanha Gaúcha” (Dom Pedrito, Rio Grande do Sul State, Brazil) during the productive cycles of 2015/16, 2016/17, 2017/18, and 2018/19. The main agronomic parameters were measured: estimated productivity per plant and hectare, and mean weight and number of clusters. All wines were elaborated by the same traditional winemaking methods. The physicochemical analyses of must and wines were performed by infrared spectroscopy using Fourier Transform Infrared Spectrometer (FTIR), and the phenolic analysis by high-efficiency liquid chromatography and UV-Vis spectrophotometry. Methoxypyrazines were quantified using headspace solid-phase microextraction (HS-SPME), followed by gas chromatography-mass spectrometry (GC-MS). The results showed that treatments did not influence agronomic parameters. However, technological maturation (sugar accumulation) had interesting results for plants managed at higher canopy heights, with respective results obtained for wine. Treatments had little influence on individual quantification of anthocyanins, although cycles had a high influence on their profile. The wines had low concentrations of methoxypyrazines and did not differ among treatments.

Keywords: *Vitis vinifera* L.; technological maturity; phenolic compounds; methoxypyrazine; Campanha Gaúcha.

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Introduction

Besides plant genetics, climate, soil, and vegetation management are determining factors of grape quality and productivity (Miele & Mandelli, 2012; Miele & Rizzon, 2013; Parker et al., 2020). Grape growers can, within certain limits, handle the latter two factors. Regarding grape quality, vegetative canopy management in vineyards is one of the most impactful (Del-Castillo-Alonso et al., 2015; Del-Castillo-Alonso et al., 2016; Leão, Nunes, & Lima, 2016).

Aside from changes in the production of photoassimilates, vegetative canopy management affects grapevine canopy microclimate, increasing concentrations of several wine chemical components and accumulating sugars, organic acids, and polyphenols, as well as impacting plant agronomic parameters (Blancquaert, Oberholster, Ricardo-da-Silva, & Deloire, 2019; Bobeica et al., 2015; Borghezan, Pit, Gavioli, Malinovski, & Silva, 2011; Parker et al., 2020; Würz et al., 2017).

Hence, in many winegrowing regions and within them, vegetative canopy structure has been managed for each wine produced, aiming to achieve the best source: sink ratio (Luciano, Albuquerque, Rufato, Miquelluti, & Warmling, 2013; Würz et al., 2020; Zhang et al., 2017). In general, this is the basic physiological principle guiding phytotechnical actions towards productivity and fruit and wine quality (Marcon Filho, Hipólito, Macedo, Kretzschmar, & Rufato, 2015; Miele & Rizzon, 2013).

In an espalier training system, canopy height is an important aspect to be defined in vineyard management so that the best source: sink ratio could be identified, and the most common figures vary from 100 to 120 cm (Borghezan et al., 2011; Brighenti, Rufato, Kretzschmar, & Schlemper, 2011; Leão et al., 2016; Würz et al., 2017). Such a management type aims to balance leaf area and grape production so that there is evenness between the main vegetative parts (branches and leaves) and reproductive part (grape clusters). This balance between leaf area (source) and productivity (sink) can be assessed by some methods, among which the Ravaz index is one of the most employed in viticulture (Naor, Gal, & Bravdo, 1997). In short, managements that generate indexes between 5 and 10 are desired, indexes above 10 indicate fruit overproduction, while values below 5 suggest excessive plant vigor (Naor et al., 1997).

Topping is the most employed practice to ensure that the above parameters are within the expected, and it consists of changing vegetative canopy dimensions (Miele & Mandelli, 2012; Würz et al., 2020). Many authors have described the influence of leaf area when topping, which affects agronomic parameters and grape quality, and hence wine quality (Borghezan et al., 2011; Miele & Mandelli, 2012; Miele & Rizzon, 2013; Würz et al., 2017). Vegetative canopy management is complex since it is closely related to climate (microclimate) and soil conditions, and climatic conditions in each growing season have a great impact on grape productivity and quality (Ferrer, Pereyra, Salvarrey, Arrillaga, & Fourment, 2020; Leão et al., 2016; Luciano et al., 2013). Given these variations and climatic characteristics, studies should be carried out during several productive cycles (harvests) to obtain more consistent data.

The “Campanha Gaúcha” (Rio Grande do Sul, Brazil) is a region considered one of the warmest and least rainy for winemaking (Tonietto, Ruiz, & Gómez-Miguel, 2012). Its main characteristics are low altitudes, flat topography with slight undulations, soil diversity (with a predominance of sandy soils), and interesting weather conditions during grape ripening, which consists of high light accumulation, high temperatures, and low rainfall (~1300 mm rainfall yearly) (Nicoll et al., 2020; Tonietto et al., 2012). In the region, wine grapes are trained in an espalier system, and ‘Cabernet Sauvignon’ is one of the main cultivars grown (Dutra et al., 2013).

‘Cabernet Sauvignon’ grapes generally produce medium to deep-coloured and medium to full-bodied wines, of which the phenolic composition must be known (Bindon et al., 2014). Another important characteristic is that, under low thermal accumulation and insulation, this cultivar can accumulate higher concentrations of methoxypyrazines (MPs), which are volatile heterocyclic nitrogen-containing compounds that contribute to vegetative or earthy aromas of various vegetables and fruits (Fontana, Rodriguez, & Cela, 2017). In grapes and wines, the most abundant MP is 3-isobutyl-2-methoxypyrazine (IBMP), which is responsible for green pepper odor and considered undesirable at concentrations close to 15 ng L⁻¹ (Mozzon, Savini, Boselli, & Thorngate, 2016). Some factors such as vineyard management, early harvest, and climatic conditions do not favor grape ripening and may result in wines with higher IBMP concentrations (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Gregan & Jordan, 2016; Lei et al., 2018).

Given the above, this study aimed to evaluate the influence of vegetative canopy management on the agronomic parameters of grapevines, on the physicochemical compositions of ‘Cabernet Sauvignon’ must and wine and their respective phenolic composition (anthocyanins, stilbenes, and flavonoids), as well as the effects on methoxypyrazine contents in wines.

Material and methods

Experimental period and edaphoclimatic conditions

The experiment was carried out in a vineyard in the city of Dom Pedrito, Rio Grande do Sul State, Brazil (31°01' S, 54°36' W, 159-m altitude), during the productive cycles of 2015/16, 2016/17, 2017/18, and 2018/19. The research site lies in the mapping unit Bexigoso, wherein the soil is classified as Typical Orthic Haplic Luvisol (Streck et al., 2008). The local climate is classified as humid subtropical, *Cfa* type according to Köeppen's classification (Tonietto et al., 2012). Accumulated monthly rainfall and average monthly temperature data were collected for the vegetative period of the four productive cycles evaluated (Figure 1).

Agronomic characteristics

The study was carried out in a 0.5-ha commercial vineyard grown with the cultivar Cabernet Sauvignon (clone R8, rootstock ‘SO4’) implanted in 2000. The grapevines were trained in an espalier system and spaced at 3.0 x 1.2 m (rows x plants) in an East-West solar orientation. The experiment was composed of two 250-m transversal rows, totaling an area of 0.08 hectare.

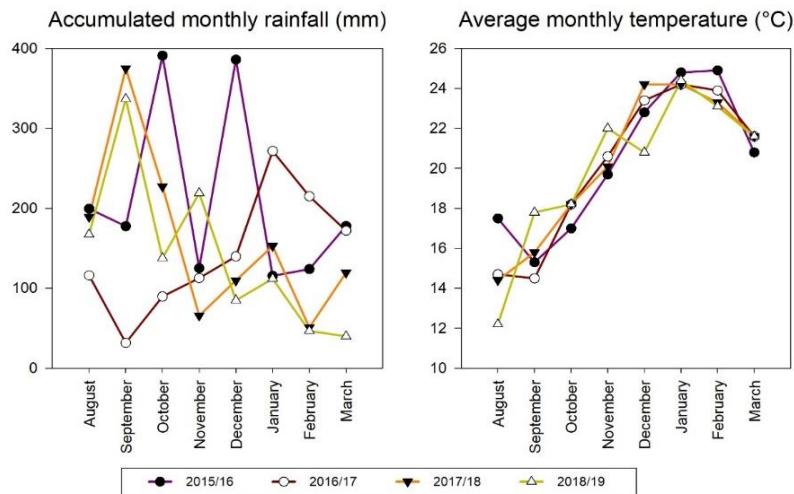


Figure 1. Accumulated monthly rainfall (in mm) and average monthly temperature (°C) in the four productive years studied: 2015/16, 2016/17, 2017/18, and 2018/19. Source: Estância Guatambu Weather Station, 2020. Available at: <http://estanciaguatambu.com.br/website/meteorologia>. Accessed on: Feb 20, 2020.

During the production cycles, phytotechnical (fertilization, mowing, and defoliation) and phytosanitary (herbicides, fungicides, and insecticides) practices were evenly applied to all treatments. Double-pruning in 'Sporonated Cord' was conducted using 2 buds per spur. The number of buds ranged from 24 to 28 per plant. The phenological cycle started in mid-September (budding) and ended in the first half of March (harvest). During ripening, protective nets were used in all treatments placed on clusters to protect grapes from bird attack and preserve productivity data.

Averages of the four productive cycles for general agronomic characteristics of the vineyard were: 124.18 g mean cluster weight, 3.25 kg mean productivity per plant, and 9.03 tons of average productivity estimated per hectare, based on the plant density of the studied vineyard (2,775 plants). Moreover, the Ravaz Index was estimated as 5.5 for T1, 4.0 for T2, 3.5 for T3, and 3.0 for T4, which were obtained in the first studied productive cycle (2015/16) (Table 1).

Winemaking

Before processing, the grapes were stored in a cold room at 6°C and 80% relative humidity for 24 hours. Afterwards, they were destemmed, crushed, and transferred to 20-L glass containers, to which was added 100 mg kg⁻¹ potassium metabisulfite (BASF SE, Ludwigshafen, Germany) and 5 g hL⁻¹ pectolytic enzyme (Colorpect VR-C®, Amazon Group Ltda, Bento Gonçalves, Rio Grande do Sul State, Brazil). To start alcoholic fermentation, 20 g hL⁻¹ active dry *Saccharomyces cerevisiae* yeast (Zymaflore FX 10®, Laffort, France) and 20 g hL⁻¹ nutrients for yeast (Gesferm Plus®, Amazon Group Ltda, Brazil) were inoculated.

The wines were made by classic winemaking procedures, with 8-day maceration with skin and seeds. During maceration/fermentation, *remontages* were performed (twice a day), and fermentation temperature was kept between 20 and 22°C. After maceration, devatting (separation of solid and liquid portions) and pressing processes were executed. Two days later, clear wine was relocated to 4.6-L bottles fully filled for malolactic fermentation. It was spontaneous (90-day duration) and, after its completion, potassium metabisulfite was re-added since it has a preservative action and prevents oxidation and proliferation of microorganisms, keeping volatile acidity levels low (adjusted concentration of 25 mg L⁻¹ free SO₂). After three months, the wines were bottled in 750 mL bottles.

Physicochemical characterization of must and wine

The physicochemical properties of must and wine analyzed were: pH, total soluble solids (TSS) in °Brix, reducing sugars (RS), total acidity (TA), and concentrations of tartaric acid and malic acid using the WineScan™ SO₂ equipment (FOSS Analytics, Hillerod, Denmark). As for the classic analysis of wines, we measured alcohol content, total acidity, pH, reducing sugar content, glycerol content, and volatile acidity (using the same equipment), color intensity and hue, and total polyphenol index (TPI) by UV-Vis spectrophotometry (UV-2000A, Instrutherm, São Paulo, São Paulo State, Brazil) (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Analysis of phenolic compounds and anthocyanins

These analyses started in the second productive cycle, wherein 200 µL wine was diluted 5 times in 800 µL methanol HPLC grade (Sigma-Aldrich, St. Louis, MO, USA) and then filtered through a 0.45 µM membrane. The process was based on the method presented by Delcambre and Saucier (2012). Once the sample was ready, an aliquot of 10 µL was injected in a high-performance liquid chromatograph (UFLC, Shimadzu, Japan), coupled with a high-resolution quadrupole-flight-time mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany). For chromatographic separation, C18 pre-column (2.0 x 4.0 mm) and C18 Luna column (2.0 x 150 mm, 100 Å, 3.0 µm) (Phenomenex Torrance, CA, USA) were used. The separation was performed with two mobile phases (eluents) to promote interaction with samples and consequently chromatographic separation. The mobile phases were: water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The separation process lasted 30 minutes for each sample and elution gradients used were: 0-2 min. 10% B; 2-15 min. 10-75% B; 15-18 min. 90% B; 18-21 min. 90% B; 21-23 min. 10% B; and 23-30 min. 10% B. Total flow and column temperature were maintained at 0.2 mL·min⁻¹ and 40°C, respectively. The mass spectrometer was operated in negative (phenolic acids and flavonoids) and positive (anthocyanins) ESI modes, acquiring spectra over a mass range of *m/z* 50-1200, with capillary voltage at 3.5 kV, nebulization gas pressure (N_2) at 2 bars, drying gas at 8 L min⁻¹, source temperature at 180°C, RF collision at 150 Vpp, transfer at 70 mS, and pre-pulse storage at 5 mS. The equipment was calibrated using 10 mM sodium formate, covering the entire range of acquisition (of *m/z* 50-1200). Automated experiments of MS/MS were executed by adjusting collision energy values as following: *m/z* 100, 15 eV; *m/z* 500, 35 eV; *m/z* 1000, 50 eV, operating nitrogen as collision gas. The MS and MS/MS data were processed using the Data Analysis 4.0 software (Bruker Daltonics, Bremen, Germany).

Anthocyanins were characterized by UV-Vis spectrum (210 - 800 nm) and exact mass, and MSn fragmentation patterns were compared to the equipment library data and databases (standard, Metlin, Mass Bank, Kegg Compound, Chem Spider) and compared with the isotopic standard. Phenolic acids and flavonoids were quantified since the second productive cycle, using an external calibration curve with the standards of each compound, and results expressed in µg mL⁻¹. Anthocyanins were quantified by comparison with a calibration curve with an external standard of pelargonidin, and results expressed in µg mL⁻¹ internal standard (reserpine).

Determination of methoxypyrazines

Methoxypyrazines were analyzed using the method of Kotseridis et al. (2008), with optimizations. For sample preparation, each wine had its pH adjusted to 6.0 using 2 mol NaOH L⁻¹. Therefore, to each 10-mL SPME flask was added 2 mL wine (pH 6.0), which was diluted in 2 mL ultrapure water and 1 g NaCl (sodium chloride). Afterwards, the samples were hermetically sealed and homogenized using magnetic stirring for 2 minutes at 37 ± 1°C, and a Divinylbenzene/Carboxen/Polydimethylsiloxane fiber (DVB/CAR/PDMS) (Supelco 57328-U®, Darmstadt, Germany) was exposed in the *headspace* for 30 minutes, under the same conditions. Thereafter, the fiber was immediately inserted into the GC-MS injector at a temperature of 250°C.

For chromatographic separation, a chromatographic column RxI-1MS (30 m x 0.32 mm x 0.25 µm) was used. The temperature program was set at 40°C for 5 minutes, with increments of 3°C every minute up to 110°C, and then holding to it for 1 minute. Finally, the increments were raised to 25°C per minute up to 230°C. Helium was used as carrier gas with a constant flow of 1 mL per minute throughout the column. The mass spectrometer was operated in selected ion monitoring (SIM) mode. Ion source temperature was set at 200°C and the interface at 290°C. An external calibration curve with a commercial standard of 2-Isobutyl-3-methoxypyrazine (Merck KGaA, Darmstadt, Germany) was performed in the matrix with a linear range of 0.5 to 7.5 ng L⁻¹ ($r^2 = 0.9974$) for quantification of the compound in the wines. The quantification was performed for a peak area base of *m/z* 124 at a retention time of 20.4 ± 0.1.

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design due to the homogeneity of the terrain and a large number of repetitions. It was composed of four treatments (different vegetative canopy heights) and six repetitions per treatment, seven plants per repetition, totaling 168 plants. The studied canopy heights were: 60 (T1), 80 (T2), 100 (T3), and 120 cm (T4).

All grapes were harvested without any sort of selection. After evaluating agronomic characters, the grapes destined for winemaking were homogeneously removed. Wine evaluations were carried out using triplicate

techniques. Must and wine agronomic and physicochemical variables were measured in all productive cycles, whereas phenolic compounds, anthocyanins, methoxypyrazines, and resveratrol were quantified only in the last three productive cycles. This was because no striking differences were detected in the wines in the first productive cycle; therefore, we decided to assess whether there might be differences in the profiles of these compounds.

Data gathered underwent analysis of variance (ANOVA) and, when significant, the averages were compared by the Tukey's test at 5% probability. For individual anthocyanin quantifications, a principal component analysis (PCA) was applied.

Results and discussion

Agronomic parameters

For the cultivar Cabernet Sauvignon, the phytotechnical management of vegetative canopy at heights of 60, 80, 100, and 120 cm did not affect grapevine responses for most of the evaluated agronomic parameters (Table 1). This result agrees with those of similar studies in which leaf area management, within a certain limit, did not affect productivity indexes (Borghezan et al., 2011; Leão et al., 2016). Our finding is important for winegrowers, especially for implementing and infrastructure of new vineyards in this region. The Ravaz index of T1 in the first productive cycle (Table 1) ranged between 5 and 10, indicating a certain balance between production and vegetation (Naor et al., 1997).

Table 1. Agronomic traits of 'Cabernet Sauvignon' grapevines managed at different vegetative canopy heights (60, 80, 100, and 120 cm) in four productive cycles (2015/16, 2016/17, 2017/18, and 2018/19).

Agronomic characteristic	Productive cycle	Treatment				CV (%)
		T1 (60 cm)	T2 (80 cm)	T3 (100 cm)	T4 (120 cm)	
Average cluster weight (g)	2015/16	78 ^{ns}	136	79	70	76.66
	2016/17	113 ^{ns}	119	123	129	22.26
	2017/18	155 ^{ns}	145	167	173	11.64
	2018/19	138 ^{ns}	118	123	121	13.78
Average cluster number per plant	2015/16	24 ^{ns}	18	20	22	23.46
	2016/17	45 ^{ns}	35	41	37	17.18
	2017/18	24 ^{ns}	26	24	24	19.29
	2018/19	23 ^{ns}	20	19	21	18.69
Productivity per treatment (kg)	2015/16	13 ^{ns}	12	12	10	36.10
	2016/17	36 ^{ns}	29	36	34	29.36
	2017/18	26 ^{ns}	26	28	28	23.16
	2018/19	21 ^{ns}	16	17	18	26.49
Productivity per plant (kg)	2015/16	1.80 ^{ns}	1.73	1.70	1.50	36.10
	2016/17	5.11 ^{ns}	4.16	5.21	4.85	29.63
	2017/18	3.76 ^{ns}	3.78	4.00	4.07	23.16
	2018/19	3.04 ^{ns}	2.33	2.39	2.61	26.49
Estimated productivity per hectare (t ha ⁻¹)	2015/16	5.142 ^{ns}	4.810	4.727	4.149	36.10
	2016/17	14.169 ^{ns}	11.541	14.457	13.464	29.63
	2017/18	10.426 ^{ns}	10.492	11.106	11.291	23.16
	2018/19	8.437 ^{ns}	6.478	6.643	7.274	26.49
Ravaz Index	2015/16	5.495 ^a	4.016 ^{ab}	3.535 ^{ab}	3.040 ^b	11.23

Legend: ^{ns} - non-significant difference, CV (%) - coefficient of variation. Means followed by distinct lower-case letters on the same line differ from each other by the Tukey's test at 5% probability.

Must physicochemical properties

Regarding technological maturity, T3 obtained the lowest levels of SST (19.83 and 19.20°Brix) and reducing sugars (195.60 and 191.00 g L⁻¹) in the 2015/16 and 2016/17 productive cycles (Table 2). This can be explained by the photosynthetic compensation in vines of T1 (60 cm) and T2 (80 cm), as these plants were topped before those in T3, which improved their recovery of photoassimilates (Leão et al., 2016).

Acidity-related parameters of must also did not show a clear effect of treatments. However, T3 had the lowest pH (3.67) in the first productive cycle (2015/16), and all treatments showed significant statistical results in the second productive cycle (2016/17). Therefore, the best treatment cannot be ascertained (Table 2). In similar studies with canopy management, both pH and total acidity were not affected (Borghezan et al., 2011; Würz et al., 2017; Zhuang, Tozzini, Green, Acimovic, & Howell, 2014); however, some studies

have shown that grapes less exposed to the sunlight have lower pH and higher acidity values (Leão et al., 2016; Zhang et al., 2017), with climate (temperature) as the main variation factor (Ferrer et al., 2020; Luciano et al., 2013). In the second productive cycle, T3 showed a higher concentration of tartaric acid (6.03 g L^{-1}), which is consistent with the other results (Table 2), e.g., higher TA and less accumulation of reducing sugars and TSS in the same productive cycle, indicating a slight delay in ripening (Rizzon & Sganzerla, 2007). Malic acid, which also has its degradation conditioned to maturation and oxidative respiration, was only changed in the second productive cycle when T2 (2.86 g L^{-1}) and T3 (2.90 g L^{-1}) obtained the highest values.

Our findings also point to a low acidity of the grape must, which is somewhat typical of the region (Zocche et al., 2017). Another important aspect is must potassium content (Table 2), which decreased throughout the productive cycles, with a consequent decrease in pH, as already explained by Ribéreau-Gayon et al. (2006).

Table 2. Physicochemical composition of grape must from ‘Cabernet Sauvignon’ grapevines managed at 60, 80, 100, and 120 cm heights in four productive cycles (2015/16, 2016/17, 2017/18, and 2018/19).

Response parameter	Productive cycle	Treatment				CV (%)
		T1 (60 cm)	T2 (80 cm)	T3 (100 cm)	T4 (120 cm)	
Total soluble solids (°Brix)	2015/16	20.90 ^a	20.70 ^a	19.83 ^b	21.33 ^a	1.27
	2016/17	19.43 ^b	19.50 ^b	19.20 ^c	19.70 ^a	0.30
	2017/18	21.80 ^{ns}	22.73	22.13	22.40	3.28
	2018/19	21.67 ^{ns}	22.07	21.57	22.03	2.21
Total acidity (g L ⁻¹ H ₂ SO ₄)	2015/16	2.70 ^{ns}	2.93	2.93	2.76	3.48
	2016/17	2.70 ^c	2.86 ^{bc}	3.13 ^a	2.90 ^b	1.96
	2017/18	2.80 ^{ns}	2.86	2.96	2.96	4.34
	2018/19	2.70 ^{ns}	2.73	2.75	2.70	3.23
pH	2015/16	3.73 ^a	3.71 ^a	3.67 ^b	3.71 ^a	1.03
	2016/17	3.55 ^a	3.53 ^b	3.50 ^c	3.47 ^d	0.09
	2017/18	3.48 ^{ns}	3.48	3.50	3.48	0.86
	2018/19	3.51 ^{ns}	3.53	3.51	3.50	0.80
Reducing sugars (g L ⁻¹)	2015/16	208.43 ^a	206.16 ^a	195.60 ^b	213.10 ^a	1.44
	2016/17	194.50 ^b	195.76 ^{ab}	191.00 ^c	197.56 ^a	0.37
	2017/18	220.43 ^{ns}	231.56	223.90	226.56	3.92
	2018/19	221.30 ^{ns}	226.50	220.30	225.30	2.56
Tartaric acid (g L ⁻¹)	2015/16	6.56 ^{ns}	6.36	6.53	6.36	1.34
	2016/17	5.53 ^b	5.53 ^b	6.03 ^a	5.66 ^b	1.01
	2017/18	6.63 ^{ns}	6.56	6.60	6.60	3.70
	2018/19	4.80 ^{ns}	4.63	4.57	4.60	3.34
Malic acid (g L ⁻¹)	2015/16	2.53 ^{ns}	2.80	2.76	2.50	5.19
	2016/17	2.60 ^b	2.86 ^a	2.90 ^a	2.66 ^b	1.60
	2017/18	1.56 ^{ns}	1.70	1.96	1.90	8.15
	2018/19	1.93 ^{ns}	1.07	2.10	1.97	4.89
Potassium (mg L ⁻¹)	2015/16	2082.33 ^{ns}	2072.66	2001.33	2053.33	4.80
	2016/17	1359.33 ^b	1363.33 ^b	1415.33 ^a	1278.00 ^c	1.53
	2017/18	1504.66 ^{ns}	1440.66	1446.33	1464.66	4.53
	2018/19	1005.67 ^{ns}	1017.00	941.00	952.00	8.54

Legend: ns - non-significant difference, CV (%) - coefficient of variation. Means followed by distinct lower-case letters on the same line differ from each other by the Tukey's test at 5% probability.

Wine physicochemical properties

Wines from grapevines with higher heights (T4, 120 cm) showed a slight trend to have higher phenolic loads (Table 3). This is because larger leaf areas increase sunlight interception, increasing the production of photoassimilates to be converted into sugars, which are precursors to phenolic compounds (Borghezan et al., 2011; Leão et al., 2016; Zhang et al., 2017). However, the phenolic composition results should be viewed with caution since not all of them are directly related to the source: sink ratio. Light and temperature, derived from the microclimate formed by treatments, are also factors that affect the phenolic composition of grape berries (Bobeica et al., 2015; Lemut, Trost, Sivilotti, & Vrhovsek, 2011; Mori, Goto-Yamamoto, Kitayama, & Hashizume, 2007).

The “harvest effect” was again noticeable mainly on contents of alcohols in wine (ethanol and glycerol) and color and structure indexes (color intensity and hue, and total polyphenol index), highlighting the 2017/18 production cycle. The volatile acidity of wines did not increase or show disparities in any of the treatments or production cycles.

Table 1. Physicochemical composition of 'Cabernet Sauvignon' wines from grapevines managed at 60, 80, 100, and 120 cm heights in four productive cycles (2015/16, 2016/17, 2017/18 and 2018/19).

Response parameter	Productive cycles	Treatment				CV (%)
		T1 (60 cm)	T2 (80 cm)	T3 (100 cm)	T4 (120 cm)	
Ethanol (% vol.)	2015/16	11.04 ^a	10.87 ^{ab}	10.54 ^b	11.14 ^a	1.49
	2016/17	10.60 ^b	11.10 ^{ab}	11.08 ^{ab}	11.43 ^a	2.02
	2017/18	12.85 ^{ab}	13.20 ^a	12.02 ^b	12.65 ^{ab}	3.41
	2018/19	12.80 ^b	13.53 ^{ab}	14.02 ^a	13.26 ^{ab}	3.13
Total acidity (in g L ⁻¹ tartaric acid)	2015/16	6.86 ^{ns}	6.66	6.70	6.90	2.01
	2016/17	5.33 ^{ns}	5.26	5.40	5.23	2.01
	2017/18	5.46 ^{ns}	5.40	5.40	5.33	1.83
	2018/19	6.90 ^c	7.16 ^{ab}	7.06 ^{bc}	7.33 ^a	0.97
pH	2015/16	3.50 ^{ns}	3.52	3.49	3.51	1.24
	2016/17	3.63 ^{ns}	3.68	3.64	3.66	1.52
	2017/18	3.80 ^{ns}	3.85	3.81	3.82	0.93
	2018/19	3.46 ^{ns}	3.57	3.53	3.44	1.93
Volatile acidity (in g L ⁻¹ acetic acid)	2015/16	0.50 ^{ns}	0.63	0.53	0.60	16.38
	2016/17	0.50 ^{ns}	0.56	0.56	0.50	8.27
	2017/18	0.46 ^{ns}	0.53	0.56	0.50	7.21
	2018/19	0.53 ^{ns}	0.60	0.63	0.70	15.05
Glycerol (g L ⁻¹)	2015/16	9.26 ^{ns}	9.13	9.23	9.06	2.23
	2016/17	7.50 ^b	7.66 ^{ab}	7.73 ^{ab}	8.06 ^a	2.32
	2017/18	9.16 ^{ns}	9.40	8.86	9.16	2.82
	2018/19	10.53 ^b	11.66 ^a	12.43 ^a	11.43 ^{ab}	3.26
Color intensity	2015/16	3.985 ^{ns}	4.145	3.749	4.448	9.00
	2016/17	2.639 ^b	3.436 ^{ab}	3.603 ^b	4.181 ^a	11.90
	2017/18	4.415 ^{ns}	4.573	4.433	4.283	7.90
	2018/19	2.565 ^{ns}	2.276	2.126	2.611	14.00
Color hue	2015/16	0.884 ^{ns}	0.901	0.914	0.875	1.70
	2016/17	0.975 ^a	0.883 ^{ab}	0.841 ^b	0.850 ^b	4.10
	2017/18	0.611 ^{ns}	0.533	0.541	0.539	11.70
	2018/19	1.245 ^{ns}	1.011	1.012	1.015	9.00
Total polyphenol index (TPI)	2015/16	23.76 ^{ns}	26.56	25.86	27.63	8.00
	2016/17	22.57 ^b	24.70 ^{ab}	25.90 ^{ab}	27.70 ^a	6.80
	2017/18	44.20 ^{ns}	44.66	45.90	43.56	4.00
	2018/19	28.93 ^b	28.23 ^b	30.43 ^{ab}	31.56 ^a	2.80

Legend: ^{ns} - non-significant difference, CV (%) - coefficient of variation. Means followed by distinct lower-case letters on the same line differ from each other by the Tukey's test at 5% probability.

Although musts were found to have distinct pH levels among productive cycles, this factor was normalized throughout winemaking, showing no significant differences (Table 3). The values found (between 3.44 and 3.85) are suitable for this cultivar. It is still important to emphasize that, especially in the Campanha Gaúcha region, the high potassium levels already detected in grapes and wines, mainly of the cultivar Cabernet Sauvignon, raise the pH in both must and wine (from 3.92 to 4.18) (Zocche et al., 2017). However, this was not observed in this study, because grapes were harvested at less advanced ripening stages when compared to what was done in the last study mentioned.

Wine color and hue were changed by treatments only in the 2016/17 production cycle. Color change was more intense in plants with 120-cm canopy height (0.445), and the least for grapevines with 60-cm height (0.264) (Table 2). This finding is coherent with the fact that, within certain limits, climatic conditions favored sugar synthesis, which also contributes to increases in pigments (Brighenti et al., 2011). Regarding the highest total polyphenol indexes (IPT) in two of the four productive cycles evaluated (2016/17 and 2018/19) for 120-cm grapevines, similar results were found in the literature, which showed that reductions in vegetative area above 26% decreased TPI values (Würz et al., 2017).

Analysis of individual anthocyanins in wine

Regarding anthocyanin composition, only two of the four anthocyanins evaluated showed significant differences (Table 4). In the 2016/17 productive cycle, petunidin and delphinidin contents were lower in grapevines with an 80-cm canopy height. It is because each treatment has its microclimate that is created by the topping height, which varies according to the climate in each productive cycle.

Part of the variability of anthocyanins and their concentrations in grapevines is associated with intrinsic factors such as genetics and enzymatic activities along the biosynthetic pathway (Dai et al., 2011). Another part is due to extrinsic factors such as light, temperature, and responses to microbiological attacks, which alter transcription factors of genes that regulate or inhibit biosynthesis pathways (Hichri et al., 2011; Sun et al., 2017), modifying the anthocyanin profile of grapes and hence wine. However, malvidin, which is the main anthocyanin found in wines and most wine cultivars (Wang, Race, & Shrikhande, 2003), did not change due to treatments.

Table 4. Individual anthocyanin concentrations (mg L^{-1}) in wines from 'Cabernet Sauvignon' grapevines with 60, 80, 100, and 120 cm canopy heights in three productive cycles (2016/17, 2017/18, and 2018/19).

Anthocyanin	Productive cycle	Treatment				CV (%)
		T1 (60 cm)	T2 (80 cm)	T3 (100 cm)	T4 (120 cm)	
Malvidin	2016/17	238.05 ^{ns}	239.24	234.69	232.43	1.49
	2017/18	243.32 ^{ns}	239.75	238.58	242.89	4.89
	2018/19	270.11 ^{ns}	266.47	272.95	275.62	4.25
Delphinidin	2016/17	13.61 ^b	12.99 ^b	14.70 ^a	14.68 ^a	1.95
	2017/18	16.02 ^{ns}	16.28	16.27	16.45	2.75
	2018/19	18.20 ^{ns}	18.24	19.33	17.94	3.62
Petunidin	2016/17	24.84 ^a	23.70 ^b	24.93 ^a	24.85 ^a	0.78
	2017/18	17.83 ^{ns}	17.59	18.36	18.64	5.82
	2018/19	15.92 ^{ns}	17.68	18.3	18.65	5.53
Peonidin	2016/17	8.74 ^{ns}	9.31	8.98	9.80	4.66
	2017/18	12.66 ^{ns}	13.43	13.14	12.88	6.70
	2018/19	11.58 ^{ns}	11.83	11.47	11.87	6.14

Legend: ^{ns} - non-significant difference, CV (%) - coefficient of variation. Means followed by distinct lower-case letters on the same line differ from each other by the Tukey's test at 5% probability.

More noticeable differences emerged throughout the production cycles, among which the wines from the 2018/19 productive cycle had higher levels of malvidin and delphinidin, but lower contents of the other anthocyanins. Conversely, the wines from the 2016/17 productive cycle, which showed less color intensity (Table 3), stood out for their petunidin contents. Finally, the wines from the 2017/18 productive cycle, whose color intensities were the highest in general, stood out above all for their peonidin contents (Figure 2).

These anthocyanin profile changes over the production cycles corroborate studies that have highlighted the importance of canopy management to regulate its exposure to sunlight and transcription of genes of anthocyanin biosynthesis enzymes (Brighenti et al., 2011; Miele & Rizzon, 2013). Added to this, anthocyanin concentrations are also strongly influenced by local edaphoclimatic conditions and grape ripening (Luciano et al., 2013).

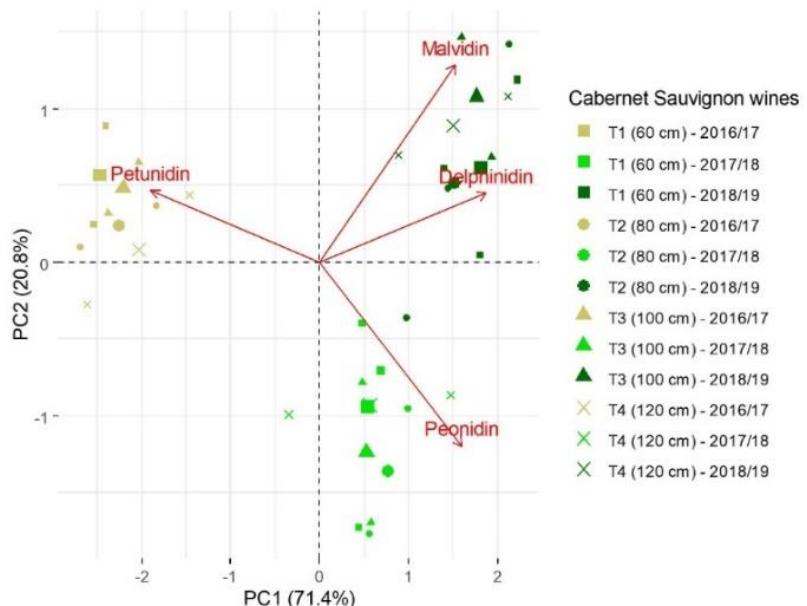


Figure 2. Principal component analysis (PCA) of the anthocyanin profile of 'Cabernet Sauvignon' wines produced in different production cycles (2016/17, 2017/18, and 2018/19) from grapevines with different vegetative canopy heights (60, 80, 100 and 120 cm).

Phenolic composition of wines

In terms of phenolic composition, only wines made in the 2017/18 production cycle from grapevines with 80 cm canopy height (T3) had higher levels of resveratrol. However, there were no differences for levels of epicatechin and catechin (Table 5).

Table 5. Individual phenolic compound concentrations (mg L^{-1}) in wines from 'Cabernet Sauvignon' grapevines with 60, 80, 100, and 120 cm canopy heights in three productive cycles (2016/17, 2017/18, and 2018/19).

Phenolic compound	Productive cycle	Treatment				CV (%)
		T1 (60 cm)	T2 (80 cm)	T3 (100 cm)	T4 (120 cm)	
Resveratrol	2016/17	1.42 ^{ns}	1.55	1.42	1.43	32.04
	2017/18	1.76 ^b	4.00 ^a	1.78 ^b	1.66 ^b	11.24
	2018/19	1.42 ^{ns}	1.55	1.42	1.43	32.00
(-)-Epicatechin	2016/17	53.32 ^{ns}	53.13	53.89	50.49	6.18
	2017/18	53.43 ^{ns}	52.54	51.58	52.16	1.94
	2018/19	52.32 ^{ns}	52.13	53.49	50.89	1.18
Catechin	2016/17	11.11 ^{ns}	11.21	10.62	11.00	8.14
	2017/18	10.68 ^{ns}	10.96	11.42	11.20	7.33
	2018/19	11.11 ^{ns}	11.21	10.62	11.00	8.14

Legend: ^{ns} - non-significant difference, CV (%) - coefficient of variation. Means followed by distinct lower-case letters on the same line differ from each other by the Tukey's test at 5% probability.

As T3 stood out for an accumulation of almost three times more resveratrol compared to the other treatments in the 2017/18 productive cycle (Table 5), one can be assumed that the association of climatic conditions in the cycle with the treatment favored stilbene metabolism. This compound has been studied for some time due to its human health benefits (Albertoni & Schor, 2015). Its synthesis is induced by biotic and abiotic factors, and concentrations increase in response to pathogen attacks (e.g., bacteria and fungi) and physical damages, mainly in fruits and leaves (Del-Castillo-Alonso et al., 2016). Blancquaert et al. (2019) also verified light and temperature effects on phenolic composition during two productive cycles and noted that canopy architecture changes in the same cultivar promoted different responses as a function of treatment.

The flavonoids evaluated here (epicatechin and catechin) have multiple photo-protective functions, and their accumulation in berry epidermis is closely associated with climate conditions, especially light and solar radiation (Agati et al., 2013). Besides that, they have a direct impact on wine quality, granting a larger structure and longer shelf life to products (Ghanem et al., 2017).

Methoxypyrazine content

Regarding the methoxypyrazine content, the wines had lower rates of 3-isobutyl-2-methoxypyrazine (IBMP), all below 2 ng L^{-1} , and did not show significant differences (Figure 3). These numbers are positive to the region, provided that the sensory detection threshold of this compound is 15 ng L^{-1} (Lei et al., 2018). Vineyard management to control its levels is also important since unfavorable conditions to grape ripening, such as lack of solar lighting in the berry area and early harvest, can result in wines with high concentrations of IBMP (Bindon et al., 2013).

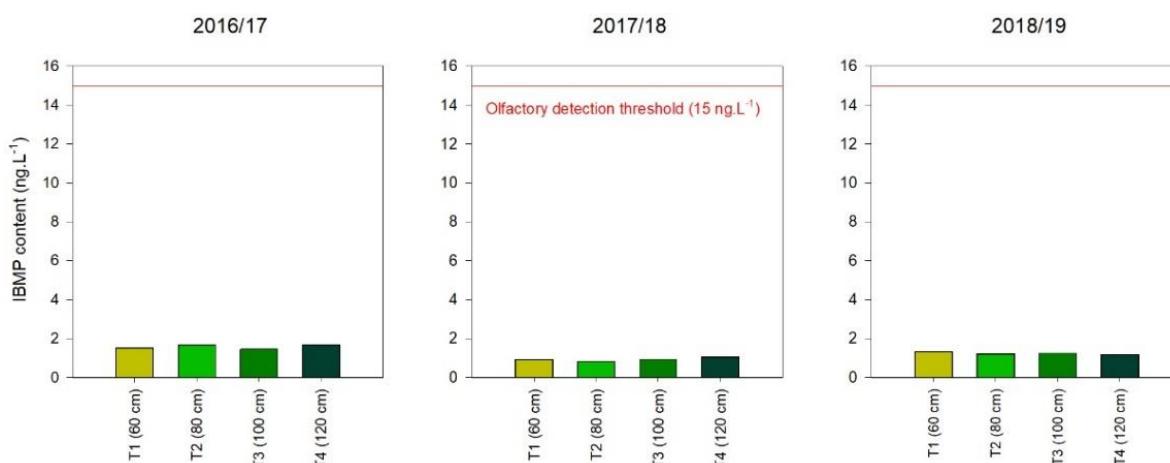


Figure 3. Quantification of 3-isobutyl-2-methoxypyrazine (IBMP) in wines produced in different production cycles (2016/17, 2017/18, and 2018/19) from 'Cabernet Sauvignon' grapevines with 60, 80, 100, and 120 cm vegetative canopy height.

As observed for must and wine quality, we found that, although some physicochemical parameters showed statistical differences among the treatments, the overall results were not affected.

Certain parameters had some advantages, and most of the results were relevant, notably from the economic point of view, as there were no significant losses in productivity in treatments with lower plants. As a result, vineyard planning costs can be reduced by changing the plant support structure, reducing wire use from 6,600 to 13,200 meters per hectare on average. Moreover, fungicide solutions can be reduced considerably, decreasing chemical pesticides necessary for phytosanitary control by about 40%.

The fact that many of the studied factors have been affected chiefly by the climatic conditions in each productive cycle elucidates grapevine plasticity and adaptability and reinforces the need for more regional studies on leaf area management.

About must and wine physicochemical quality, in higher vegetative canopies, mainly 120 cm (T4), sugars had a slight tendency to accumulate more in berries, thus increasing alcohol contents. Moreover, color intensity and hue, as well as total polyphenol index, were also superior in higher canopy plants.

For anthocyanin composition, the treatments showed no statistical difference. Malvidins were one of the anthocyanins found in greater amounts in the wines from all productive cycles. However, the other anthocyanins showed lower levels, each in a production cycle, namely: petunidin in 2016/17, peonidin in 2017/18, and delphinidin in 2018/19.

In general, the phenolic composition was little affected by treatments, showing greater accumulation of resveratrol and stilbene in T2 (80 cm in height) in the 2017/18 production cycle. The studied flavonoids showed no difference.

Differences in the amount of 3-isobutyl-2-methoxypyrazine (IBMP) were not identified, and its levels were low for wines from all production cycles. No other methoxypyrazines were detected.

Conclusion

Grapevines have phenotypic adaptation and plasticity as the result of millions of years of evolution, which was corroborated in this experiment since the agronomic parameters evaluated showed non-statistical differences. Regarding must and wine, grapevines were mostly influenced by the productive cycle (i.e., "harvest effect"), with each canopy height promoting different results in each of the four cycles studied.

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