Article

# Planting density interferes with strawberry production efficiency in southern Brazil

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**Abstract**: In the search for more efficient production systems, many changes have occurred in the strawberry production sector. Planting density is one of the management techniques that most interferes with the quality of fruits and production aspects. This study aimed to evaluate the effect of different planting densities on the photosynthetic characteristics, fruit quality, and production of the strawberry cultivar Pircinque. The study was conducted in the 2018/2019 and 2019/2020 harvests in Lages, Santa Catarina, Brazil. The treatments consisted of plant spacing of 5, 10, 15, 20, 25, and 30 cm. The experimental design was in randomized blocks, with four blocks and plots of 20 plants. Plant spacing interfered with fruit quality, photosynthetic efficiency, production, productivity, and economic return. Due to the fruits of 'Pircinque' with higher quality than other cultivars, the planting spacing between 5 and 15 cm allows meeting the fruit's main production and quality requirements. However, it is up to the producer to adapt the management if opting for higher planting densities, which allow greater economic viability of the business.

**Keywords:** Fragaria X ananassa; planting spacing; photosynthetic efficiency; productive viability; fruit quality

## 1. Introduction

The strawberry (*Fragaria X ananassa* Duchesne) is produced and appreciated throughout the world. It is a culture of great economic importance, standing out among producers and consumers as the main species among small fruit groups. [1]. That is mainly due to the high-quality characteristics of the fruits and their nutritional properties, highly appreciated by consumers, especially for the presence of polyphenols, ellagic acid, anthocyanins, and vitamin C [2, 3]. Worldwide, 8.3 million tons were produced in 372.3 thousand cultivated hectares in 2018. As the world's largest strawberry producers, China (111 thousand hectares), Poland (47.9 thousand hectares), Russia (29.8 thousand hectares), the United States (19.9 thousand hectares), Turkey (16.1 thousand hectares), Germany (13.9 thousand hectares) and Mexico (13.6 thousand hectares) are worth mentioning [4].

Even though not being among the leading strawberry producers globally, Brazil stands out as the largest producer in South America, with about 4.5 thousand hectares cultivated and 165 thousand tons produced in 2019 [5,6]. The leading Brazilian strawberry producers are the states of Minas Gerais, Paraná, the Rio Grande do Sul, São Paulo, Brasília, Espírito Santo, Bahia, and Santa Catarina. Together, Minas Gerais, Paraná, and the Rio Grande do Sul represent approximately 75% of the cultivated area and national production. Due to the marketing of fruit's high added value and

considered a microclimatic culture, strawberry cultivation has an outstanding characteristic of social and economic involvement, becoming a principal activity of small rural properties [6, 7, 8, 9, 10, 11].

Many authors refer to numerous factors that may directly influence strawberry cultivation and their soil and climatic conditions and management responses. However, among these factors, we can highlight that the correct choice of a cultivar can determine the success in cultivation since it had interaction with the cultivation environment [12]; the temperature, photoperiod, and the interaction between them; the phyllochron and thermal sum also influence the production phase and the seasonal production cycle [13,14]. The plants must have high vigor, physiological, and phytosanitary quality, capable of promoting a high survival rate and vegetative growth after planting [15, 16, 17]. Different cultivation systems can also interfere with planting density, productivity, and cultivar indication [18].

It is also relevant to validate that in Brazil, about 70% of strawberry crops are carried out with the cultivars Albion, Camarosa, and San Andreas [8] and that in the states of Santa Catarina and the Rio Grande do Sul, about 80% of new strawberry crops are carried out in soilless cultivation systems [6,8]. Thus, we observe that there are still deficiencies for suitable indications in the management techniques for the soilless strawberry cultivation and understanding the cultivars recently introduced in Brazil to enable new alternatives to producers.

The interaction between cultivar and soilless cultivation system is an essential factor to be studied, combined with different planting densities. It is necessary to optimize structure usage to obtain a greater yield per area [18].

Planting density may interfere with plant growth, biomass distribution between plant organs, fruit production and size, the qualitative characteristics of fruits, and total yield [18]. It should also be noted that aspects related to leaf appearance [19], floral induction, flower formation, and the growth and development of the fruits [20] can be changed. In turn, these factors also interact with each other, requiring attention throughout the productive season, as they end up interfering in the capture of sunlight and directly influencing the photosynthesis of the leaves [19].

The critical balance between the green area's vigor in the vegetative phase and the production of fruits during the crop's productive cycle may determine its success [20, 21, 22]. Few reports in the literature relate the balance between the green area with fruit load and planting density, turning spacing an isolated study. This factor's influence is described in the literature [23] correlated with the fruits' size. In general, the greater the spacing, the larger fruits are expected to be harvested. That is done to the detriment of greater sun exposure, less competition for nutrients, and lower incidence of diseases [24].

Given the above, and bearing in mind that the cultivar of strawberry Pircinque has grown to be a new option for Brazilian producers, mainly due to the high quality of the fruit (sweetness, taste, size, crispness, and firmness), and its increased vigor and resistance of plants to pathogens [25, 26], it is essential to carry out studies aiming to understand better the influence of planting density in the cultivar mentioned above.

Thus, this study aims to evaluate the interference of different planting densities in the photosynthetic, productive, and quality aspects of the fruits of the strawberry cultivar Pircinque in cultivation carried out in the mountain region of the state of Santa Catarina, Brazil.

#### 2. Materials and Methods

## 2.1 Plant Material and Experimental Design

The research was conducted at the Center for Agroveterinary Sciences of the University of Santa Catarina (CAV-UDESC), based in Lages, mountain region of Santa Catarina, Brazil. The evaluations were carried out during two consecutive agricultural harvests (2018/2019 and 2019/2020). The experimental field was located at the coordinates 27°47' south latitude and 50°18' west longitude, at an altitude of 923 meters above sea level. The climate of the region is classified as humid subtropical mesothermal, Cfb, according to Köppen, with an average annual temperature around 15.6 °C, and average yearly precipitation of 1,400 mm, with rainfall well distributed throughout the year [27].

The conventional cultivation was carried out in a soilless cultivation system, with a structure of troughs filled with a commercial substrate (40% carbonized rice chaff + 40% decomposed pine bark + 20% sphagnum peat; and pH 6.0), and fixed greenhouse, covered with transparent polyethylene film with 100 microns thickness. The soilless cultivation system for strawberry cultivation is widely studied, as it has become alternative cultivation in protected environments. It is a cultivation technique growing strongly in Brazil and represents more than half of strawberry crops in Santa Catarina and the Rio Grande do Sul [8, 28].

Plants type "plug plants" of the Pircinque cultivar (Short Day) were transplanted in a single row, and the treatments in six different spacing between plants (5, 10, 15, 20, 25, and 30 cm); thus, a planting density equivalent to 240, 120, 80, 60, 48, and 40 thousand plants per hectare, respectively, in the ascending order from the smallest to the largest spacing of the planting between plants. The strawberry plants were produced by a commercial nursery located in Farroupilha (29°12' S, 51°19' W, and 720 meters of altitude), State of Rio Grande do Sul, Brazil. The type of plant "plug plant" was obtained from unrooted runners (tips) grown in trays containing peat, for 4 weeks [29]. The experimental design was in randomized blocks, with four blocks, the experimental unit consisting of 20 useful plants. The cultivation trough was 15 cm wide by 20 cm deep, supported one meter high to the ground.

Irrigation and fertigation were carried out by a localized system, using an irrigation tape, with drippers spaced 10 cm apart. The nutrient solution incorporated in the irrigation water was adjusted so that the nutrient solution's electrical conductivity was approximately 1.1 dS m<sup>-1</sup> and pH around 6.0 [30]. The nutrient solution's pH and electrical conductivity were monitored and adjusted according to the necessity to maintain the proposed levels. Fertigation was performed according to the culture phase, and it was performed twice a day in the vegetative stage. The frequency was increased by up to five times a day during the reproductive phase when temperatures are higher in the summer. The planting of the plants was carried out in the first half of May of each year. The harvests were carried out between September to January in both year, with an interval of 3 to 5 days, when the fruits' epidermis reached 80% uniform red color. The climatic data regarding the average temperature (°C), relative humidity (%), and precipitation (mm) were monitored through the meteorological station of the National Institute of Meteorology [31].

## 2.2 Analyzed Variables

# 2.2.1 Productive Parameters

At the end of each harvest, the fruits were counted and weighed with a semi-analytical precision scale (0.01 g), certified by the National Institute of Metrology, Quality, and Technology [32]. The results were summed, and from these, was estimated the number of fruits per plant (UN Plant<sup>-1</sup>), obtained by dividing the total number of fruits harvested by the total number of plants that composed each of the experimental units. The total production per plant (g plant<sup>-1</sup>) was obtained from the division of the total weight of the harvested fruits by the number of plants contained in each repeat. The fresh fruit mass (g fruit<sup>-1</sup>) was estimated by the ratio obtained between the commercial production variables (g plant<sup>-1</sup>) and the number of commercial fruits in each experimental unit. The percentage of commercial fruit production in relation to the total output per plot was obtained by dividing commercial production by the total output of each repeat, multiplying by 100. Commercial fruits were considered those with no damage (rot or deformity) and a fresh mass greater than or equal to 10 g [33]. Based on the fruits' average monthly marketing price, they estimated the gross and net yields that each planting density could allow a strawberry producer in the state of Santa Catarina.

## 2.2.2 Qualitative Parameters

For the fruit quality analyzes, they considered uniform samples of 10 fruits per plot, in a total of four times per cycle.

The luminosity (L), Chroma (C), and hue angle (°hue) parameters were quantified for the coloration of the fruit epidermis using a Konica Minolta® digital colorimeter. For each fruit, two readings were performed on opposite sides in the equatorial region. L\* values may range from 0 (darker) to 100 (lighter). For Chroma or color purity, the lower the value obtained, the higher the degree of color impurity, that is, the lower the pigments' saturation. Higher values increase the saturation generating purer colors. The hue angle (°hue) defines the hue of the epidermis and can vary from 0 to 360, in which smaller values correspond to the shades closer to the intense red and larger values to the shades closer to the orange-red [43].

The pulp firmness variable, expressed in Newton, was measured by a Texture Analyser TA.T.plus texturometer, with a tip of 2 mm, two penetrations of 10 mm in each fruit, on opposite faces in the equatorial region. The total soluble solids' concentration was quantified from a digital refractometer (Atago PR-101A, with automatic temperature correction). The concentration of sugars in the fruits was obtained, measured in °Brix. Titratable acidity was expressed by the citric acid content for 100 g of fresh fruit mass and was determined by an automatic TITRONIC ® titrator. The sample was formulated from a dilution of 5 ml of juice in 45 ml of distilled water and then titrated with 0.1 N NaOH solution until PH 8.1 [35]. Finally, the total soluble solids/titratable acidity ratio was calculated by dividing the sugar and acidity levels obtained.

# 2.2.3 Gas Exchange

The photosynthetic parameters were determined using the IRGA-Infrared Gas Analyzer, model Li-6400 XT from Licor. Forty readings per treatment were performed on the mature leaves most exposed to the sun, with no senescence sign. In both years, the readings were performed in the second half of October, in periods with higher photosynthetic rates (from 11h to 13h). The chamber's internal flow was fixed at 400 μmol s<sup>-1</sup> and the internal photosynthetically active radiation (PAR) at 1500 μmol s<sup>-1</sup>m<sup>-2</sup>, as recommended by the manufacturer for C3 plants [36]. Through the evaluations were obtained the data of liquid assimilation of CO2 or photosynthetic yield (A, μmol CO2 m-2 s<sup>-1</sup>), the intercellular concentration of CO2 (Ci, μmol mol<sup>-1</sup> ar), stomatal conductance (Gs, mol CO2 m-2 s<sup>-1</sup>) and transpiration rate (E, mol CO2 m<sup>-2</sup> s<sup>-1</sup>). Through the relation between the CO2 assimilation and transpiration rate (A/E), the water-use efficiency (WUE, mmol H2O<sup>-1</sup>) was calculated, while the carboxylation efficiency (A/Ci) was due to the CO2 assimilation and intercellular CO2 concentration ratio in the leaf [37].

## 2.3 Statistical Analysis

The data of each harvest, evaluated separately, were submitted to the variance analysis (ANOVA) through the F test. For the data that presented significance, quadratic polynomial regression analysis was performed. To better visualize the relation between the different spacing and the variables analyzed, a principal components analysis (PCA) was also performed [38]. All statistical analyses were performed with the help of statistical software R [39].

## 3. Results and Discussion

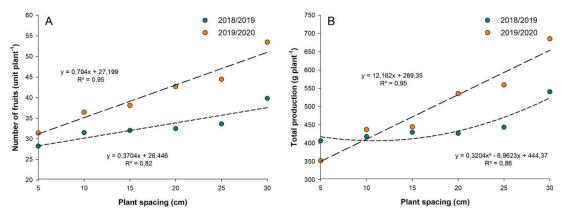
# 2.2.1 Productive Parameters

For the number of fruits per plant, the increase in spacing between plants and lower planting density resulted in increments for this variable adjusted in linear models in both harvests (Figure 1a). The highest average number of fruits harvested per plant in both crops were in the largest spacing (30 cm), with average values of 40.0 and 53.5 fruits per plant, respectively (Figure 1A). The number of fruits per plant achieved in this study, with the spacing between 10 and 30 cm in the 2018/2019

harvest, varied from 31 to 40 fruits per plant. The 2019/2020 harvest ranged from 36.5 and 53.5 fruits per plant, higher than other studies [40]. Other authors reported variations from 21.3 to 44.9 fruits in soil and substrate cultivations [41]. The number of fruits was higher in the 2019/2020 harvest than in the 2018/2019 harvest. These variations in the number of fruits per plant are mainly due to factors such as cultivars, date of planting [42, 43], cultural practices [43, 44, 45], and also by temperature fluctuations, water regime, and incidence of diseases during the growing seasons [12, 46].

Other authors reported the increase in the number of fruits verified in the present study due to increased spacing between plants and in several cultures, such as larger fruit vegetables [47, 48]. Usually, the increase in planting density and the reduction of spacing between plants lead to a reduction in the proportional partition of dry and fresh matter for the fruits and, consequently, the reduction in the number of fruits harvested and the individual production of the plants, as verified in the present study (figures 1A and 1B). They also point out that the decrease in spacing between plants reduces leaf expansion and provides higher shading, which causes a reduction in solar radiation interception and consequently affects the production of assimilated by the plant [49].

The highest averages for total production per plant were obtained with the largest spacing between plants (30 cm), 540.45 g plant<sup>-1</sup> in the 2018/2019 harvest and 685.50 g plant<sup>-1</sup> in the 2019/2020 harvest (Figure 1B). The average productions in the two evaluated crops were below the potential of the cultivar Pircinque, 1 kg per plant, considered appropriate to provide good profitability to the producer [26], and also when compared to another study that obtained the average production of 848.9 g plant<sup>-1</sup>, testing four cultivars in suspended cultivation [44].



**Figure 1.** The response of the number of fruits per plant (A) and the total production (B) of strawberry plants cv. 'Pircinque', in the different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2018/2019 and 2019/2020 harvests.

In both agricultural harvests of 2018/2019 and 2019/2020, there was a decrease in productivity due to the increase in spacing between plants (Figure 2A). The highest productivity values were in the smallest spacing (5 cm), no matter the harvest, and consequently in the highest planting density (240 thousand ha<sup>-1</sup> plants), where the yields were 97.5 ton ha<sup>-1</sup> in 2018/2019 and 84.4 ton ha<sup>-1</sup> in 2019/2020.

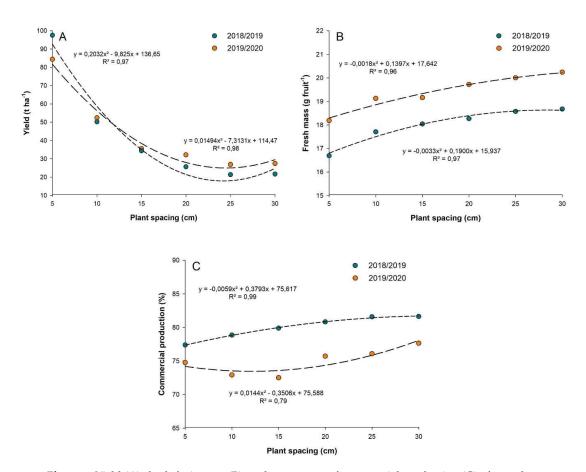
The increase in the number of fruits harvested per unit area obtained in the smaller plant spacing and higher planting densities was decisive to achieve high values of this yield component. The highest yields in the present study were obtained with a plant spacing of 5 to 15 cm, ranging in

the first harvest from 97.5 ton ha<sup>-1</sup> to 34.3 ton ha<sup>-1</sup>, and in the second harvest from 84.4 ton ha<sup>-1</sup> to 35.5 ton ha<sup>-1</sup>, values above average when compared to other studies [39]. However, when analyzing the spacing of 10 cm between plants, regardless of the harvest, there are also high yields, with values above 50 ton ha<sup>-1</sup> and increases of 38.8% (2018/2019) and 45.1% (2019/2020), when compared to the Brazilian average of 36.1 t ha<sup>-1</sup> [8]. The 2019/2020 harvest exceeded the strawberries' world average yield of 22.7 t ha<sup>-1</sup>, regardless of the spacing [4].

The use of smaller spacing and higher planting density optimized the use of semi-hydroponic structures and the cultivation area, allowing higher yields (Figure 2A) and better use per unit area. High yields are essential to ensure the economic return and enable cultivation, especially in crops typically exploited in small areas, such as strawberries. A study conducted with the cultivar Camarosa also indicated that the higher density of plants considered (200 thousand ha<sup>-1</sup> plants) led to higher productivity [50].

In the 2018/2019 harvest, there was an increase in the average fresh mass of commercial fruits, with a maximum value of 18.7 g fruit-1, obtained at the spacing of 28.8 cm between plants (Figure 2B). As the spacing between plants increased in the 2019/2020 harvest, there was an increase in the fresh mass of commercial fruits, with an estimated maximum value of 20.3 g fruit-1, at the spacing of 38.8 cm. These results corroborate with other studies, stating that the smaller spacing between plants increased the total dry mass and fruits and higher productivity, resulting from more fruit harvested per unit of the cultivated area [18]. There were oscillations of fresh mass among the treatments and the harvests in both harvests, with the lowest values observed in 2018/2019. That may be due to factors that interfere with cultivation, such as photoperiod and temperature, which affect the productive stage's hormonal regulation [51], and oscillations in temperature patterns and water regime, which always occur from one year to another [52].

There was a small oscillation between the different treatments for the percentage of production classified as commercial (Figure 2C). However, there was a significant difference between them during the evaluated harvests. However, in the 2018/2019 harvest, commercial fruit production was higher than the 2019/2020 harvest. That is possibly due to the higher number of fruits per plant obtained in the 2019/2020 harvest, which resulted in higher production of fruits below the commercial standard, of 10 g per fruit, due to the need to distribute the assimilates to a larger number of fruits. The maximum percentage of commercial production was obtained with the spacing of 32.1 cm for the 2018/2019 harvest and the minimum point in the spacing of 12.2 cm for the 2019/2020 harvest.



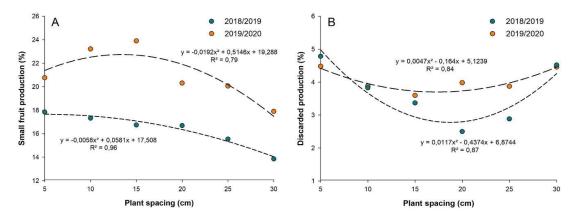
**Figure 2.** Yield (A), fresh fruit mass (B), and percentage of commercial production (C) of strawberry fruit cv. 'Pircinque', depending on the different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm), in the 2018/2019 and 2019/2020 harvests.

Spacing between plants also interfered with the percentage of fruits classified as small (Figure 3A). For both harvests, the portion of small fruits was lower with the decrease in planting density, with the maximum values for the spacing 5 cm in the 2018/2019 harvest and 13.4 cm for the 2019/2020 harvest. These results indicate that the spacing above 5 cm and 13.4 cm for the respective years would be relevant to promote the decrease in the small fruits' production. Studies report that with the increase in planting density, there may be a decrease in the crown's fresh mass [50]. In turn, it decreases the concentration of carbohydrates and consequently also causes interference in fresh fruit mass. That may occur due to the competition for carbohydrates and assimilates.

The increase in spacing in strawberry planting increased the yield per plant by about 40% and promoted the increase in fruit size [53]. Other studies also indicate positive correlations between the number of fruits per plant to the percentage of small fruits. The higher the number of fruits, the higher the percentage of fruits classified as small [54], a fact also observed in this study. Other studies also report an increase in commercial fruit production and a decrease in small fruits' percentage as the spacing between plants increases [55,56].

Another critical piece of information is related to the percentage of discarded fruits (Figure 3B) due to fungi or pests' attack, making the commercialization of fruits impossible. And for this factor, the lowest index of discarded fruits was verified for the 2018/2019 harvest at 18.6 cm spacing between

plants. For the 2019/2020 harvest, the lowest index was verified at 17.4 cm spacing. The smaller spacing between plants probably resulted in a more deficient aeration of plants, increasing the vegetation's moisture indexes. Excess moisture, in turn, collaborates with the increased occurrence of pathological diseases. The smaller spacing and the incidence of rot diseases relation are positively correlated, more severe in smaller spacing between plants [57].

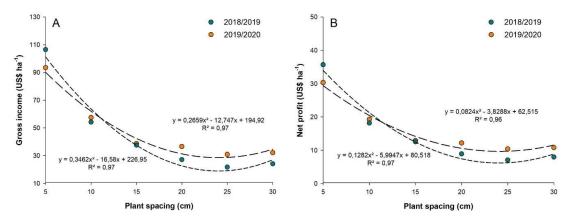


**Figure 3.** The percentage of small fruit (A) and fruit classified as discard (B) of strawberry cv. 'Pircinque', depending on the different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm), in the 2018/2019 and 2019/2020 harvests.

Concerning gross income (US\$ ha<sup>-1</sup>), estimated by the production trading, and considering the average price over the last five years from the Companhia de Entrepostos e Armazéns Gerais de São Paulo (the largest center of the food supply in Latin America) [58], there was a tendency toward a decrease in income due to the increase of the spacing (up to 25 cm) in both growing seasons since the spacing of 5 cm was the one that allowed the highest yield per hectare (US\$ 161.000,00 and 143.000,00), associated to higher productivity. The 5 cm spacing obtained a better estimate of financial gain at the end of the two harvests (Figure 4A), respectively 339% and 199% higher than the larger spacing (30 cm).

For both years, minimum values were obtained with the estimated spacing of 23.9 cm between plants, equivalent to a density of 50.2 thousand ha<sup>-1</sup> plants. That indicates that the yield's viability would be related to spacing lower than 23.9 cm between plants, resulting in planting densities higher than 50.2 mil ha<sup>-1</sup> plants. Studies with planting density for the cultivation of Camino Real strawberry in the soilless cultivation system indicate a density of 125 thousand plants ha<sup>-1</sup> as the point of balance between production, fruit quality, and the number of plants required [18].

For the net profit variable (figure 4B), the estimated production costs for the state of Santa Catarina (U\$ 1 kg<sup>-1</sup> strawberry) [59] were discounted, and the costs of the plants of type "plug plants" (U\$ 0.14 per plant - Pasa nursery), according to planting density for each planting spacing. Thus, it was observed in the two harvests that the spacing of 5 cm was the one that allowed higher net profit (U\$ 56,000 and U\$ 47,000), respectively 278% and 123% higher in relation to the lower planting density for both harvests evaluated. The minimum values were verified for the spacing of 23.2 (2018/2019) and 23.3 (2019/2020) for the evaluated crops. It is recommended planting spacing of less than 23.2 cm between plants to obtain greater net profits, which directs to a density of 51.7 thousand ha<sup>-1</sup> plants.



**Figure 4.** The response of gross income (A) and net profit (B) of strawberry production cv. 'Pircinque', depending on the different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm), in the 2018/2019 and 2019/2020 harvests.

For the survival of any economic activity, it must be profitable to cover the value invested and the labor employed in the production, obtaining the return on investment [60]. Therefore, it should be considered that lower planting densities allow higher productivity and net profit per area. However, the producer must also consider the increased demand for labor because very dense plants require more time in some activities, such as harvesting and other required cultivation practices [61]. Therefore, studies that observe the values of income and net profit under specific conditions provide elements that measure the probability of return for particular investments, thus enabling the correct decision making on the choice of the feasible and appropriate spacing for each cultivation condition.

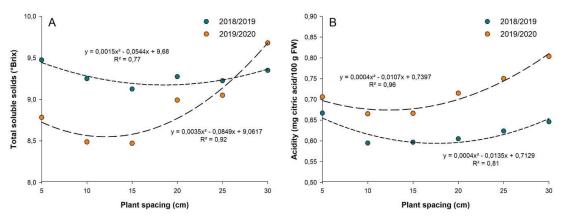
## 2.2.2 Qualitative Parameters

Regarding fruit sweetness parameters, represented by the content of total soluble solids (figure 5A), in the 2018/2019 harvest, a lower concentration of sugars was found in the spacing of 18.1 cm, corresponding to 9.2 °Brix. In the 2019/2020 harvest, although the averages in most of the tested spacing were smaller than those obtained in the previous harvest, the largest tested spacing (30 cm) produced fruits with higher sugar content (9.7 °Brix). It exceeded the averages obtained in the 2018/2019 harvest. For the 2019/2020 harvest, a minimum value was verified in the 12.1 cm spacing between plants, resulting in soluble solids estimated at 8.1 ° Brix.

With the increase of the spacing between plants, there is a higher interception of sunlight, increasing the biomass production, and, consequently, the photosynthetic rate, due to the higher number of leaves, directly affecting the fruits' quality [18]. Besides, this higher sun exposure conditioned by the higher distance between plants raises net photosynthesis levels by plants, which results in a higher accumulation of sugars, responsible in its vast majority for fruits' sweetness [62].

As for the titratable acidity (figure 5B), a slight decrease was observed in the 2018/2019 harvest due to increased spacing. A minimum acidity value (0.60 g 100g citric acid-1) was achieved with the spacing of 16.8 cm between plants. In the 2019/2020 harvest, the lowest acidity value (0.67 g 100g citric acid-1) was found in the spacing of 13.3 cm. However, there was an increase in the averages, with maximum acidity value (0.78 g 100g citric acid-1), obtained in the largest spacing used (30 cm). The sweetness of strawberry fruits is determined by the soluble sugar contents, mainly glucose, sucrose, and fructose. Concerning titratable acidity, its principal constituent is citric acid [63].

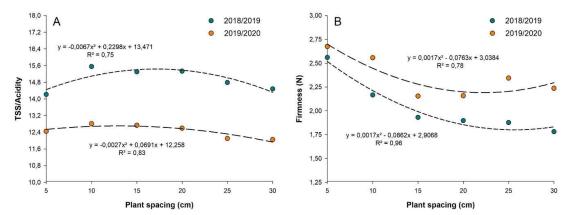
According to [64], as fruit ripening progresses, the content of soluble sugars in fruits tends to increase, and acids' concentration tends to reduce. This process accelerates in conditions of higher sun exposure, provided by the lower plant density. That explains the positive correlation (0.95) obtained between these two variables since a balance of both is necessary to get good-tasting fruits [65]. Positive correlations between these two parameters have already been verified by other authors [66, 67], which proves these compounds' importance in the final flavor composition of strawberry fruits.



**Figure 5.** The response of total soluble solids value(A) and acidity (B) of strawberry plants cv. 'Pircinque', in different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2018/2019 and 2019/2020 harvests.

Regarding the total soluble solids/titratable acidity ratio, increases were observed in different intensities among harvests. (Figure 6A). In the 2018/2019 harvest, the maximum soluble solids and titratable acidity ratio (15.4) were obtained at a spacing of 17.1 cm. In the 2019/2020 harvest, this relation was less intense, with a maximum rate of 12.7, obtained at a spacing of 12.8 cm. The fruits' flavor is mainly determined by the total soluble solids/titratable acidity ratio, so the higher the value obtained for this ratio, the greater the possibility of acceptance of fruits by consumers [68], since it contains one of the main ways of quantifying the fruits' flavor [69]. Thus, in the 2018/2019 harvest, due to the higher sugar levels and lower acidity concentrations, the fruits were sweeter, configuring fruits with better flavor.

For fruit pulp firmness, a significant effect was observed, provided by the different planting spacing. In both crop harvests, the regressions obtained generated minimum point when used approximately 26 cm of spacing between plants in the 2018 / 2019 harvest and 22 cm spacing in the 2019 / 2020 harvest. The average pulp firmness in the fruits harvested in 2018/2019, in turn, according to different spacing, was generally lower than the averages obtained in 2019/2020. We can also notice high firmness values in the smallest spacing tested, spatially 5, 10, and 15 cm, due to the higher production of small fruits in these spacing (Fig.6B).



**Figure 6.** The response of total soluble solids value and titrable acidity ratio (A) and pulp firmness (B) of the fruits of strawberry plants cv. 'Pircinque', in different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2018/2019 and 2019/2020 harvests.

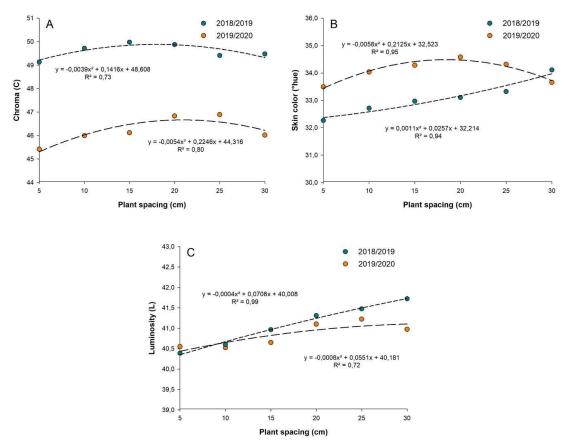
Small fruits have lower cell content, making the cells more compact and rigid, with a less internal dilution of calcium, which allows higher calcium availability for the cell wall composition, increasing its resistance and consequently the firmness of the fruit pulp [52, 70]. However, the pulp firmness correlated positively with the percentage of commercial production. That explains the importance of this parameter in the fruits' quality, which has already been verified in other studies [71]. It highlights the importance of this parameter for obtaining commercial fruits since the pulp firmness is directly related to the strength and durability of fruits, especially during harvest and post-harvest [72].

The lower pulp firmness in fruits is related to the enzyme polygalacturonase's action, stimulated by ethylene's production in the maturation process, responsible for degrading the calcium pectates constituent of the cell wall [73]. In the present study, the decrease in the firmness of the fruits in the larger spacing may be related to the larger fruit size since larger fruits tend to have more expanded cells due to the higher accumulation of water. It ends up diluting the calcium concentrations in the cells, decreasing the firmness of the pulp [52, 70]. Besides, with the higher spacing of plants, the exposure of the fruits to sunlight is higher. It increases the ripening speed, accelerating the degradation of calcium pectates in the cell wall due to ethylene's high production [73].

For evaluating the epidermis saturation or Chroma (Figure 7A), a higher saturation of the fruit epidermis was observed in the first year, and the first and second year averages were 49.6 and 46.2, respectively. For the 2018/2019 harvest, the saturation index's highest response was verified at spacing 18.1 and spacing 20.7 in the 2019/2020 harvest. Smaller spacing than these indicates the production of fruits with less pure coloring. For the epidermis color tone (Figure 7B), the spacing of 20 and 25 cm resulted in fruits with a higher indication of epidermis directed to the red color. In the 2018/2019 harvest, a minimum point trend was observed at 11.6 cm spacing, and for the 2019/2020 harvest, a maximum curve was observed at 18.3 cm spacing. We can conclude that spacing between 11.6 and 18.3 promotes redder fruits' production by these harvests results.

The larger hue angle, along with the luminosity, can result in brighter fruits. Characteristics that make the fruit more attractive and a favorite to consumers, according to [74]. For color brightness (figure 7C), there was a tendency of larger spacing to favor fruit production with a higher solar incidence in both harvests. The lowest results for Chroma, hue angle and luminosity were found in

the 5 cm spacing. A similar description was also found by other studies [75], probably due to the lower incidence of light inside the plants.



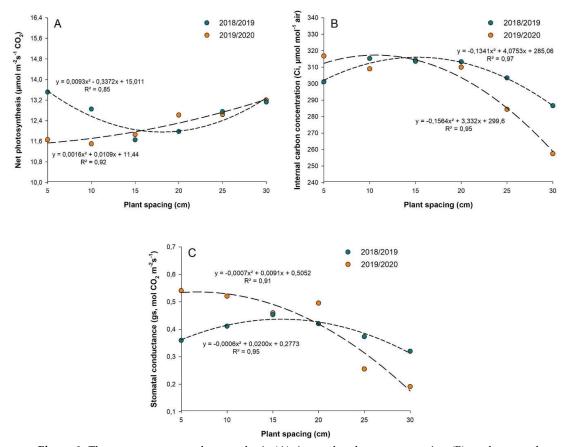
**Figure 7.** The response to Chroma(A), skin color (°hue) (B), and luminosity (C) parameters of strawberry plants cv. 'Pircinque', in different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2018/2019 and 2019/2020 harvests.

## 2.2.3 Gas Exchange

Strawberry plants use approximately 95% of the absorbed water for temperature regulation. They have specialized mechanisms such as the closure of the stomata, which can result in low absorption of carbon dioxide (CO2) [75], leading to a reduction in the flow of water out of the leaf than in the flow of CO<sub>2</sub> that reaches the chloroplasts, thus affecting net photosynthesis (figure 8A) [77,78].

The variable internal carbonic concentration (Ci) (Figure 8B) and stomatal conductance (gs) (Figure 8C) demonstrated similar behavior in both years of cultivation concerning the different spacing. This similarity is because several environmental factors control the stomatal opening. Luminosity, temperature, and carbon dioxide concentration are the most important ones [69]. In this case, the smaller spacing allowed higher average values for both years of Ci (308.9 µmol mol<sup>-1</sup> ar) and gs (0.45 mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), reaching a maximum point at spacing 15.19 cm for the 2018/2019 harvest (figure 8C). Spacing higher than 15.19 cm between plants reduced Ci. It is expected that under conditions of CO<sub>2</sub> limitation, Ribulose oxygenation reactions (RuBP) are initiated, thus affecting the

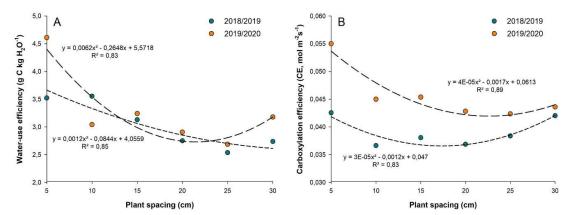
efficiency of the Calvin cycle and consequently reducing the values [69]. The Ci becomes a good indication regarding the physiologic mechanism that plants use to control the temperature being proportional to transpiration [79, 80, 81].



**Figure 8.** The response to net photosynthesis (A), internal carbon concentration (B), and stomatal conductance (C) of strawberry plants cv. 'Pircinque', in different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2018/2019 and 2019/2020 harvests.

Understanding that the assimilation of carbon dioxide from the external environment can promote a loss of water and that this decrease can restrict the entry of CO2 [82]. The water-use efficiency (WUE) indicates that the observed values relate to the amount of carbon that the plant fixes [82], and thus it is verified decline in the rates of WUE, to the planting spacing increment (figure 9A). These are reflections of the increases observed in photosynthesis. Research indicates that microclimate conditions can influence gas exchange, so the decreases recorded in the WUE may be linked to the increases in intercepted solar radiation, that is, in the different spacing microclimate [70].

When there are high Ci and gs values, it can be stated that the carboxylation efficiency (CE) suffers an increase (figure 9B) due to the increased availability of ATP and NADPH and by-products for rubisco [70]. Thus more dense spacing (>20 cm) directly interferes with the availability of CO<sub>2</sub> in the leaf mesophyll, amount of light, temperature, and enzymatic activity so that there is photosynthesis. If the Ci is low, all flow is interrupted due to the absence of essential compounds in the leaf mesophyll cells. To maintain the minimum photosynthesis activities, the plant uses CO<sub>2</sub> from respiration, limiting the whole process [70].



**Figure 9.** The response to water-use efficiency (WUE) (A), carboxylation efficiency (B) of strawberry plants cv. 'Pircinque', in different planting spacing between plants (5, 10, 15, 20, 25, and 30 cm) in the 2017/2018 and 2018/2019 harvests.

## 3.4 Multivariate analysis

When we observe the multivariate analyses, the spacing 30 and 5 cm form different groups and the other treatments (Figure 10). They can be represented by variables such as total soluble solids and titratable acidity for the spacing of 30 cm and pulp firmness, percentage of commercial fruits, and pH for the spacing 5 cm between plants. On the other hand, the 10 and 15 cm spacing form a group represented by small fruits' production and a higher relation between total soluble solids and titratable acidity. The spacing of 20 and 25 cm contributed to forming a fourth group, represented by epidermis coloration variables and productive variables, such as fruit numbers per plant, production per plant, and fresh fruit mass. The variables obtained through gas exchange analysis (IRGA) did not influence the PCA, demonstrating a low contribution to variation indexes.

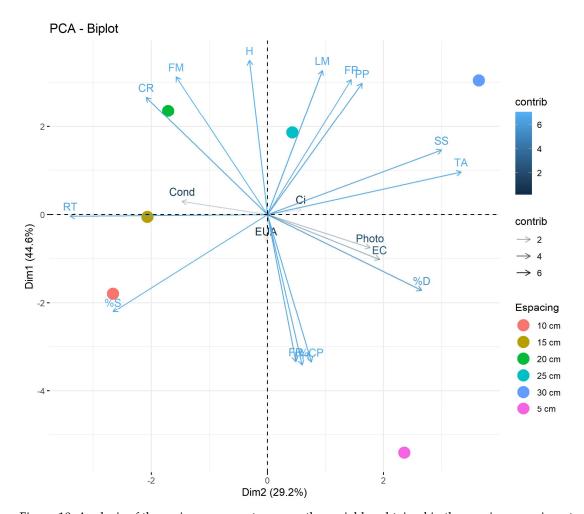


Figure 10. Analysis of the main components among the variables obtained in the spacing experiment for the cultivar "Pirncinque" evaluated in Lages-SC, Brazil in the 2018/2019 and 2019/2020 harvests. Caption: FP = fruits per plant. PP = production per plant. PH = productivity per hectare. FM = fresh mass. "CP = commercial percentage. "S= small fruits percentage. "D=discard percentage. LM = epidermis luminosity. CR = epidermis Chroma. H = epidermis hue angle. FR = firmness. SS = total soluble solids. TA = titratable acidity. RT = soluble solids/titratable acidity ratio.

When using four dimensions, it is possible to explain 97% of the variation obtained in the experiment (Figure 11). Being the first axis (X) with greater representativeness (44.6%), the variables evaluated with more significant influence were: hue angle, epidermis luminosity, pulp firmness, production per plant, Chroma, and total soluble solids, ordered in increasing order by weight in the component. On the other hand, the Y-axis (29.2%) is composed of the variables: titratable acidity, percentage of discarded fruits, stomatal conductance, photosynthesis, pulp firmness, and production per plant.

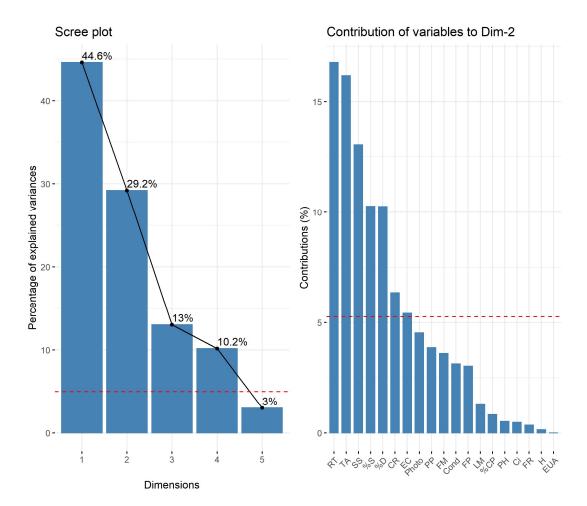


Figure 11. Decomposition of the main component analysis for the six different spacing (5, 10, 15, 20, 25, and 30 cm) in the production and quality of strawberry fruits cv. 'Pircinque'. Caption: FP = fruits per plant. PP = production per plant. PH = productivity per hectare. FM = fresh mass. %CP = commercial percentage. %S= small fruits percentage. %D=discard percentage. LM = epidermis luminosity. CR = epidermis Chroma. H = epidermis hue angle. FR = firmness. SS = total soluble solids. TA = titratable acidity. RT = soluble solids/titratable acidity ratio.

# 4. Conclusions

Based on the study conducted, it is concluded:

Planting spacing of 5 to 15 cm between plants is recommended for growing the Strawberry cultivar Pircinque;

The use of higher plant density allows higher productivity, yield, and net profit;

For higher densities (< 10 cm), the producer may adapt management that allows increasing quality in the fruits produced, which may result in the use of more labor and technologies;

In conditions of lack of workforce and technologies, the producer can opt for larger planting spacing (10 to 15 cm), aiming to balance production and fruit quality.

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administration, funding acquisition, A.A.K., L.R., and G.B. All authors have read and agreed to the published version of the manuscript.

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