Bud Load and Its Influence on Agronomic Performance and Wine Aromatic Composition of 'Fiano' Grapevines in Southern Brazil

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The objective of this study was to evaluate the influence of bud load on the agronomic performance and composition of volatile compounds of the 'Fiano' grapevine cultivated in Southern Brazil. The experiment was conducted in a commercial vineyard in the municipality of Campo Largo, Paraná (Southern Brazil) in the 2019/2020 and 2020/2021 seasons. Vines were Guyot-pruned and evaluated according to different bud loads: 10, 20, 30 and 40 buds per plant. Agronomic performance, technological maturity and the volatile compounds were evaluated, and the data were submitted for variance analysis. The mean values were compared using the Tukey test ($p < 0.05$). The volatile compounds in the wines were identified using gas chromatography (GC-MS/HS-SPME). The loads of 30 and 40 buds per plant showed an increase in productivity and the maintenance of the physical characteristics and technological ripeness of the grapes. A higher bud load resulted in a larger leaf area and better values for the Ravaz Index. However, the highest bud load (40 buds per plant) reduced the sprouting and shoot length, while a bud load of 30 buds per plant presented the best agronomic performance in the subtropical region of Southern Brazil. The physical characteristics of the bunches and the technological ripeness were not affected, and the increase in bud load did not alter most of the volatile compounds of 'Fiano' wines. However, some volatile compounds, such as diethyl succinate and linalool, presented higher concentrations in wines with the lowest yields (10 buds per plant).

INTRODUCTION

Brazil experienced an increase in its wine production in 2022, with an increase of 14% from 2017, with record volumes registered in 2020 and 2021. Brazil is the second-largest wine market in South America (International Organisation of Vine and Wine [OIV], 2022; Roca, 2022). In vines, as in most fruit species, the balance between fruit load and leaf area influences the quantity and quality of production. This balance between these two parameters is decisive for the composition and maturation of the grapes, and any technique that modifies the relation between leaf area (source) and fruit load (sink) has a direct impact on grape quality (Lopes Fernandes de Barros *et al*., 2019). The balance between production and leaf area can be maintained through management techniques, such as winter pruning, defoliation and bunch thinning (Aru *et al*., 2022; Alshallash *et al*., 2023).

Pruning is one of the most important horticultural processes in viticulture due to its impact on fruit yield and quality (Gutiérrez-Gamboa *et al*., 2021). Pruning includes the selective removal of canes, shoots, leaves and other vegetative parts of the grapevine (Gatti *et al*., 2016). Winter pruning has a significant effect on the nature of the crop during the growing season through its effect on bud load, bud fertility and nutrients stored in the vine (Qiu *et al*., 2019).

Bud load is the number of buds left on a grapevine after pruning (Poni *et al*., 2016). The ideal bud load for a grapevine depends on the cultivar, the growing conditions, and the desired yield (Dobrei *et al*., 2016). However, it may be necessary to adjust the bud load up or down, depending on the bud fruitfulness, to meet the productive objective (Monteiro *et al*., 2021). The adaptive processes by which vines respond to increased bud numbers have been described, and include reduced vegetative growth, reduced bud fertility, shorter shoots with shorter internodes, greater productivity,

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and a greater number of bunches per plant, in addition to having longer bunches and smaller berries (Greven *et al*., 2014, 2015).

The volatile compounds play an essential role in shaping the typical flavours of grapes and wines. These compounds contribute to the aroma "bouquet" of wines when analysing the head-space, as well as to the odour/aroma component (palate aroma) of the overall flavour perceived on drinking (Bakker & Clarke, 2011). These compounds provide enough chemical foundation for forming the non-invasive approach known as "volatolomics", which involves the study of volatile organic compounds (Giannoukos *et al*., 2019; Tian *et al*., 2023). The concept of single-vineyard wine has become more and more popular (Lu *et al*., 2023). Usually, a single-vineyard wine represents the highest quality of wines produced from a certain region, which reflects the winemakers' pursuit of precision viticulture (Bramley *et al*., 2011).

The available literature contains a lack of information on the effect of bud load on yield, grape composition, and wine quality of *Vitis vinifera L*. cultivars in subtropical regions, especially in Southern Brazil. The current work aimed to evaluate the influence of bud load on the agronomic and oenological performance of the grapevine 'Fiano' grown in Southern Brazil.

MATERIALS AND METHODS

Experimental area

The experiment was carried out in a vineyard located in the municipality of Campo Largo, Paraná, Brazil (25°23'41" S and 49°30'12" W) in the 2019/2020 and 2020/2021 seasons. This region is characterised by an altitude of 975 meters above sea level and is part of the Cfb climate (subtropical with temperate summer) according to the Köppen classification (Alvares *et al*., 2013), with rainfall distributed throughout the year and the possibility of severe frost.

'Fiano' is a white grape from South Italy (Campania region) that has high potential for winemaking. 'Fiano' vines were grafted onto Paulsen 1103 rootstock and planted in 2010. Vines were trained to a vertical trellis system with three wires, at 1.2 m from the ground, with a spacing of 2.7 m between rows and 1.2 m within the row for all treatments (3 086 plants per ha). Treatments were loads of 10 buds, 20 buds, 30 buds, and 40 buds per plant (Fig. 1), with only the buds of the fruiting canes evaluated. Each cane was pruned with 10 buds and arched. Four to five spurs were left per plant to form new shoots, which were pruned in the following season. Guyot pruning was carried out, with spurs and unilaterally or bilaterally arched canes, depending on the treatment.

Climate parameters were assessed according to the World Meteorological Organization (WMO), and average monthly data on air temperature (minimum and maximum) and rainfall from 2019-08-31 to 2021-04-30 were provided by the Paraná Meteorological System (SIMEPAR) from the closest weather station to the vineyard (Fig. 2). The BBCH scale (Lorenz *et al*., 1995) was used to define the phenological stages of the vine. After pruning, 4% hydrogen cyanamide was applied to induce and standardise sprouting. Disease control, canopy management (weeding, defoliation and pruning), and fertilisation were carried out according to the technical recommendations for the crop.

Productive and morphological variables

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

The leaf area was measured using 150 leaves, randomly collected during the grape harvest, and was determined using a WinRHIZO area meter (LA1600, Regent Instruments Inc). The number of shoots and the number of leaves per shoot were counted on five plants per treatment to estimate the vine leaf area of each treatment. The balance between vegetative and productive growth was estimated by the ratio of the vine leaf area per kilogram of grapes produced (m^2/kg).

In samples of 10 bunches per plot, the mass of the bunch (g) and the mass of the rachis (g) were obtained using a semi-analytical balance, the length of the bunch (cm) was

FIGURE 1
Treatments with different bud loads on the 'Fiano' grapevine. (A: 10 buds; B: 20 buds; C: 30 buds; and D: 40 buds). Campo Largo, PR. Brazil.

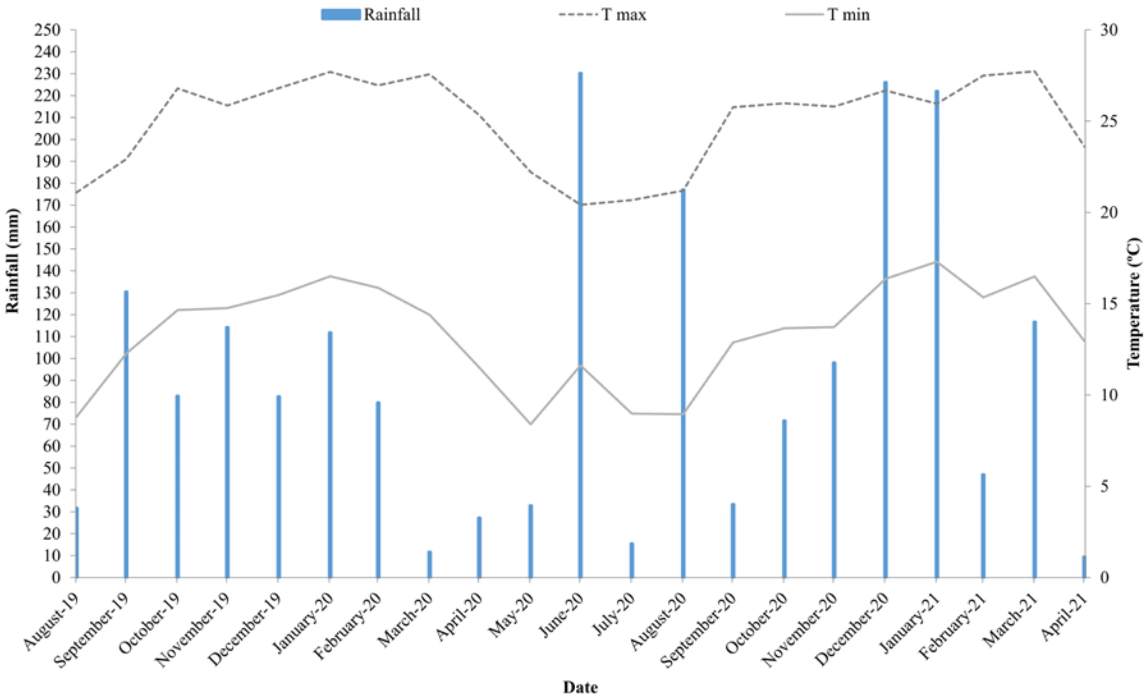

FIGURE 2

Cumulative rainfall (mm) and mean minimum and maximum temperatures (ºC) in the municipality of Campo Largo, PR, Brazil, during the 2019/2020 and 2020/2021 vine seasons. Source: SIMEPAR, Lapa, PR, Brazil.

measured with a ruler, and the number of berries per bunch was counted. After the start of ripening, the length of the shoots (cm) was assessed weekly using a tape measure. After harvesting, the number of leaves per shoot were counted. The internode length (cm) and the diameter of the shoots (cm) at the first and tenth internode were measured using an analogue calliper.

Maturation evolution and microvinification

From the beginning of the ripening until harvest, 100 berries were collected every week to monitor and determine the technological ripeness. The berries were taken to the laboratory for weighing, separation of the skins for analysis, and maceration. The must was used to determine total soluble solids (°Brix), total titratable acidity and pH, according to the methodology proposed by the OIV (2020).

Total soluble solids (TSS) were determined using a refractometer (ITREFD 45 model, Instrutemp, São Paulo, SP, Brazil). The device was calibrated with distilled water, after which the must was distributed over the prism, and the reading was taken directly in °Brix. Total titratable acidity (TTA) was obtained by titration of a solution of 5 mL of must dilute in 50 mL of distilled water and a standardised alkaline solution of 0.1 N sodium hydroxide, using phenolphthalein (1%) as an indicator. The volume of NaOH consumed was used to determine the TTA in mEq/L, and then converted into g/L of tartaric acid. The hydrogen potential (pH) was recorded from the samples collected on the day of harvest using a pH meter (BEL Engineering, model: w3b pH meter, Monza, Milan, Italy), after calibration in known buffer solutions of pH 4.0 and 7.0. A sample of 30 kg of grapes were manually harvested from each treatment. Microvinifications were carried out in a commercial winery according to the protocol adapted from Makhotkina *et al*. (2013) and Pszczolkowski (2014). Subsequently, the volatile compounds of the wines from the different treatments were identified using headspace solid-phase microextraction/gas chromatography with mass spectrometry detection (HS-SPME-GC-MS).

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

An already optimised methodology, previously described by Tao *et al*. (2008), was used for the extraction of the volatile components of the samples. In a 15 mL vial containing a magnetic stirring bar, 7.5 mL of sample, 0.7500 ± 0.005 g of NaCl and 2 uL of α-pinene solution, used as internal standard, were added. The vial was inserted into a container with a glass jacket that was placed on a magnetic stirring plate and connected to a thermostatic bath with water circulation (SOLAB SL 152, Piracicaba, SP, Brazil). The vial was kept in a water bath at 40 ± 0.2°C, and the contents were stirred for 5 min. The solid-phase microextraction (SPME) fibre was exposed to the headspace of the vial, which was kept at the same temperature for 30 minutes. SPME fibre, composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with a 50/30 μm thick and 1 cm long (SUPELCO, Bellefonte, PA, USA) coating, was used. The compound α-pinene was used as an internal standard because it is not a typical volatile compound in wine. It showed a location-perfect ion peak that differed from the peaks found in the volatile compounds in wines.

The SPME fibre containing the adsorbed volatile components was inserted manually into the gas chromatography with mass spectrometry detection (GCMS)

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injector at 250°C (splitless mode, equipped with glass liner, 0.75 mm I.D.) and held for 5 min. The desorbed components were separated on an Agilent 7890A GCMS using the methodology adapted from Tao and Li (2009). An Agilent HP-5MS column (30 m x 0.25 mm x 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 maintained at 40°C for 5 min, followed by a heating ramp from 40°C to 260°C with a heating 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 range of 30 m/z to 400 m/z. The mass spectrometer was operated with the electron impact at 70 eV. The peaks were manually integrated into the G1701EA GC/MSD Chemstation software. The volatile substances were characterised by comparing the mass spectrum and the experimental Kovats Index (KI) for each component with the respective mass spectra and theoretical Kovats Index of the standards described by Adams (2017). Experimental KI values were obtained by injecting a sample of saturated hydrocarbons (Sigma-Aldrich) under the same conditions used for the samples and calculating them according to Van den Dool and Kratz (1963). Each sample was analysed in at least triplicate, and the percentage of the volatile compounds was obtained based on the area of the specific compound compared to the total area of all the volatile measured compounds. Compounds not identified in the literature were identified by characterisation, based on NIST software with over 90% similarity.

Experimental design and statistical data analysis

The experiment was laid out in a randomised complete block design, with four blocks and 10 plants per plot. The data was submitted to analysis of variance (ANOVA), and the means were compared using the Tukey test at a 5% probability of error, using Sisvar software version 5.6 (Ferreira, 2019).

RESULTS

Vine morphological components and yield

The sprouting (%) of the 'Fiano' under the different bud loads presented similar behaviour in both seasons evaluated, but for the 2019/2020 season, the load of 10 buds per plant with 100% sprouting was significantly higher than the 89.2% for 30 buds and 80.3% for the load of 40 buds (Table 1).

The increase in the number of buds caused a reduction in the percentage of sprouting of the 'Fiano' in both seasons, although the number of bunches per plant and the yield were higher for the 30 (2.27 kg/plant) and 40 (2.34 kg/plant) bud loads in the first season. The treatments of 30 buds per plant and 40 buds per plant presented more bunches than the 10- and 20-bud treatments, with 21.8 and 24.2 bunches in the first season and 23.2 and 26.6 bunches in the second, respectively. The yield per hectare in the 2020/2021 season was higher for the bud loads of 20, 30 and 40 buds than for the 10-bud treatment.

To characterise the balance between vegetative growth and production, the Ravaz Index was calculated using the ratio of yield and pruning mass for the same plant. The

TABLE 1
Effect of bud load on the yield components of the 'Fiano' grapevine (*Vitis vinifera* L.) in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR. Brazil.

Parameter	Bud load				p-value
	10	20	30	40	
		2019/2020			
Number of shoots	12.7d	19.25c	26.9b	32.1a	< 0.001
Sprouting (%)	100.0a	96.2ab	89.2b	80.3c	< 0.001
Number of bunches	8.1b	10.1b	21.8a	24.2a	< 0.001
Yield (kg/plant)	0.73b	1.00b	2.27a	2.34a	< 0.001
Yield per hectare (t/ha)	2.26b	3.09b	7.02a	7.21a	< 0.001
Pruning mass (kg)	0.36b	0.44ab	0.54a	0.47ab	0.021
Ravaz Index	2.44b	2.87ab	4.74ab	5.22a	0.002
		2020/2021			
Number of shoots	11.8d	19.9c	27.6b	31.5a	< 0.001
Sprouting (%)	100.0a	99.4a	92.8b	78.2c	< 0.001
Number of bunches	11.3c	18.7b	23.2a	26.6a	0.002
Yield (kg/plant)	0.91b	1.84a	2.05a	2.72a	0.028
Yield per hectare (t/ha)	2.70b	5.47a	6.10a	8.07a	0.028
Pruning mass (kg)	0.40b	0.45b	0.54a	0.53a	0.002
Ravaz Index	2.26b	4.20a	4.01a	5.44a	0.044

Values followed by different letters in the same line differ significantly (Tukey test, $p < 0.05$). ns = not significant by analysis of variance (ANOVA) at a 5% error margin.

Ravaz Index reached better values for the 30- and 40-bud loads in both harvests, varying between 4.01 and 5.44, and indicating a better vegetative-productive balance for these treatments in our study.

In both seasons, the 10-bud treatment presented longer shoots compared to 30 and 40 buds per plant (Table 2). For the 2019/2020 season, shoot length was higher in the 10-bud treatment (147.3 cm), followed by the 20- and 30-bud treatments (126.8 cm and 134.1 cm), which did not differ from each other, and the shortest shoots were found in the 40 bud-treatment (112.4 cm). In the second season, the length of the shoots was longer for the treatments with lower bud loads (10 and 20 buds), and significantly longer compared to the treatments with 30 and 40 buds. The internode length and the shoot diameters had no statistical differences among the treatments in both evaluation seasons, although the number of leaves per shoot was higher for the 10-bud treatment than for the other treatments in the second season.

The vine leaf area showed similar behaviour in the two seasons evaluated, with the treatment with 40 buds per plant presenting a higher leaf area due to the greater number of shoots and leaves, being significantly higher than the other treatments. A higher vine leaf area was found in 2020/2021, with 7.3 m^2 for the 40 buds per plant, followed by 30 buds (5.5 m^2), 20 buds (4.1 m^2) and 10 buds (2.5 m^2). However, the ratio between leaf area and yield reached better values for 30 and 40 buds per plant and differed from the others in 2019/2020. In the second evaluation season, all the treatments presented better values for vine leaf area and production ratio (between 2.21 m^2/kg and 2.74 m^2/kg), and these did not differ significantly from each other.

There was no significant difference n all physical parameters of the bunches (Table 3), showing that the different bud loads had minimal influence on the physical parameters of the bunches of 'Fiano' under the conditions of this study. The bunch mass varied from 104.2 g to 127.1 g, and the bunch length from 13.8 cm to 15.8 cm over the two evaluation seasons. In the 2019/2020 season, the number of berries per bunch for 10 buds (91), 20 buds (86), 30 buds (105) and 40 buds (83) showed no significative differences, as well as in 2020/2021, when the numbers were 101 berries for 10 buds, 102 berries for 20 buds, 103 berries for 30 buds and 107 berries for 40 buds. Berry mass and rachis mass were evaluated and also did not present significant differences in both seasons.

Technological maturity

The ripeness of the berries was evaluated weekly after véraison, and the different bud loads presented minimal interference on the technological maturity for total soluble solids (TSS), total titratable acidity (TTA), and pH for both seasons (Table 4). A higher content of TSS and TTA was found for all treatments in the 2020/2021 season, although the behaviour among the treatments was similar, with no differences in the content of TSS and TTA for the different bud loads. TTA values varied from 7.69 g/L to 8.03 g/L of

TABLE 2

Effect of bud load on the vegetative components of the 'Fiano' grapevine in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR. Brazil.

Parameter	Bud load				p-value
	10	20	30	40	
			2019/2020		
Shoot length (cm)	147.3a	126.8b	134.1b	112.4c	< 0.001
Number of leaves per shoot	16.4 ns	14.9	15.5	15.1	0.158
Internode length (cm)	8.7 ns	8.4	8.6	7.5	0.442
Diameter 1ª internode (cm)	0.39 ns	0.33	0.34	0.30	0.696
Diameter 10ª internode (cm)	0.35 ns	0.29	0.31	0.27	0.109
Vine leaf area (m^2)	3.1d	4.3c	6.3b	7.2a	< 0.001
Leaf area per yield (m^2/kg)	4.3a	4.2a	2.8b	3.1b	< 0.001
			2020/2021		
Shoot length (cm)	137.1a	129.3a	124.1b	116.0b	0.004
Number of leaves per shoot	14.7a	13.2b	12.5b	13.1b	0.037
Internode length (cm)	9.34 ns	9.92	10.01	8.94	0.379
Diameter 1st internode (cm)	0.32 ns	0.31	0.30	0.33	0.710
Diameter 10th internode (cm)	0.24 ns	0.23	0.25	0.24	0.966
Vine leaf area (m^2)	2.5d	4.1c	5.5b	7.3a	< 0.001
Leaf area per yield (m^2/kg)	2.74 ns	2.21	2.73	2.65	< 0.001

Values followed by different letters in the same line differ significantly (Tukey test, $p < 0.05$). ns = not significant by analysis of variance (ANOVA) at a 5% error margin.

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tartaric acid in the first season, and 8.26 g/L to 9.04 g/L in the second season, with no statistical differences in the different bud loads in both evaluation seasons. The pH when berries were ripe for harvest was 3.08 (10 buds), 3.10 (20 buds), 3.14 (30 buds) and 3.05 (40 buds) in the 2020/2021 season, with similar behaviour in the previous season. Due to the effect on the physical characteristics of bunches, the technological maturity of 'Fiano' was minimally affected by the different bud loads. The evolution of the technological ripeness of 'Fiano' under the different bud loads was monitored weekly, and small variations were observed for the TSS and TTA variables throughout the season, but the treatments did not present differences between the different bud loads for the technological ripeness variables (TSS, TTA and pH) at harvest.

Wine volatile compounds

The volatile compounds present in the wines from the different bud loads of 'Fiano' showed no significant differences for 18 of the 26 aromatic compounds identified (Table 5). The volatile compounds were identified by gas chromatography, and their corresponding retention times and theoretical and experimental Kovats Index. The compounds identified were esters, alcohols, monoterpenes, aldehydes, phenols, medium-chain fatty acids and norisoprenoids.

After evaluating all the identified volatiles and their relative area in the chromatogram, isoamyl alcohol was found to be the compound of the alcohol class present in

TABLE 3
Effect of bud load on the physical characteristics of bunches and berries for 'Fiano', in 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Physical parameters	Bud load				CV (%)
	10	20	30	40	
		2019/2020			
Bunch mass (g)	118.2 ns	109.4	121.3	116.6	20.7
Number of berries	91 ns	86	105	83	15.8
Berry mass (g)	1.32 ns	1.26	1.15	1.39	13.4
Rachis mass (g)	6.3 ns	5.8	5.9	6.8	11.5
Bunch length (cm)	15.2 ns	13.9	15.8	14.2	8.2
		2020/2021			
Bunch mass (g)	122.8 ns	113.5	104.2	127.1	16.0
Number of berries	101 ns	102	103	107	14.1
Berry mass (g)	1.21 ns	1.22	1.19	1.17	9.0
Rachis mass (g)	5.4 ns	5.2	5.2	5.0	15.8
Bunch length (cm)	14.3 ns	13.8	14.7	14.6	17.2

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

TABLE 4
Effect of bud load on the technological maturity of 'Fiano' in the 2019/2020 and 2020/2021 seasons. Campo Largo, PR, Brazil.

Parameters	Bud load				CV (%)
	10	20	30	40	
		2019/2020			
Total soluble solids (°Brix)	18.0 ns	17.6	18.4	17.6	8.1
Total titratable acidity (g/L)	8.03 ns	7.76	7.69	7.88	3.9
pH	3.11 ns	3.17	3.15	3.12	2.4
		2020/2021			
Total soluble solids (°Brix)	19.0 ns	18.8	18.6	18.8	3.06
Total titratable acidity (g/L)	9.04 ns	8.70	8.26	9.30	5.5
pH	3.08 ns	3.10	3.14	3.05	2.1

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

TABLE 5
Identified volatile compounds, p-values, chromatogram relative area and aroma descriptors for 'Fiano' wines and for the different bud loads. Campo Largo, PR, Brazil.

Compound (%)	Bud load					Aroma descriptors
	10	20	30	40	p	
Isoamyl alcohol	37.40 ns	33.42	31.43	32.43	0.105	Honey, fruit [a]
Ethyl butanoate	0.38 ns	0.48	0.52	0.47	0.160	Fruit, pineapple [b]
Ethyl lactate	0.32 b	1.43 a	1.44 a	1.42 a	<0.001	Solvent, acetone [s]
4-methyl pentanol	0.28 ns	0.26	0.21	0.23	0.104	No descriptor [d]
Ethyl-2-methylbutanoate	0.74 ns	1.20	0.97	0.90	0.165	Strawberry, fruit candy [e]
Ethyl isovalerate	0.83 ns	0.84	0.93	0.95	0.854	Fruit, apple [e]
Hexanol	0.50 ns	0.46	0.47	0.42	0.292	Flower, grass, resin, herbaceous [b]
Isoamyl acetate	0.32c	0.97b	1.50a	0.93b	< 0.001	Banana, pineapple [l]
2-Methyl butyl acetate	0.12b	0.38a	0.46a	0.33a	0.002	Pineapple, banana, fruit [f, g]
Ethyl hexanoate	7.26b	8.18ab	9.35ab	10.04a	0.058	Fruit, green apple, spices [h, i]
Hexyl acetate	0.05 ns	0.08	0.09	0.06	0.044	Apple, pear, fruit [i, j, l]
2-Ethyl hexanol	0.40b	0.52b	0.90a	0.83a	< 0.001	Citrus, herbaceous [t]
Ethyl-2-hexanoate	0.10b	0.11b	0.20a	0.22a	0.003	Pepper, sweet, earthy [s]
2-Nonanone	0.29 ns	0.10	0.19	0.14	0.548	Herbaceous, fruit [r]
Linalool	0.04a	0.01b	0.01b	0.01b	0.048	Floral, lavender [e, q]
Nonanal	0.10 ns	0.20	0.14	0.16	0.570	Citrus, herbaceous [t]
Phenylethyl alcohol	0.16 ns	0.20	0.17	0.19	0.593	Floral, rose flower, perfume [b, n]
Diethyl succinate	22.75a	20.46b	19.23b	20.38b	0.022	Wine, fruit [t]
Ethyl-octanoate	10.86 ns	12.64	13.01	12.38	0.349	Melon, wood [s]
Citronellol	0.52 ns	0.61	0.64	0.52	0.404	Spices, lemon [p, q]
Ethyl-2-phenylacetate	9.20 ns	9.76	10.37	10.19	0.751	Tropical fruit, pineapple [p]
2-Phenyl ethyl acetate	0.19 ns	0.37	0.34	0.34	0.160	Floral, sweet [o]
4-Ethylguaiacol	0.33 ns	0.47	0.61	0.52	0.226	Animal, contaminant [u]
Caprylic acid	0.34 ns	0.33	0.35	0.36	0.929	Animal, solvent [n]
β-Damascenone	0.84 ns	1.51	1.21	1.11	0.110	Floral, apple pie, honey [l, m]
Ethyl decanoate	0.53 ns	0.48	0.65	0.57	0.389	Fruit, grapes [v]

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*. (2021), [b] Wu *et al*. (2019), [c] Molina *et al*. (2009), [d] Lijun *et al*. (2021), [e] Pereira *et al*. (2014), [f] Jiang & Sun (2018), [g] Yu *et al*. (2019), [h] Chen *et al*. (2013), [i] Gambeta *et al*. (2014), [j] Noguerol-Pato *et al*. (2009), [l] Peng *et al*. (2013), [m] Escudero *et al*. (2007), [n] Feng *et al*. (2017), [o] Zhao *et al*. (2020), [p] Lu *et al*. (2022), [q] Tao *et al*. (2008), [r] Li *et al*. (2008), [s] Mayr *et al*. (2014), [t] Wang *et al*. (2016), [u] Milheiro et al. (2019), [v] Welke et al. (2014).

the largest quantities. This alcohol is a typical compound of young wines and, for 'Fiano', is related to tropical fruits and sweet aromas. Some esters (ethyl-octanoate and ethyl-2-phenylacetate) showed no differences between the treatments, although the ester ethyl hexanoate was more prevalent in 40-bud wines in comparison with the 10-bud treatment. The treatments with 20, 30 and 40 buds per plant presented higher levels of ethyl lactate and 2-methyl butyl acetate. Finally, wines from the lowest bud load (10 buds per plant), and consequently lowest productive yield, showed higher contents of diethyl succinate and for the monoterpene linalool.

DISCUSSION

In this work, the main objectives were to determine if an increase in the bud load affects the growth of the shoots, the technological maturation of the bunches and the volatile compounds of the wines for the different treatments.

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Bud load affects the sprouting, the growth of the shoots and the balance between vegetative growth and production

In relation to the results shown in Table 2, the data corroborates a study carried out by Wurz *et al*. (2020), where the authors found that an increase in the number of buds per plant increased the number of shoots and bunches per plant, resulting in an increase in yield, as well as enabling a better vegetative-production balance. According to O'Daniel *et al*. (2012), increasing bud load per plant results in an increase in the number of shoots per hectare, and consequently a reduction in the spacing among shoots.

In this work, the treatment with the highest bud load (40 buds) presented no differences in yield when compared to the treatment with 30 buds per plant, and better yields than the 10- and 20-bud treatments. According to Greven *et al*. (2015), the increase from 24 to 72 buds per plant resulted in increasing the yield from 4.8 t/ha to 12.7 t/ha. The same authors suggest that, as bud load increases over the years, grapevines tend to promote changes in their behaviour through compensation of the vegetative canopy and productivity, mainly due to changes in cluster architecture and reduced bud fertility (Greven *et al*., 2014). In the same work, it was observed that an increase in bud load resulted in a reduction in shoot length and diameter, number of leaves, as well as internode length. These studies presented similar results as the results for 'Fiano'. Several studies agree that Ravaz Index (RI) values between 4 and 7 are indicative of balanced vines capable of producing quality fruit (Silva *et al*., 2009). An RI higher than 7 indicates excessive fruit production, and values lower than 4 show excessive plant vigour (Howell, 2001). Analysing both the cycles evaluated, the treatments with 30 and 40 buds per plant had the most balanced values for the Ravaz Index.

The treatment with 10 buds per plant presented longer shoots than the 30 and 40 buds per plant treatments in both seasons. Nevertheless, for internode length and shoot diameter, these differences were not significant. Shoots with the greatest length were those from the treatments with the lowest bud load per plant, indicating that increasing the bud load can be an alternative to control the excessive vegetative vigour, causing a reduction in shoot length. However, as observed for sprouting, the length of the branches was lower in 40 buds per plant, indicating that this treatment can cause excessive shading due its greater number of branches and leaves. Regarding the vine leaf area, the behaviour was the same in both seasons, with 2.5 m^2 (10 buds), 4.1 m^2 (20 buds), 5.5 m^2 (30 buds) and 7.3 m^2 (40 buds) in the 2020/2021 season, and the treatment with the higher number of buds presented the higher total leaf area. The leaf area (m^2) was higher with higher bud loads due to the greater number of shoots, and consequently the total number of leaves and leaf area.

According to the literature, values considered adequate between vine leaf area and production ratio were from 7 cm^2/g to 20 cm^2/g (0.7 m^2/kg to 2.0 m^2/kg) (Howell, 2001; Kliewer & Dokoozlian, 2005). In this work, all treatments presented values higher than 2.0 m^2/kg for this ratio, indicating that vegetative growth is slightly excessive for 'Fiano' under these climatic conditions. Several studies in Southern Brazil indicate that the region's climate and soil conditions favour vegetative growth and plant vigour. Borghezan *et al*. (2011), evaluating the vegetative and productive behaviour of the 'Cabernet Sauvignon', 'Merlot' and 'Sauvignon blanc' in a high-altitude region (São Joaquim, SC, Southern Brazil), reported that the ratio of total leaf area to production varied from 3.7 m^2/kg to 8.4 m^2/kg, which indicates greater imbalance, especially for the vines with the highest values for this ratio. In another study on 'Merlot' in Sao Joaquim (SC, Southern Brazil), a ratio of 4.5 m^2/kg was observed in plants that did not receive topping and defoliation management throughout the harvest (Brighenti *et al*., 2010). Values lower than 0.6 m^2/kg are generally insufficient to fully ripen the fruit, and values higher than 2.0 m^2/kg usually indicate excessive vigour. The higher the value of this ratio, the greater the vigour, which ends up causing delays in ripening and a reduction in the polyphenol content, and provides a microclimate more favourable to the development of diseases due to the greater shading.

In 2019/2020, the treatments with 30 and 40 buds had better values for this ratio, indicating a better balance between vegetative growth and production. In the 2020/2021 season, this ratio varied between 2.21 and 2.74, with no statistical difference between all treatments. A properly balanced plant has sufficient vegetative growth to supply nutrients in adequate quantities to complete the ripening of the grape, develop fertile or productive buds for the following year, and store nutritional reserves, so determining an adequate leaf area per unit of production must always consider the cultivar, and especially the soil and climate conditions of each region (Jackson, 2008). Under the conditions in this study, vine leaf area and production ratio were slightly high, and management practices such as topping and green pruning could help to improve the performance of this cultivar. However, the 30-bud treatment presented better results for its equivalent yield compared to the 40-bud treatment, and less reduction of sprouting and shoot growth.

Bud load effects on the physical and chemical composition of the bunches

For the physical characteristics and technological maturity of the bunches, these parameters were not affected by the increase in the bud load in both seasons. These results are similar to those found by Wurz *et al*. (2019), namely that technological ripeness was not influenced by the increase in bud load (15 to 75 buds per plant) for 'Cabernet Franc', evaluated in São Joaquim, Southern Brazil. Several studies indicate that a higher number of buds per plant leads to higher yields without affecting fruit quality (Intrieri, 2011; Poni *et al*., 2016; Wurz *et al*., 2019), which corroborates the results of this study. In the second season, the TSS and TTA were higher for all treatments compared with 2019/2020, probably caused by the climatic conditions of warmer temperatures during grape ripening.

Bud load and its effects on volatile compounds in the wines

Regarding the volatile compounds in wines obtained from different vine bud loads, generally, the most abundant chemical class was higher alcohols, esters, and acids. These results corroborate several authors who have studied volatile compounds in wines from *Vitis vinifera* L. cultivars (Yu *et al*., 2019; Lijun *et al*., 2021; Alba *et al*., 2022).

Most of the volatile compounds identified in this work presented no differences between the treatments, although some important volatiles for fruit and floral aroma in wines (diethyl succinate and linalool) presented higher values in the treatment with the lowest yield (10 buds per plant). These results corroborate a study carried out by Alba *et al*. (2022), evaluating 'Sangiovese' with higher and lower production loads. These authors observed that the lowest yields (reduction in yield due to thinning of bunches) had wines with higher levels of diethyl succinate. However, the majority of volatile compounds showed no significant difference between treatments. In a study carried out by Škrab *et al*. (2021), the authors found that the thinning of bunches, and consequently lower yield loads for 'Ribolla Gialla', had a small positive effect on the volatile composition of the wines, where the highest concentration of aromatic compounds (citronellol and linalool) was observed for the treatments with reduced yields. In this work, the volatile linalool presented a higher concentration than the other treatments, therefore citronellol showed no differences. These results suggest that the bud load can affect specific volatile compounds in the wines, although most of the identified compounds were not affected by the increase in the load.

CONCLUSIONS

For 'Fiano', using the Guyot pruning system with 10, 20, 30 and 40 bud loads, the treatment with 30 and 40 buds per plant presented better results for yield and technological maturity. These treatments can increase productivity and maintain the physical characteristics of the bunches, along with the technological ripeness of the grapes.

Higher bud loads result in higher yields, greater vine leaf area, and a better relationship between vegetative growth and production (Ravaz Index). However, the highest bud load (40 buds per vine) can reduce the sprouting and the shoot length. Therefore, in the subtropical regions in Southern Brazil, the 30-bud load presented the best agronomic performance.

The increase in bud load did not affect 18 of the 26 identified volatile compounds in 'Fiano' wines. Some compounds were affected minimally by the different treatments (2-methyl butyl acetate, ethyl hexanoate and 2-ethyl hexanol), and some volatiles occurred in greater quantities in wines with lower yields (10 buds per plant), such as diethyl succinate and linalool.

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