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Agronomic performance of 'Viognier' grapevines in subtropical conditions and wine aromatic compounds under different bud numbers retained at pruning

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ABSTRACT

Managing bud load is a major concern for vine growers, affecting vine balance and thus, fruit quality. Achieving the right bud number requires consideration of vineyard conditions and desired outcomes, and it is normally controlled through pruning. Subtropical climates often have longer growing seasons, and finding the optimal bud load is even more important, as vines tend to have increased growth. This study evaluated the influence of different bud numbers on the agronomic performance and aromatic composition of 'Viognier' grapevines grown under subtropical conditions. The experiments were conducted in a commercial vineyard in the municipality of Campo Largo, Paraná, Brazil, during the 2019/2020 and 2020/2021 growing seasons. Vines were pruned using the Guyot system and evaluated under different bud numbers at pruning: 10, 20, 30, and 40 buds per plant. Agronomic performance, grape technological maturity, and wine volatile compounds using Gas Chromatography and Mass Spectrometry detection (HS-SPME-GC-MS) were assessed. Higher bud numbers (30 and 40 buds per plant) resulted in increased yield, larger leaf area, and balanced Ravaz index values. The morphology of the bunches and technological ripeness were not significantly different between the treatments. However, the highest bud number (40 buds) increased the percentage of blind buds and decreased shoot length. Some volatile compounds, such as citronellol and β-damascenone, were found in higher concentrations in wines from the lowest bud number (10 buds per plant), while hexanol, ethyl octanoate, and ethyl-2-phenylacetate were more prevalent in treatments with higher loads.

KEYWORDS: grapevine pruning, viticultural practices, volatile compounds, gas chromatography, technological maturity

INTRODUCTION

Since 2018, the global wine and vine sector has faced significant challenges, including reductions in wine production, consumption, and vineyard surface area. However, Brazil has emerged as an exception, with a 12.1 % increase in wine production in 2023 compared to the previous year and a 31.4 % rise above the average of the last five years. Brazil is now the second-largest wine market in South America, with wine consumption growing by 11.6 % between 2022 and 2023 (OIV, 2024).

To ensure economic viability and environmental sustainability, modern viticulture must effectively manage a range of complex objectives (Theocharis *et al.*, 2024). Effective vineyard management is crucial for maximising production efficiency while maintaining high grape quality. Key practices such as defoliation, topping, and pruning can be optimised to improve vineyard performance (Alatzas *et al.*, 2024; Würz *et al.*, 2019).

Pruning plays a critical role in producing high-quality grapes, but crop size must be carefully managed to balance fruit quality and vegetative growth for consistent productivity (Al-Saif *et al.*, 2023). Winter pruning is typically carried out during the dormant season, when the vine is leafless, and involves the removal of a significant portion of the previous season's growth to shape the vine for the upcoming growing season. This practice includes the removal of dead, damaged, and diseased wood, as well as excess canes and spurs. Additionally, it entails the careful selection and retention of an appropriate number of buds to ensure a balanced crop load. Winter pruning, in particular, significantly influences grapevine performance during the growing season by affecting bud number and the vine's stored nutrient reserves (Qiu *et al.*, 2019).

Vine balance was defined by Gladstones (1992) as the state in which vegetative vigour and fruit load are in equilibrium, ensuring optimal fruit quality. Maintaining this balance is essential for effective vineyard management in modern viticulture (Poni *et al.*, 2018; Theocharis *et al.*, 2024). Adjusting bud number, either increasing or decreasing it based on bud fruitfulness and vine vigour, is necessary to achieve productive objectives and maintain 'vine balance' (Monteiro *et al.*, 2021). Vine responses to increased bud numbers include reduced vegetative growth, lower bud fertility, shorter shoots with shorter internodes, higher productivity, and a greater number of bunches per plant (Greven *et al.*, 2014; Greven *et al.*, 2015; Würz *et al.*, 2023; Bonin *et al.*, 2024).

In subtropical viticulture, managing the higher vine vigour is particularly important to avoid dense canopies related to shading of clusters, disease susceptibility, and poor wine quality. Among canopy management techniques, winter pruning is one of the most powerful, although it has received little attention in recent literature. This study directly addresses this gap by evaluating the influence of bud number on the agronomic and oenological performance of the 'Viognier' grapevine grown in Brazil to equip growers with essential insights to refine their vineyard strategies and maximise grape production within their distinct environmental conditions.

MATERIALS AND METHODS

1. Experimental area

The experiment was conducted in a vineyard located in the municipality of Campo Largo, Paraná, Brazil (25° 23' 41" S 49° 30' 12" W) during the 2019/2020 and 2020/2021 growing seasons. The region is situated at an altitude of 975 metres above sea level and is characterised by a Cfb climate (subtropical with temperate summers) according to the Köppen classification (Alvares *et al.*, 2013). This region had an average annual temperature of 17.8 °C, with monthly means ranging from 14.2 °C in July to 21.3 °C in February, and annual rainfall averaging 1,450 mm, according to data from the Paraná Meteorological System (SIMEPAR). The vineyard soil is classified as clay loam, and irrigation was not employed, as rainfall is evenly distributed throughout the year.

'Viognier' variety, originating from the northern part of the Côtes du Rhône in France, is known for its early budburst, and this cultivar has shown strong adaptability and growth in warm climate regions (Palladini *et al.*, 2021). The 'Viognier' vines, grafted onto Paulsen 1103 rootstock and planted in 2010, were trained on a vertical trellis system with three wires, the first positioned 1.2 metres above the ground. Vine spacing was 1.2 metres within rows and 2.7 metres between rows, yielding a planting density of 3,086 vines per hectare. Four rows were selected within the vineyard for the study, each row constituting one block subdivided into four plots, with each plot containing five vines per treatment. Ten vines per treatment were randomly selected across the four blocks for evaluations, and the experiment was arranged in a randomised complete block design, comprising four blocks and ten vines per treatment, totalling 40 vines. Guyot pruning was applied, using spurs and either unilaterally or bilaterally arched canes, depending on the treatment. Four treatments were established: 10 buds (one cane), 20 buds (two canes), 30 buds (three canes), and 40 buds (four canes), with each cane pruned to retain 10 buds and arched. Only buds on fruiting canes were evaluated. Additionally, four to five renewal spurs were retained per vine to ensure adequate shoot development for the subsequent season. Climate data, including average monthly air temperature (minimum and maximum) and rainfall from 31 August 2019 to 30 April 2021, were obtained from the Paraná Meteorological System (SIMEPAR) based on the closest weather station to the vineyard (40 km from Campo Largo), following the World Meteorological Organisation (WMO) standards (Figure S1). The phenological stages (Tables S1, S2, and S3) of the vines were defined using the BBCH scale (Lorenz *et al.*, 1995). After pruning, 4 % hydrogen cyanamide was applied to induce and standardise sprouting. Disease control, canopy management (including

weeding, defoliation, and pruning), and fertilisation were uniformly applied across all treatments in accordance with technical recommendations for vine cultivation.

2. Vegetative growth and yield components

At commercial harvest, 10 plants were harvested. All treatments were harvested simultaneously. The number of shoots and bunches per plant was counted, and the total mass of bunches per plant (kg/plant) was determined. Production per plant was determined by directly weighing all bunches using a commercial digital scale. Yield (t/ha) was obtained by multiplying the production per plant by the planting density (3,086 plants/ha). The Ravaz index was determined as the ratio between fruit production per plant (kg/plant) and the mass of pruned material per plant (kg/plant) (Brighenti *et al.*, 2011).

For each replication, 10 bunches were sampled to determine the bunch mass (g), rachis mass (g), bunch length (cm), and the number of berries per bunch. Bunch mass and rachis mass were measured using a semi-analytical balance, while bunch length was measured with a ruler. After harvesting, in four canes for replication, the shoot length (cm) was measured using a tape measure, and the number of leaves per shoot was counted. The internode length (cm) and shoot diameter (cm) at the first and tenth internodes were measured using an analogue calliper in the same canes.

Leaf area was estimated following an adapted methodology proposed by Uzokwe *et al.* (2012). A total of 150 leaves per treatment were randomly collected during the grape harvest from the upper, middle, and lower thirds of the vines. Leaf area measurements were obtained using a WinRHIZO area meter (LA1600, Regent Instruments Inc.), and the average leaf area (cm^2) was determined through scanning. The number of shoots and leaves per shoot was counted on 10 vines per treatment to estimate the total leaf area per vine for each treatment, calculated by multiplying the average leaf area by the total number of leaves per plant. The balance between vegetative and productive growth was assessed using the ratio of vine leaf area to grape yield (m^2/kg).

3. Maturation and microvinification

From the onset of ripening, 100 berries per treatment were collected weekly to monitor and determine technological ripeness (Figures S2 and S3). The berries were transported to the laboratory for weighing, skin separation for analysis, and maceration. The must obtained was used to determine total soluble solids (TSS, °Brix), total titratable acidity (TTA), and pH, following the methodology proposed by the International Organisation of Vine and Wine (OIV, 2020).

Total soluble solids were measured using a refractometer (model ITREFD 45, Instrutemp, São Paulo, SP, Brazil). The device was calibrated with distilled water, and the must was distributed over the prism for direct reading in °Brix. Total titratable acidity was determined by titrating a solution of 5 mL of must diluted in 50 mL of distilled water with a standardised 0.1 N sodium hydroxide (NaOH) solution, using phenolphthalein (1 %) as an indicator. The volume of NaOH consumed was used to calculate TTA in mEq/L, which was then converted to g/L of tartaric acid. pH was measured using a pH meter (BEL Engineering, model W3B pH-meter, Monza, Milan, Italy). The pH meter was calibrated with buffer solutions of pH 4.0 and 7.0 before recording the pH of the samples collected on the day of harvest.

A total of 30 kg of grapes were manually harvested from each treatment. Microvinifications were carried out in a commercial winery following a protocol adapted from Pszczółkowski (2014) and Makhotkina *et al.* (2013). The harvested grapes were stored in a cold room for 24 hours at a temperature of 5 to 8 °C. Subsequently, 20 kg of grapes per treatment were standardised by discarding bunches affected by pests or diseases to ensure consistent processing across all four treatments. The grape bunches were destemmed to separate the berries from the rachis and transferred to a hydropneumatic press. The must was drained into a 12.5-litre glass container, and 20 mg/L of SO_2 was added to the must using a 10 % potassium metabisulfite solution. The container was sealed and stored in a cold room at 2 °C for 72 hours to allow coarse particles to settle and clarify the must.

Following this period, the clarified must was transferred into 750 mL green glass bottles. Three bottles (replicates) per treatment were prepared for both the 2020 and 2021 seasons. The must samples were uniformly prepared across all treatments, with the remaining must volume discarded. Active hydrated yeast *Saccharomyces cerevisiae* (Fermol Blanc, AEB, Italy) was inoculated into each replicate at a concentration of 0.2 g/L. Upon completion of fermentation, 60 mg/L of SO_2 was added to each replicate using a 10 % potassium metabisulfite solution. The wines were then stored in a cold room at 0 to 1 °C for 21 days to achieve tartaric stabilisation. Finally, the wines were stored in a light-free environment (approximately 16 °C) until analysis.

4. Analysis of volatile compounds by gas chromatography (HS-SPME-GC-MS)

The volatile compounds of the wines from the different treatments were subsequently identified using Headspace Solid-Phase Microextraction coupled with Gas Chromatography and Mass Spectrometry detection (HS-SPME-GC-MS).

For the extraction of volatile components from the samples, an optimised methodology previously described by Tao *et al.* (2008) was employed. In a 15 mL vial containing a magnetic stirring bar, 7.5 mL of the sample, 0.7500 ± 0.005 g of NaCl, and 2 µL of α-pinene solution (used as an internal standard) were added. The vial was placed in a glass-jacketed container positioned on a magnetic stirring plate and connected to a thermostatic bath with water circulation (SOLAB SL 152, Piracicaba, SP, Brazil). The vial was maintained in a water bath at 40 ± 0.2 °C, and the contents were stirred for 5 minutes. A Solid-Phase Microextraction (SPME) fibre composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with a 50/30 µm thick coating and 1 cm length (SUPELCO, Bellefonte,

PA, USA) was exposed to the vial's headspace at the same temperature for 30 minutes. The compound α-pinene was chosen as the internal standard because it is not typically found in wine and exhibits a distinct ion peak location, separate from the peaks of other volatile compounds in wines.

The SPME fibre containing the adsorbed volatile components was manually inserted into the Gas Chromatography with Mass Spectrometry detection (GC-MS) injector at 250 °C (splitless mode, equipped with a glass liner, 0.75 mm inner diameter) and held for 5 minutes. The desorbed components were separated using an Agilent 7890A GC-MS system following a methodology adapted from Tao and Li (2009). An Agilent HP-5MS column (30 m × 0.25 mm × 0.25 µm) composed of dimethyl/diphenyl polysiloxane (95 %/5 %) was used, with a helium gas flow rate of 1.0 mL/min. The oven temperature was initially maintained at 40 °C for 5 minutes, followed by a heating ramp from 40 to 260 °C at a rate of 9 °C/min. The interface and ion source temperatures were set at 300 °C. Data were acquired in full-scan mode with a mass range of 30-400 m/z. The mass spectrometer was operated in electron impact mode at 70 eV.

Peaks were manually integrated using the G1701EA GC/MSD ChemStation software. Volatile compounds were identified by comparing their mass spectra and experimental Kovats Index (KI) with the corresponding mass spectra and theoretical KI of standards described by Adams (2017). Experimental KI values (Table S4) were determined by injecting a sample of saturated hydrocarbons (Sigma-Aldrich) under the same conditions used for the samples and calculated according to Van den Dool and Kratz (1963). Each sample was analysed in triplicate for each treatment over both seasons. Volatile compounds were quantified based on the area of the specific compound relative to the total area of all measured volatile compounds. Compounds not identified in the literature were characterised using NIST software, with identification requiring a similarity threshold of over 90 %. The volatile compounds were analysed in triplicate from three bottles of wine per treatment across both seasons, and the results were expressed as the mean for each volatile compound.

5. Statistical analysis

The means of the variables were subjected to analysis of variance (ANOVA), followed by the *F*-test ($p \leq 0.05$). Significant differences among bud number treatments were subsequently compared using the Tukey test at a 5 % probability of error, using the Sisvar software version 5.6 (Ferreira, 2019).

RESULTS AND DISCUSSION

1. Bud number affects the sprouting, the growth of the shoots, and the balance between vigour and production

For the 2020 season, the 10-bud treatment showed 100 % sprouting, while the 40-bud number had 80 % (Table 1). This response was similar in the 2021 season. Several studies show a drop in the percentage of sprouting as the number of buds increases (Greven *et al.*, 2015; Würz *et al.*, 2019; Bonin *et al.*, 2024). In a study with the Muscat cultivar, Archer and Fouché (1987) observed that the sprouting percentage was 93 % in vines pruned with 40 buds per plant, compared to 100 % sprouting in vines pruned with a number of 16 buds per plant. The lower sprouting percentage led to an increase in the number of 'blind buds' (buds where no shoot growth was observed), likely due to a change in sink-source relationships.

The number of bunches was higher for the higher bud numbers treatments (30 and 40 buds), which were not statistically different from each other in both evaluated cycles. The lowest bud number (10) had the lowest number of bunches in both seasons and, consequently, the lowest yields. In the 2019/2020 season, the numbers of 30 and 40 buds yielded 2.16 and 2.45 kg per plant, respectively (Table 1). In the 2021 harvest, yields were higher for all treatments, but the behaviour remained similar, with the highest yields for the treatments with 30 and 40 buds per plant, at 3.40 and 3.82 kg per plant, respectively (Table 1). Pruning mass was not statistically affected by bud number in the first year.

Ravaz Index (RI) values between 4 and 7 are indicative of balanced vines capable of producing quality fruit (Da Silva *et al.*, 2009). RI values higher than 7 indicate excessive fruit production, and values lower than 4 show excessive plant vigour (Howell, 2001). During the 2019/2020 cycle, the higher bud numbers showed RI values closer to the ideal range, with 3.93 (40 buds) and 3.70 (30 buds) being statistically greater than the other treatments. For the second year, the plants had higher values for RI due to the higher yields, and the treatments of 20, 30, and 40 buds presented higher and balanced values than the lowest bud number treatment (10 buds). The treatment with 40 buds per plant showed an RI value of 6.26, along with 5.28 (30 buds) and 4.42 (20 buds), indicating that the plants in these treatments presented a good balance between vigour and production under the conditions of this study.

Shoots were longer in the lowest bud number (156.37 cm) during the first season and statistically higher compared to the 40-bud treatment (121.12 cm). This behaviour was similar in the second evaluation cycle, but with shorter shoots for all treatments (Table 2). The number of leaves per shoot was also higher in the treatment with 10 buds per plant in both cycles. The adaptive processes by which the vine responds to an increase in bud number have been described in several studies with other vine cultivars and include reduced bud fertility, shorter shoots, shorter internodes, higher yields, and a greater number of bunches per plant (Greven *et al.*, 2015; Würz *et al.*, 2023; Bonin *et al.*, 2024).

Greven *et al.* (2014) reported that increasing bud numbers (from 24 to 72 buds per vine) led to significant reductions in shoot length, shoot diameter, number of leaves per shoot, and internode length across multiple seasons, reflecting heightened competition for resources among shoots. In contrast, the present study, which examined lower bud

TABLE 1. Effect of bud number on the vegetative growth and yield components of the 'Viognier' grapevine variety, in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Parameter	Bud number (buds/vine)				*p*-value
	10	20	30	40	
2019/2020					
Number of shoots	10d	18.50c	27.25b	32.37a	< 0.001
Sprouting (%)	100a	92ab	86ab	80b	0.020
Number of bunches	8.12c	13.12b	19.8a	22.6a	< 0.001
Yield (kg/plant)	0.84c	1.52b	2.16a	2.45a	< 0.001
Yield per hectare (t/ha)	2.6c	4.7b	6.6a	7.5a	< 0.001
Pruning mass (kg)	0.43ns	0.55	0.58	0.62	0.432
Ravaz index	1.98c	2.77b	3.70a	3.93a	0.004
2020/2021					
Number of shoots	10.75d	20.87c	29.62b	34.62a	< 0.001
Sprouting (%)	100a	100a	98a	81b	0.023
Number of bunches	9.25c	16.25b	21.87a	26.62a	< 0.001
Yield (kg/plant)	1.04c	2.23b	3.40a	3.82a	< 0.001
Yield per hectare (t/ha)	3.08c	6.62b	10.08a	11.33a	< 0.001
Pruning mass (kg)	0.45b	0.52b	0.70a	0.71a	0.049
Ravaz index	2.69b	4.42a	5.28a	6.26a	0.015

Values followed by different letters on the same row differ significantly according to the Tukey test ($p < 0.05$). ns = not significant.

numbers (10 to 40 buds per vine), observed similar trends for shoot length and number of leaves per shoot, with reductions generally occurring as bud load increased (*e.g.*, shoot length decreased from 156.37 cm at 10 buds to 121.12 cm at 40 buds in 2019/2020, and from 147.37 cm to 126.12 cm in 2020/2021; $p < 0.05$ in both seasons). However, effects on internode length and shoot diameter were less consistent and of smaller magnitude, potentially owing to the lower overall bud loads reducing vine competition. For instance, internode length showed a significant but modest decline only in 2019/2020 (from 7.87 cm at 10 buds to 7.23 cm at 30 buds; $p = 0.002$), with no significant differences in 2020/2021. Shoot diameter remained largely unaffected, except for the distal (10th) internode in 2020/2021, where the highest bud load (40 buds) resulted in a smaller diameter (0.22 cm) compared with lower loads ($p = 0.030$). These findings suggest that vegetative growth parameters may exhibit threshold responses, with pronounced reductions primarily at higher bud loads as observed by Greven *et al.* (2014), whereas lower loads in the present study supported more vigorous growth without substantial compromise.

The significant variation in total leaf area indicates that bud number influenced the vegetative growth of 'Viognier' vines. This variation was primarily driven by treatment-induced differences in two key parameters: the number of shoots per vine and the number of leaves per shoot. Higher bud numbers, particularly the 40-bud treatment, resulted in a greater number of shoots, which, combined with a moderate number of leaves per shoot (14.3 to 15.7 across seasons), contributed to significantly larger total leaf areas (6.21 m^2 in 2019/2020 and 7.22 m^2 in 2020/2021) compared with lower bud loads (2.55 m^2 and 2.51 m^2 for 10 buds in 2019/2020 and 2020/2021, respectively). These differences were statistically significant ($p < 0.001$) and consistent across both seasons, as shown in Tables 1 and 2.

TABLE 2. Effect of bud number on the vegetative growth of the 'Viognier' grapevine variety, in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Parameter	Bud number (buds/vine)				*p*-value
	10	20	30	40	
2019/2020					
Shoot length (cm)	156.37a	142.78ab	136.50ab	121.12b	0.034
Number of leaves per shoot	17.6a	15.1b	16.3ab	14.3b	0.007
Internode length (cm)	7.87a	7.53ab	7.23b	7.55ab	0.002
Diameter 1st internode (cm)	0.38ns	0.36	0.33	0.32	0.696
Diameter 10th internode (cm)	0.37ns	0.32	0.34	0.28	0.109
Vine leaf area (m^2)	2.55c	3.77b	5.92a	6.21a	< 0.001
Leaf area/Yield (m^2/kg)	3.09b	2.46ab	2.75ab	2.53a	0.017
2020/2021					
Shoot length (cm)	147.37a	120.35b	124.50b	126.12b	0.009
Number of leaves per shoot	17.3a	15.0b	14.1b	15.7b	0.005
Internode length (cm)	7.89ns	8.03	8.81	8.13	0.421
Diameter 1st internode (cm)	0.5ns	0.46	0.42	0.39	0.261
Diameter 10th internode (cm)	0.41a	0.37a	0.36a	0.22b	0.030
Vine leaf area (m^2)	2.51d	4.15c	5.55b	7.22a	< 0.001
Leaf area/Yield (m^2/kg)	3.15ns	2.85	2.65	3.01	< 0.001

Values followed by different letters on the same row differ significantly according to the Tukey test ($p < 0.05$). ns = not significant.

The literature suggests that optimal leaf area-to-yield ratios for various grape cultivars range from 0.6 to 2.0 m^2/kg (Howell, 2001; Kliewer & Dokoozlian, 2005; Jackson, 2020). An imbalance in this ratio, such as excessive leaf area relative to yield, can lead to preferential allocation of photoassimilates to vegetative structures, resulting in increased vigour, canopy shading, and delayed fruit maturation (Jackson, 2020). In contrast, an optimal balance supports both vine health and fruit quality. In the present study, increasing bud number from 10 to 40 buds significantly enhanced yield, with the 40-bud treatment consistently exhibiting the highest leaf area. In the 2019/2020 season, the 40-bud treatment achieved a leaf area to yield ratio of 2.53 m^2/kg, which, although slightly above the optimal range, was significantly lower ($p = 0.017$) than the 10-bud treatment (3.09 m^2/kg), indicating a better vegetative-reproductive balance. The 20- and 30-bud treatments, with ratios of 2.46 and 2.75 m^2/kg, respectively, did not differ significantly from other treatments. In the 2020/2021 season, no significant differences in leaf area-to-yield ratios were observed across treatments. This outcome is likely attributable to the reduced precipitation peaks and more stable temperatures (Figure S1), which may have moderated vegetative growth and yield responses, contributing to the observed uniformity in leaf area-to-yield ratios.

In Slovenia, Šuklje *et al.* (2013) reported leaf area-to-yield ratios of 0.63-1.85 m^2/kg for Sauvignon blanc, lower than the 2.53-3.15 m^2/kg observed in this study. Higher ratios are typical in southern Brazil's subtropical climate, often leading to vigorous vineyards. For instance, Borghezan *et al.* (2011) found ratios of 3.7-8.4 m^2/kg in high-altitude regions of southern Brazil for Cabernet-Sauvignon, Merlot, and Sauvignon blanc, indicating excessive vigour and photoassimilate imbalance. Similarly, Brighenti *et al.* (2010), studying Merlot on subtropical conditions, reported a ratio of 4.5 m^2/kg, suggesting high vegetative growth. Ratios below

0.6 m^2/kg may impair fruit ripening, while values above 2.0 m^2/kg, as observed here, can delay ripening, reduce polyphenol content, and increase fungal disease risk due to bunch shading (Kliewer & Dokoozlian, 2005). Higher bud numbers effectively moderated vegetative growth, achieving a more optimal balance between yield and vigour. These findings align with Würz *et al.* (2020), who observed reduced shoot vigour and length in 'Cabernet franc' under high bud numbers in São Joaquim, Santa Catarina, Brazil. In this study, 'Viognier' 30- and 40-bud treatments exhibited significantly shorter and less vigorous shoots compared to the 10-bud treatment (Table 2), reflecting improved resource allocation and vine balance in subtropical conditions.

2. Bud number effects on the morphological and chemical composition of the bunches

The evaluation of bud number on the morphological and chemical composition of 'Viognier' bunches revealed no significant differences across the assessed attributes for the 2019/2020 and 2020/2021 harvests (Table 3). Bunch mass ranged from 107.3 g to 123.6 g, number of berries from 68.2 g to 83.4 g, berry mass from 1.48 g to 1.67 g, rachis mass from 4.8 g to 5.8 g, and bunch length from 12.3 cm to 15.0 cm, with all parameters showing non-significant variation ($p > 0.05$) across bud numbers of 10 to 40 buds per vine. Similarly, for technological ripeness, total soluble solids (TSS), total titratable acidity (TTA), and pH exhibited no significant differences between treatments in either cycle, except for TTA in 2019/2020, where the 10-bud treatment (7.17 g/L) displayed a higher value compared to the 20-bud (6.59 g/L) and 30-bud (6.67 g/L) treatments ($p = 0.018$; Table 4).

The lack of significant changes in bunch morphology and chemical composition contrasts with findings from other studies with different bud numbers (Greven *et al.*, 2014; Teocharis *et al.*, 2024). The absence of similar trends in the current study may be attributed to the subtropical climate of southern Brazil, characterised by frequent rainfall and higher temperatures (Figure S1). These conditions likely supported consistent vegetative and reproductive growth, mitigating the competitive effects of higher bud numbers on bunch morphology and berry composition that are typically observed in temperate regions. The stable temperature and precipitation patterns may have buffered resource allocation, maintaining uniform bunch characteristics across treatments, and potentially overshadowing the influence of bud number on technological ripeness parameters. This adaptive response of 'Viognier' vines under subtropical conditions highlights the need for region-specific management practices to optimise yield and quality. These results suggest that varying bud number (from 10 to 40 buds) had a minimal impact on the technological ripeness of 'Viognier' grapes in the conditions of this study.

TABLE 3. Effect of bud number on the physical characteristics of bunches and berries of 'Viognier' grapevine variety, in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Physical parameters	Bud number (buds/vine)				*p*-value
	10	20	30	40	
2019/2020					
Bunch mass (g)	107.3ns	104.1	120.2	119.8	0.738
Number of berries	68.2ns	72.6	78.8	76.2	0.958
Berry mass (g)	1.62ns	1.57	1.67	1.65	0.058
Rachis mass (g)	4.8ns	5.1	5.4	5.2	0.940
Bunch length (cm)	12.9ns	13.6	14.9	13.4	0.656
2020/2021					
Bunch mass (g)	108.4ns	114.2	122.5	123.6	0.906
Number of berries	72.2ns	73.4	80.1	83.4	0.809
Berry mass (g)	1.50ns	1.52	1.51	1.48	0.965
Rachis mass (g)	4.9ns	5.1	5.7	5.8	0.591
Bunch length (cm)	13.8ns	14.1	15.0	12.3	0.541

ns = not significant by analysis of variance (ANOVA) at 5 % error probability.

TABLE 4. Effect of bud number on the total soluble solids (TSS), total titratable acidity (TTA), and pH of 'Viognier' musts, in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Parameters	Bud number (buds/vine)				*p*-value
	10	20	30	40	
2019/2020					
TSS (°Brix)	17.8ns	17.6	18.0	18.2	0.511
TTA (g/L)	7.17a	6.59b	6.67b	6.78ab	0.018
pH	3.21ns	3.22	3.20	3.18	0.651
2020/2021					
TSS (°Brix)	17.2ns	17.3	16.8	17.0	0.378
TTA (g/L)	8.22ns	8.04	8.16	8.41	0.634
pH	3.14ns	3.13	3.16	3.12	0.725

ns = not significant by analysis of variance (ANOVA) at 5 % error probability.

3. Bud number affects some specific aromatic compounds in the wines

Out of the 26 aromatic compounds identified in 'Viognier' wines, bud number did not affect the concentrations of 17 of them (Table 5).

However, the compounds that stood out were found preferentially in the treatment with 10 buds per plant. The lowest bud load significantly enhanced the concentrations of citronellol (10.51 %, $p < 0.001$), linalool (0.10 %, $p = 0.037$), and β-damascenone (2.65 %, $p = 0.003$), which contribute lemon, spice, floral, and honey-like aromas (Tao *et al.*, 2008; Peng *et al.*, 2013). These findings align with Škrab *et al.* (2021), who reported higher levels of citronellol, linalool, and β-myrcene in 'Ribolla Gialla' wines from lower-yield treatments, suggesting that reduced yields enhance varietal aroma expression.

In contrast, higher bud numbers (30 and 40 buds per plant) significantly increased hexanol concentrations (0.79 % and 0.75 %, respectively) compared to the 10-bud treatment (0.41 %; $p = 0.002$; Table 5), contributing to herbaceous and grassy notes, likely due to denser canopies reducing berry exposure to sunlight (Torres *et al.*, 2020 ; Martínez-Lüscher and Kurtural, 2023). Similarly, ethyl octanoate and ethyl-2-phenylacetate were more prevalent in higher bud number treatments, contributing melon, wood, and tropical fruit aromas (Mayr *et al.*, 2014; Lu *et al.*, 2022). These findings indicate that bud number can selectively modulate specific volatile compounds in 'Viognier' wines under subtropical conditions, offering a strategic tool for tailoring wine sensory profiles.

CONCLUSIONS

This study on 'Viognier' grapevines under the Guyot pruning system in subtropical conditions provides clear insights into optimising bud numbers for vine balance, yield, and wine quality. Overall, bud numbers of 30 buds per plant emerged as the most suitable under the experimental conditions, based on criteria such as the Ravaz index, enhanced yield, and moderated vegetative vigour without compromising bunch morphology, technological ripeness, or sprouting. Higher loads (30 and 40 buds) achieved a favourable equilibrium between vegetative and reproductive growth, as evidenced by increased leaf area and reduced shoot length, which helped mitigate excessive vigour typical in subtropical climates.

In contrast, lower bud number (10 buds per plant) prioritised wine quality attributes, yielding higher concentrations of desirable floral and fruity volatile compounds such as citronellol, linalool, and β-damascenone, likely due to greater photosynthate allocation per berry and reduced competition among sinks. However, this came at the expense of lower yields and suboptimal vine balance, with the Ravaz index values indicating excessive vigour. Higher bud loads, while promoting herbaceous and tropical notes like hexanol, ethyl octanoate, and ethyl-2-phenylacetate, potentially linked to denser canopies, supported greater productivity, making them preferable for commercial objectives focused on volume.

The subtropical climate of southern Brazil is characterised by high humidity, even rainfall distribution, extended growing seasons, and amplified vine vigour. Unlike temperate regions, where lower loads might suffice for quality, these conditions favoured elevated loads to harness excess growth for yield without evident declines in technological maturity, highlighting region-specific adaptations in viticultural practices.

TABLE 5. Volatile compounds and aroma descriptors in 'Viognier' wines across bud number treatments in subtropical conditions.

Compound (%)	Bud number (buds/vine)				*p*-value	Aroma descriptors
	10	20	30	40		
Alcohols						
2-Ethyl hexanol	0.67ns	0.59	0.70	1.46	0.135	Citrus, herbaceous[r]
4-Methyl pentanol	0.12b	0.18ab	0.23a	0.19ab	0.021	No descriptor[c]
Hexanol	0.41b	0.63a	0.79a	0.75a	0.002	Flower, grass, resin, herbaceous[b]
Isoamyl alcohol	38.32ns	43.91	48.82	35.63	0.053	Honey, fruit[a]
Phenylethyl alcohol	4.95ns	4.21	1.39	1.03	0.056	Floral, rose flower, perfume[b,l]
Esters						
2-Methyl butyl acetate	0.26ns	0.29	0.30	0.37	0.481	Pineapple, banana, fruit[e,f]
2-Phenyl ethyl acetate	0.36a	0.21b	0.12b	0.15b	< 0.001	Floral, sweet[m]
Diethyl succinate	7.76ns	10.64	8.88	9.03	0.054	Wine, fruit[r]
Ethyl butanoate	0.55ns	0.56	0.79	0.83	0.466	Fruit, pineapple[b]
Ethyl decanoate	0.35ns	0.66	0.52	0.43	0.141	Fruit, grapes[t]
Ethyl hexanoate	15.74a	10.11ab	6.41b	10.50ab	0.016	Fruit, green apple, spices[g,h]
Ethyl isovalerate	0.44ns	0.67	0.43	0.56	0.372	Fruit, apple[d]
Ethyl lactate	0.46ns	0.27	0.43	0.35	0.851	Solvent, acetone[q]
Ethyl-octanoate	1.68b	6.16ab	13.10a	12.26a	0.005	Melon, wood[q]
Ethyl-2-hexanoate	0.12ns	0.14	0.07	0.13	0.233	Pepper, sweet, earthy[q]
Ethyl-2-methylbutanoate	0.20ns	0.30	0.17	0.50	0.167	Strawberry, fruit candy[d]
Ethyl-2-phenylacetate	1.42b	6.73ab	8.27ab	16.72a	0.395	Tropical fruit, pineapple[n]
Hexyl acetate	0.12ns	0.13	0.07	0.09	0.084	Apple, pear, fruit[h,i,j]
Isoamyl acetate	1.16ns	0.98	1.09	0.71	0.180	Banana, pineapple[i]
Terpenes						
Citronellol	10.51a	4.92b	2.93c	2.75c	< 0.001	Spices, lemon[n,o]
Linalool	0.10a	0.05b	0.03b	0.03b	0.037	Floral, lavender[d,o]
Ketones						
2-Nonanone	0.11ns	0.72	0.13	0.25	0.062	Herbaceous, fruit[p]
β-Damascenone	2.65a	0.49b	0.16b	0.91b	0.003	Floral, apple pie, honey[i,k]
Aldehydes						
Nonanal	0.12ns	0.14	0.16	0.11	0.319	Citrus, herbaceous[r]
Phenols						
4-Ethylguaiacol	0.09ns	0.22	0.10	0.17	0.166	Animal, contaminant[s]
Acids						
Caprylic acid	0.27ns	0.23	0.11	0.26	0.315	Animal, solvent[l]

Data are mean values from 2019/2020 and 2020/2021 wines. Letters show significant differences from another treatment in the same row ($p < 0.05$). [a] Carpena *et al.* (2020), [b] Wu *et al.* (2019), [c] Nan *et al.* (2021), [d] Pereira *et al.* (2014), [e] Jiang and Sun (2018), [f] Yu *et al.* (2019), [g] Chen *et al.* (2012), [h] Gambeta *et al.* (2014), [i] Noguerol-Pato *et al.* (2009), [j] Peng *et al.* (2013), [k] Escudero *et al.* (2007), [l] Feng *et al.* (2017), [m] Zhao *et al.* (2020), [n] Lu *et al.* (2022), [o] Tao *et al.* (2008), [p] Li *et al.* (2008), [q] Mayr *et al.* (2014), [r] Wang *et al.* (2016), [s] Milheiro *et al.* (2019), [t] Welke *et al.* (2014).

For growers, bud load selection should be guided by production goals and market demands in 'Viognier' production. Thirty to forty buds per plant to maximise yield in vigorous subtropical vineyards. Lower loads if wine profiles with enhanced varietal aromas are targeted, as in niche markets valuing floral and citrus notes. Criteria such as the Ravaz index, yield, aromatic preferences, and vigour assessments can inform decisions.

Future research should focus on higher bud numbers (*e.g.*, 45, 50, 60, or 75 buds/plant) to determine thresholds where bunch quality or ripeness might degrade in subtropical environments. Moreover, assessing the economic impacts of different crop loads could guide growers in optimising profitability while maintaining quality in developing wine regions like Brazil.

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