# The influence of different training systems and rootstocks on 'Sauvignon Blanc' grapes

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**ABSTRACT:** In viticulture, training systems and rootstocks are crucial factors in the growth and development of plants, thus affecting the production and quality of grapes. Therefore, the present study aimed to determine the best combination of rootstock and training system for 'Sauvignon Blanc', in Jundiaí, state of São Paulo, Brazil. To assess the data, a randomized block design in a 2 × 2 factorial scheme was used, that is, two rootstocks ('IAC 766 Campinas' and '106-8 Mgt') and two training systems (low and high espaliers), with four replications. The characteristics assessed were production, physicochemical analysis and concentration of bioactive compounds. With regard to the physicochemical composition of the grape must, the results indicated that the tested combinations are within the standard requirements for the winemaking industry; outcomes may change according to the rootstock used, i.e., an increase in production or an increase in the nutritional quality of the fruits. Nevertheless, '106-8 Mgt' rootstock showed great yield in both training systems, that is, an increase of up to 80%. Finally, 'Sauvignon Blanc' must be grafted on to 'IAC 766 Campinas' rootstock through a low espalier system to achieve a high content of phenolic compounds and flavonoids, and high antioxidant activity.

Key words: bioactive compounds, antioxidant capacity, yield, '106-8 Mgt', 'IAC 766'.

## INTRODUCTION

The *Vitis vinifera* L. 'Sauvignon Blanc' cultivar, originally from France, is extensively cultivated across the world, such as in the United States, New Zealand, Australia, Chile, Argentina, South Africa and Brazil (Camargo et al. 2015). The grapes of 'Sauvignon Blanc' are known to produce white wines with noticeable acidity. The region of Jundiai in the State of São Paulo, Brazil, is the country's biggest grower of fresh table grapes (Hernandes et al. 2010), but local grape producers have gained interest in producing wines.

Grapevine cultivars (*V. vinifera*) generally demand high-tech care when compared to American grapevines (*Vitis labrusca* L.) and hybrids (*V. vinifera*  $\times$  *V. labrusca*), as they are more susceptible to fungal infections; therefore, they need intensive care. Adjustment of agricultural management practices is needed to optimize and identify different training systems, as well as the use of rootstocks to enhance production and, consequently, to improve the quality traits of the grapes (Dias et al. 2017; Vršič et al. 2015).



In viticulture, the wire laying system is successfully used across the globe. In espalier systems, vines are trained to grow along a wire or trellis at different heights, regarding the distance from soil to plant canopy. The espalier system alters plant architecture, which has an impact on the vegetative and productive development of the vine. Rootstocks play an important role in the adaptation of vines to the environmental conditions, affecting production by modifying vegetative and root growth; besides that, rootstocks affect the production and physicochemical composition of the fruits (Silva et al. 2017; Vršič et al. 2015).

Wine growers are presently using a great variety of rootstocks and different training systems. Each of them has advantages and disadvantages, but the growing area must be taken into consideration before selecting a rootstock and training system, since it is crucial to adopt the most appropriate agricultural management practices for the 'Sauvignon Blanc' cultivar. In this context, the objective of this work was to assess the production, physicochemical traits and bioactive compounds in 'Sauvignon Blanc' grapes grafted onto two rootstocks ('IAC 766 Campinas' and '106-8 Mgt') in high and low espalier systems under subtropical climate conditions.

#### MATERIAL AND METHODS

A field experiment was conducted over two crop seasons (2015 and 2016) in an experimental 'Sauvignon Blanc' (*V. vinifera* L.). vineyard at the Advanced Fruit Research Centre of the Agronomic Institute (IAC) in Jundiai, state of São Paulo, Brazil (located at 23°06'S, 46°55'W, at an altitude of 745 m above sea level). According to the Köppen classification, the climate of the area is the Cwa type (Cepagri 2017). According to Embrapa (1999), the soil of the experimental area is classified as cambisol dystrophic red. The maximum temperature ranged from 23.2 to 31.9 °C in 2015 and from 23.7 to 30.8 °C in 2016 (Fig. 1).



**Figure 1.** Precipitation (mm·month<sup>-1</sup>) and average monthly temperatures (°C) recorded during the test period in the years 2015 (a) and 2016 (b). Jundiaí (SP).

In September 2009, the vineyard was installed by growing the rootstocks. In July 2010, 'Sauvignon Blanc' cultivars were grafted on to them. The trees were planted at 2.5 m spacing between rows and 1.0 m between vines.

Annual fertilization of the vineyard was based on prior soil analysis that followed the recommendations of Terra et al. (1998), that is, using 130 kg·ha<sup>-1</sup> of nitrogen, 310 kg·ha<sup>-1</sup> of P2O5 and 190 kg·ha<sup>-1</sup> of K<sub>2</sub>O, with the following sources: simple superphosphate, potassium chloride and 20-5-20 formulation. For the organic matter source, 10 t·ha<sup>-1</sup> of poultry litter was applied annually. This fertilization was conducted in three stages, the first of which was done one month before pruning and contained the poultry litter application associated with the recommended doses of 100% of P<sub>2</sub>O<sub>5</sub> and 50% of K<sub>2</sub>O.

To assess the data, a randomized block design in a  $2 \times 2$  factorial arrangement was used as a statistical model, with three vines per plot and four replications. Thus, treatments consisted of combining 'Sauvignon Blanc' cultivar grafted onto two rootstocks ('IAC 766 Campinas' [106-8 Mgt × *Vitis caribaea*] and '106-8 Mgt' [*Vitis riparia* × {*Vitis rupestris* × *Vitis cordifolia*]) in high and low espalier systems.

For low-espaliered plants, three wires were placed at heights of 1, 1.3 and 1.6 m above ground level, while four wires were installed at heights of 1.0, 1.3, 1.6 and 2.0 m above ground level for high-espaliered plants.

The production per vine was determined in each season, considering the bunch mass at harvest (kg-vine<sup>-1</sup>), the number of grape clusters per vine and yield (ton·ha<sup>-1</sup>), considering a planting density of 4,000 vines·ha<sup>-1</sup>.

The physical traits of grape clusters and berries were evaluated: 10 grape clusters were randomly sampled in each plot to measure cluster fresh mass (CFM), the number of berries per cluster (NBC) and berry fresh mass (BFM); the latter was performed in a sampling of 10 berries collected from the top, middle and bottom of the grape cluster (3:4:3), totalling 100 berries per plot.

The chemical properties of the grape must were assessed: the content of soluble solids (SS), titratable acidity (TA), pH, SS/TA ratio and the content of reducing sugars (percentage of glucose). The SS content was determined by direct refractometry, using a digital refractometer (ATAGO, Kirkland, WA, USA), expressed in °Brix. Titratable activity was determined by means of titration, with 5 g of grape must diluted in 100 mL of distilled water, titrated with a standardized solution of sodium hydroxide at 0.1 N, up to pH 8.2, the results being expressed in mEq·L<sup>-1</sup> and calculated as the percentage of tartaric acid. The maturation index was obtained through the ratio between the values for SS and TA (SS/TA). The pH was determined through direct reading by using a titrator (Micronal Titrator, model B274, São Paulo, Brazil). All analyses followed the methodology proposed by the Adolfo Lutz Institute (IAL 2005).

The content of reducing sugars was determined according to the colorimetric method proposed by Somogyi–Nelson, which is based on an analytical glucose curve at absorbance of 510 nm; the results were expressed as a percentage (Nelson 1944).

Regarding the bioactive compounds in grape berry skin, the content of total flavonoids was determined according to the methodologies described by Awad et al. (2000); determination of total phenolic compounds followed the method proposed by Singleton and Rossi (1965) and that of antioxidant activity followed the ferric reducing antioxidant power (FRAP) method, which consists of measuring the ability of antioxidants to reduce ferric iron (Fe<sup>3+</sup>) (Benzie and Strain 1996).

The mean values obtained over the two production seasons were subjected to analysis of variance, then submitted to F test ( $p \le 0.05$ ); means were compared by Tukey's test ( $p \le 0.05$ ) using the computer program SISVAR (Ferreira 2011). Subsequently, multivariate analyses were performed, such as the correlation between variables (p < 0.01) and principal component analysis (PCA), in order to characterize all treatments. The statistical analysis was performed using the SAS system (Statistical Analysis Software, version 9.2).

#### **RESULTS AND DISCUSSION**

There was no interaction between training system and rootstock for the number of clusters per vine, number of grape berries per cluster, grape berry fresh mass, production or yield of 'Sauvignon Blanc' grapevines (Table 1).

Treatments	Yield per vine (kg∙vine⁻¹)	Yield (t∙ha⁻¹)	Number of grape clusters per vine	Number of grape berries per cluster	BFM (g)
High espalier	1.37	5.50	13.30	43.30	2.10
Low espalier	1.48	5.90	12.50	44.30	2.14
IAC 766	1.02b	4.10b	7.40b	37.15b	2.16
106-8 Mgt	1.84a	7.30a	18.40a	50.53a	2.07
CV (%)	7.2	26.5	10.6	12.9	9.2

**Table 1.** Yield per plant, yield, number of grape clusters per plant, number of grape berries per cluster and BFM the 'Sauvignon Blanc' in training system (high espalier and low espalier) and rootstocks ('IAC 766 Campinas' and '106-8 Mgt'). Jundiaí, SP, 2015 and 2016.

Means followed by the same lower-case letter in the columns indicates that the results do not differ significantly by Tukey's test (p < 0.05).

Analysing the factors separately, an isolated effect of the rootstocks was observed. The 'Sauvignon Blanc' cultivar, when grafted on '106-8 Mgt' rootstock, showed an increase of approximately 11 clusters per vine and 13 berries per cluster in relation to the rootstock 'IAC 766 Campinas', resulting in an 80% increase in production and 78% increase in yield. According to Bascuñán-Godoy et al. (2017), yield is mainly correlated to the number of grape clusters, but also the traits of grape clusters and berries, as well as the number of grape berries per cluster. In this experiment, this can be seen from the PCA in Fig. 2, where the yield components were grouped in PC1+.



**Figure 2.** Principal component analysis biplot of production attributes (yield [PROD], grape cluster fresh mass [CFM], grape berry fresh mass [BFM], number of grape cluster per vine [NC] and number of berries per grape cluster [NBC]), physicochemical (soluble solids content [SS], titratable acidity [TA], Ratio SS/TA, pH) and biochemicals (flavanols content [FLA], phenols and antioxidant capacity by FRAP [FRAP]) in 'Sauvignon Blanc' grape grafted onto 'IAC 766 Campinas' and '106-8 Mgt' rootstocks in high and low espaliers systems. Treatments: Mgt\_L – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; Mgt \_H- 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_L – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in low espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in high espalier; IAC\_H – 'Sauvignon Blanc' grafted onto 'IAC 766 Campinas' in hig

Overall, the results show better productive performance of 'Sauvignon Blanc' grafted onto '106-8 Mgt' rootstock. Nonetheless, the literature has already evidenced the effects on plant development and production depending on the interaction between canopy and rootstock; consequently, there is a physiological mechanism based on their compatibility (Tecchio et al. 2019; Vršič et al. 2015). These mechanisms are, therefore, involved in endogenous growth factors (carbohydrates and hormones), nutrients, water uptake and translocation (Nawaz et al. 2016).

Most vigorous rootstocks have a greater capacity for water and nutrient uptake and translocation; besides that, they have the potential to produce growth-promoting substances that contribute to the canopy performance. Several studies have already shown grape clusters with higher average yields when grapevines are grafted onto 'IAC 766 Campinas', which vigour is considered as moderate (Tecchio et al. 2020). Although '106-8 Mgt' rootstock has less vigorous activity, it showed better canopy compatibility in the present study. Still, production is not only associated with canopy and rootstock interactions, but also climate and soil conditions that can fully affect the interaction (Vršič et al. 2015).

There was a significant effect of the interaction between rootstock and training system on the grape CFM (Table 2). In vines grafted onto 'IAC 766 Campinas' rootstock, the low espalier conduction system provided more fresh mass of the grape clusters. In contrast, in the plants grafted onto '106-8 Mgt' rootstock, greater fresh mass of the bunches was obtained in the high espalier system (Table 2). This outcome has also been described in some studies across different Brazilian wine regions (Dias et al. 2017; Miele and Rizzon 2019). This may have occurred because, among other factors, conduction systems and rootstocks affect the capacity of the canopy to capture light, which can directly increase carbon assimilation and storage and, consequently, yield (Bascuñán-Godoy et al. 2017; Palliotti 2012).

Table 2. The effects of training system (high espalier and low espalier) and rootstocks ('IAC 766 Campinas' and '106-8 Mgt') on fresh mass the 'Sauvignon Blanc' grape, ANOVA significance. Jundiaí (SP), 2015 and 2016.

	Roots	stocks	ANOVA (Epropobility)		
Treatments	IAC 766	106-8 Mgt	ANOVA (F probability)		
	Grape cluster fresh mass (g)		Training systems (DS)	NS	
High espalier	73.1bB	109.4aA	Rootstocks (R)	< 0.001	
Low espalier	87.5bA	101.9aB	DS × R	0.004	

Means followed by distinct letters, uppercase in the column and lowercase in the row, differ by the Tukey's test at 5% probability. 'indicates a significant difference between training systems and the mean values followed the same lower-case letter in the columns indicates that the results do not differ significantly by Tukey's test. NS: not significant (p < 0.05).

There was no interaction between training system and rootstock for reducing sugar content, SS content, TA, maturation index or pH in the grape berries (Table 3).

**Table 3.** Reducing sugar (RS), soluble solids content (SS), titratable acidity (TA), SS/TA (ratio) and pH the 'Sauvignon Blanc' grape grown in training system (high espalier and low espalier) and rootstocks ('IAC 766 Campinas' and '106-8 Mgt'), and ANOVA significance. Jundiaí (SP), 2015 and 2016.

Treatments	RS (%)	SS (°Bx)	TA (% tartaric acid)	SS/TA	рН	
High espalier	19.8a	18.7	0.92	20.8	3.6	
Low espalier	18.0b	17.9	0.97	19.0	3.6	
IAC 766	19.9a	18.6	0.91	20.9	3.5	
106-8 Mgt	18.0b	18.0	0.98	19.0	3.6	
ANOVA (F probability)						
Training system (DS)	0.007	NS	NS	NS	NS	
Rootstocks (R)	0.006	NS	NS	NS	NS	
DS x R	NS	NS	NS	NS	NS	
CV (%)	5.7	7.5	20.0	17.5	6.8	

Means followed by the same lower-case letter in the columns indicate that the results do not differ significantly by Tukey's test. NS: not significant (p < 0.05).

The reducing sugar content ranged from 18.0 to 19.9%, within the requirements related to the production of wine, as it can contain up to 80 g of glucose per litre. These factors, however, individually affected the mentioned characteristics, since high-espaliered plants presented an increase in reducing sugars (1.8%) in grape berries when compared to low-espaliered plants. Therefore, the high espalier system promoted a greater leaf area that enhanced canopy light interception, in consequence affecting the photosynthetic activity of the grapevine (Bavougian et al. 2012; Palliotti 2012). Also, bud differentiation occurs when leaves are more exposed to sunlight, thus enabling grape berries to accumulate great reserves, as well as the salification of organic acids.

The results indicate that 'IAC 766 Campinas' promoted a 1.9% increase in the sugar content in relation to '106-8 Mgt'. In addition to productive traits, the interaction between canopy and rootstock can also affect the quality of the fruits.

There was no interaction between training system and rootstock for SS, TA, SS/TA or pH. Although these characteristics did not present any significant differences, flavour is a complex trait, composed of SS and volatile substances that are stored in fruit during its development. The concentration of sugars in grape berries affects their sensory properties, since sugars can generate precursors for the synthesis of organic acids and phenolic and aromatic compounds during ripening (Kuhn et al. 2013). In grape must, the SS content ranged from 17.9 to 18.6 °Bx, which is in accordance with the commercial standard, thus corroborating the literature on 'Sauvignon Blanc' (Würz et al. 2019).

Regarding pH, the values varied between 3.5 and 3.6, that is, within the range recommended for winemaking; when the pH in grape must is below 3.3, it can negatively affect the quality of the wine (Rizzon and Miele 2002). Titratable acidity varied between 0.91 and 0.98% tartaric acid. The maturation index ranged between 19.0 and 20.9, which is also within the ideal target for grapes intended for winemaking, i.e., from 15 to 45 (Brazil 2004).

In addition to the importance of the physicochemical properties of grape berries and grape must for the quality of wine, the presence of bioactive molecules in grapes can add value to the product. Lately, there has been a trend for finding and studying these natural substances (Silva et al. 2015). There was a significant effect of the interaction between rootstock and training system on the total phenol content (Fig. 3a). By combining 'IAC 766 Campinas' rootstock and the low espalier system, a 73.04% increase in the total phenol content was observed when compared to grapevines trained in the high espalier system. Yet there were no significant differences between '106-8 Mgt' rootstock and the two training systems for this trait, since the values ranged between 55.02 and 57.70 mg gallic acid 100  $g^{-1}$  (Fig. 3a). Phenolic compounds are involved in the adaptation of plants to the environmental and climate conditions. The molecules of phenolic compounds are responsible for the colour, aromas and flavour of the grapes; consequently, they have a significant impact on the structural properties and sensorial properties of grapes and, in particular, astringency in wines (Teixeira et al. 2013).

Overall, the 'Sauvignon Blanc' vines trained in the low espalier system showed an increase in total phenol content when grafted onto 'IAC 766 Campinas'. However, '106-8 Mgt' presented a high total phenol content in high espalier-trained vines (Fig. 3a). Yet, Silva et al. (2017) did not observe any significant differences for the same cultivar when evaluating total phenol content by comparing 'IAC 766 Campinas' and '106-8 Mgt' in a low espalier system. According to Flamini et al. (2013), the content of phenolic compounds can change depending on the variety, maturation stage, vegetative vigour and agricultural management practices.

Regardless of the rootstock, the total flavonoid content was higher in grapevines trained in the low espalier system than in the high espalier system, with differences between these systems of up to 88.0% ('IAC 766 Campinas') and 74.7% ('106-8 Mgt') (Fig. 3b). Canopy architecture interferes with the synthesis of bioactive compounds, since grape clusters are formed by a set of fruits; biochemical processes are not fully synchronized as they depend on the position of grape berries (Pagay and Cheng 2010). Thus, canopy temperature and incident light can change depending on the training system, consequently affecting metabolic pathways and thus leading to a greater or lesser amount of these compounds (Teixeira et al. 2013). Yet, the relationship between canopy and rootstock directly affects primary metabolism and, so, productive traits, but also specialized metabolism and, consequently, the biochemical traits of grapevines (Silva et al. 2017) by directly influencing the antioxidant capacity of grapes.

With regard to the antioxidant activity of grape berry skins, the mean for low-espaliered wines was 1.7 times higher with 'IAC 766 Campinas' than with high-espaliered ones and, therefore, similar findings were demonstrated for phenolic and flavonoid compounds (Fig. 3c). However, the 'Sauvignon Blanc' cultivar grafted onto '106-8 Mgt' showed greater antioxidant activity in high-espaliered plants (i.e., 29.5% higher). In grapes, the recognition of these antioxidant properties of phenolic compounds, such as flavonoids, has stimulated consumption due to their beneficial health effects (Savalekar et al. 2019). These molecules have been identified and quantified and then included in the category of free radical neutralizers, being effective in preventing self-oxidation (Gomez-Gomez et al. 2018). Although the antioxidant capacity is of great importance in grape quality and is widely discussed in current studies, comparing data has been a challenge due to the studies already available in the literature. This can be attributed to the use of different analytical methods (FRAP, DPPH and ORAC, among others), reference units and even the material analyzed (whole grape berries, grape berry skin, grape seeds) (Flamini et al., 2013; González-Centeno et al. 2013; Miele and Rizzon 2019).



**Figure 3.** Analysis of the content of phenols (a), flavonoids (b) and antioxidant capacity by FRAP (c) the 'Sauvignon Blanc' in training system (high espalier and low espalier) and rootstocks ('IAC 766 Campinas' and '106-8 Mgt'). Lower-case letters indicate difference between rootstocks and upper-case letters indicate difference between training systems, both by the Tukey's test (p < 0.05). Jundiaí (SP), 2015 and 2016.

The PCA led to better visualization of outcomes and characterization of treatments for the traits evaluated in the 'Sauvignon Blanc' cultivar grafted onto 'IAC 766 Campinas' and '106-8 Mgt' rootstocks in the two different training systems (high and low espaliers) (Fig. 2).

The PCA explained 93.21% of the data variation with the first two components (PC1 + PC2) (Fig. 2). The first component (PC1) was associated with production components such as yield, grape CFM, number of grape clusters per vine and number of grape berries per cluster, as well as maturation index and antioxidant capacity. The PC1 explained 56.25% of the total data variation. The PC2 was associated with the content of phenols and flavonoids and BFM, and explained 36.96% of the data variation (Fig. 2).

The 'Sauvignon Blanc' grafted onto '106-8 Mgt', cultivated on both high and low espaliers, showed better productive performance with higher productivity, more clusters per plant and greater fresh mass of clusters and NBC. But these

treatments also showed less SS, lower pH and, consequently, low SS/TA. Besides that, these treatments presented less antioxidant capacity, as well as a low total phenol and flavonoid content. The 'Sauvignon Blanc' cultivar grafted onto 'IAC 766 Campinas' in high-espaliered plants had a high SS content and pH and, consequently, a high maturation index (ratio) and a low berry fresh mass. Yet, smaller berries tend to concentrate solutes and therefore may have had a higher SS content. When 'Sauvignon Blanc' was grafted onto 'IAC 766 Campinas' in the high espalier system, the results indicated the highest levels of phenolic compounds, flavonoids and antioxidant capacity and the highest berry fresh mass of all, thus indicating a good option by adding value to the end product due to improvements in the nutritional traits, regardless of productive performance.

#### CONCLUSION

Regardless of the training system, 'Sauvignon Blanc' grafted on to '106-8 Mgt' showed great productive characteristics, that is, an increase in crop yield of up to 80%. In order to obtain a higher content of phenolic compounds and flavonoids, and higher antioxidant activity, 'Sauvignon Blanc' must be grafted on to 'IAC 766 Campinas' rootstock using a low espalier system.

## **AUTHORS' CONTRIBUTION**

**Conceptualization:** Tecchio M. A. and Simonetti L. M.; **Methodology:** Lima G. P. P., Simonetti L. M., Sousa M. C., Diamante M. S., Silva M. B. and Moura M. F.; **Investigation:** Simonetti L. M., Callili D., Moura M. F. and Silva M. J. R.; **Writing – Original Draft:** Simonetti L. M., Nunes J. G. S. and Sousa M. C.; **Writing –Review and Editing:** Simonetti L. M., Silva M. B., Silva M. J. R., Sousa M. C., Lima G. P. P. and Tecchio M. A.; **Funding Acquisition:** Tecchio M. A. and Lima G. P. P.; **Supervision:** Tecchio M.A..

## DATA AVAILABILITY STATEMENT

Data will be available upon request.

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