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RESEARCH

Assessment of stingless bee densification to improve pollination service: a case study in strawberry cultivation in field conditions

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Background: Pollination is an ecosystem service of utmost importance for agriculture. In this context, the study aimed to evaluate the pollination service of naturally occurring bees and after densification with colonies of stingless bees (*Nannotrigona testceicornis* and *Tetragonisca angustula*) in commercial strawberry plantation. The study was carried out in the four seasons of the year and under two experimental conditions: natural pollination (NP) and pollination under bee densification. The supply of flowers and bee density were estimated. For bee densification, four boxes of colonies of Iraí bees and four of Jataí bees were placed near the strawberry plantation. Five treatments were established for each condition, considering NP and pollination with bee densification on different days from flower anthesis, ranging from the 1st to the 5th day. Fruits were harvested, quantified, and submitted to evaluations to determine biometry, degree of deformity, and percentage of fertilized achenes.

Results: There was a higher density in summer compared to other seasons in both conditions evaluated. The weight was greater (20.19 g) when fruits were pollinated on the first day after floral anthesis under pollination conditions with bee densification. It was observed that free pollination provided fruits with greater a greater of deformation of flowers on the fourth and fifth day after anthesis. However, when bee density became higher, the greatest degree of deformity was observed only in fifth-day fruits. The densification with stingless bees provided a 50% increase in the number of bees present on strawberry plants in summer and also a 15% increase in fruit weight and the occurrence of a lower degree of fruit deformity.

Conclusions: Density with stingless bees provided a 50% increase in the number of bees present on strawberry plant in the summer, with an improvement in fruit quality and aggregate commercial value, providing a 15% increase in fruit weight and a lower degree of deformity under the conditions of densification of Iraí (*N. testceicornis*) and Jataí (*T. angustula*) bees. Under the evaluated conditions, the introduction of stingless bee boxes in the summer is recommended.

Keywords: cv. San Andreas, *Fragaria × ananassa*, *Nannotrigona testaceicornis*, pollination, *Tetragonisca angustula*

Introduction

Ecosystem services guarantee human beings and the environment benefits related to food production, conservation of biological diversity, recovery of agroecosystems and an economic development model that values and preserves these services can contribute to achieving social and economic well-being for more people (CGEE 2017). Among these services, pollination is an ecosystem service of utmost importance for agriculture, contributing to food production in addition to increasing environmental quality ensuring the reproduction and genetic variability of plant species. Insects are among the main groups of pollinating animals, which are considered the main pollinators of the planet's flora, especially bees, which are the main pollinating agents (Inouye 2013).

While bees obtain the nectar and pollen necessary to feed themselves and produce honey, agriculture benefits from pollination, which increases productivity and guarantees higher quality fruits and, consequently, higher market value. Studies have highlighted the great importance of bees in pollinating agricultural crops (Garibaldi et al. 2013; Giannini et al. 2015). Due to their abundance in different ecosystems, bees appear first when it comes to pollinators for agriculture (Klein et al. 2007). According to research in the areas of interaction, pollination, and agriculture, pollinated fruits have a more seeds, greater homogeneity in maturation, more uniform shape, greater nutritional value, and a longer shelf life (Klatt et al. 2014).

Adequate management of pollinators is an alternative to increasing agricultural production and overcoming the pollination deficit in cultivated plants. Some studies have shown the efficiency of stingless bees as pollinators, and genera such as *Geotrigona*, *Melipona*, *Nannotrigona*, *Partamona*, *Plebeia*, *Scaptotrigona*, and *Trigona* were observed pollinating several agricultural crops (Slaa et al. 2006), such as: coffee, avocado, strawberry, pepper, tomato, among others. Stingless bees have great potential for agricultural pollination, are social, and form perennial and populous colonies with a large flow of activity, not posing risks to human health as they have atrophied stinger (Michener 2007). From these perspectives, stingless bees of the species Iraí (*Nannotrigona testceicornis*) and Jataí (*Tetragonisca angustula*) are promising pollinators for different agricultural crops, promoting an increase in fruit production and quality (Malagodi-Braga and Kleinert 2004; Silva et al. 2020). In Brazil, some studies with Iraí have been published, for example, on pollination in protected cultivation for cucumber crops (Nicodemo et al. 2013; Santos et al. 2008). In strawberry cultivation, Roselino et al. (2009) observed that, in protected cultivation, the quality of fruits improved. The same authors also reported the long time spent visiting flowers to collect pollen and nectar, favoring the pollination process. According to Lorenzon and Morado (2014), the Jataí bee is of paramount importance for several crops, citing is as a forager of mango, guava, ingazeiro, among others. Ferreira et al. (2020) observed that in all climatic seasons of the year, Jataí bees foraged on plant species in diverse vegetable crops, various species of fruit trees and medicinal, spicy, and aromatic plants, among other species, which flowered in the climatic season. autumn, followed by winter, spring, and summer.

The strawberry fruit is mainly sold fresh, and because of this, the market becomes more demanding with its quality every day. However, to obtain good quality strawberries and to guarantee good productivity, pollination by bees is an important factor in strawberry production (Malagodi-Braga 2018). The study by Klatt et al. (2014) found in nine strawberry cultivars that flowers pollinated by bees resulted in fruits with high commercial value, with bee pollination increasing on average the commercial value per fruit by 38.6% compared to wind pollination and by 54.3% compared to self-pollination. In this way, the commercial valuation of strawberry fruits for the fresh market is directly related to the pollination service.

In this context, the study aimed to evaluate the pollination service of naturally occurring bees and after densification with colonies of stingless bees (*N. testceicornis* and *T. angustula*) in commercial strawberry plantation.

Material and Methods

Characterization of the study area

The study was carried out in the municipality of Barra do Choça, in the "Planalto da Conquista" region, with latitude 14° 52' south and longitude 40° 34' west and an average altitude of 900 m above sea level (City Hall of Barra do Choça 2020).

Experiments were carried out on a commercial planting farm with semi-organic management (use of fertigation whenever necessary) in an open field under the protection of low strawberry tunnels of the 'San Andreas' cultivar (planted area of 30 m × 30 m). Assessments were developed in the four seasons of the year 2022: summer (January–February), autumn (April–May), winter (July–August), and spring (October–November). Being applied over thirty days of each season, with the same conditions for all treatments, with the following climatic data obtained from the Meteorological Station of the State University of Southwest Bahia (Fig. 1).

Density of flowers and bee density

The density of flowers was estimated by counting the number of open flowers/m^2 and bee density by counting the number of bees in 100 flowers of planting, based on the FAO protocol (Vaissière et al. 2011), being in four plots on strawberry flowers before and after the installation of the colonies. Observations were carried out every day of field

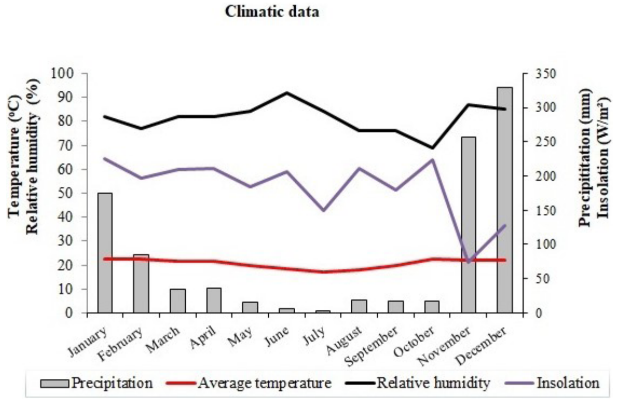

Fig. 1 Climatic data (temperature [ºC], relative humidity [%], precipitation [mm] and insolation [W/m^2]), generated by the meteorological station at the State University of Southwestern Bahia in the year 2022.

observation with four randomized replicates.

Densification of stingless bees

Four boxes of populated colonies with approximately 2,000 individuals each of Iraí bees (*N. testceicornis*) and four boxes with approximately 2,000 individuals each of Jataí bees (*T. angustula*) were placed close to the strawberry plantation (5 m). The eight bee boxes were inserted into the strawberry plantation, where all treatments were applied, so that all treatments that were evaluated on this plantation were exposed to the same environmental conditions and bee density. After placing the colonies, 15 days were allowed for them to adapt to the field, and only after this period experimental evaluations carried out.

Bee colonies were provided by the Universidade Estadual do Sudoeste da Bahia (UESB) Beekeeping and Meliponiculture Sector. These species occur naturally in the region and are easily managed in rational boxes.

Selection of flower buds for pollination

Flower buds were randomly chosen for all treatments, with only primary buds of inflorescences being selected one day before their anthesis, which were protected with voile fabric. On the day of each treatment, protection was removed, leaving flowers available for visitors for 24 hours and protected again according to treatments.

Pollination experiments

Treatments were carried out in beds with two rows and eleven tunnels, using a completely randomized experimental design with six replicates each.

In the first experiment, under natural planting conditions, five treatments were carried out with natural pollination (NP), varying pollination in relation to the day of floral anthesis, organized as follows: NP1 (1st day of anthesis); NP2 (2nd day of anthesis); NP3 (3rd day of anthesis); NP4 (4th day of anthesis); and NP5 (5th day of anthesis).

In the second experiment, with densification of Iraí and Jataí bees, five treatments similar to the first treatment were carried out, also varying the day of floral anthesis: pollination with bee densification (PBD) - PBD1 (1st day of anthesis); PBD2 (2nd day of anthesis); PBD3 (3rd day of anthesis); PBD4 (4th day of anthesis) and PBD5 (5th day of anthesis).

Harvest and evaluations

Fruits resulting from treatments were harvested and quantified when 75% of their external surface was red and fully ripe. Fruits were taken to the Laboratory of Semiarid Biodiversity at the UESB in order to determine their physical parameters.

Evaluations were carried out on the same day of harvest, and the biometry of fruits was determined based on the following parameters: fresh mass (g), longitudinal length (mm), and equatorial diameter (mm).

Fruits were classified into categories, according to the degree of deformation, adopted by standards of the Brazilian Program for the Modernization of Horticulture and Integrated Strawberry Production (PBMH, PIMo 2009): well formed (0), mild deformation (up to 30%), medium deformation (between 30% and 60%), and severe deformation (above 60%). At the end, fruits were separated to determine the fertilization rate of achenes using the methodology proposed by Thompson (1971), which consisted of removing achenes with the aid of tweezers and placing them in containers with water to determine their buoyancy capacity. Achenes that sank in an aqueous solution were considered viable, and those that floated were considered non-viable.

The percentage of fertilized achenes (PFA) was calculated according to the formula:

$$PFA = (\text{number of fertilized achenes} / \text{number of achenes per fruit}) \times 100.$$

Statistical analysis

Data from the different evaluations (supply of flowers, bee density, fruit biometry) were submitted to normality and homogeneity tests, proceeding to analysis of variance. Subsequently, these variables had the means of their treatments compared using the Scott Knott test at 5% probability, using the statistical software SISVAR version 5.8. The percentages of fruit formation, deformation, and fertilized achenes were analyzed using the chi-square test with a probability of less than or equal to 5% using the PAST software (Hammer et al. 2001).

Collectively, data were submitted to principal component analysis (PCA) using the XLSTAT® software version 19.2.2 (Addinsoft, Paris, France) to determine the relationship between variables (bees, weight, diameter, length, PFA, and degree of deformity) and different treatments.

Results

Density of flowers and bee density

The supply of flowers varied throughout the seasons, with autumn and winter having the highest average supply (average values above 20 flowers/m^2), and in the summer, the supply was the lowest (average values below 10 flowers/m^2) (Table 1).

Regarding the presence of bees in flowers, there was a higher density in summer compared to other seasons in both conditions evaluated, with an increase of 50% in the density of bees in flowers after the introduction of boxes in the cultivation area (Table 1).

Considering that only summer showed a significant increase in the number of bees, the parameters of fruits from

Table 1 Average number of flowers (m²) and bees (100 flowers) in the cultivation of the strawberry cultivar ± standard deviation, under natural conditions and density bees

Seasons	Flowers (m²)		Bees (100 flowers)	
	No densification	Densification	No densification	Densification
Summer	8.25 ± 3.77^{aB}	9.55 ± 4.15^{aC}	16.35 ± 7.49^{bA}	24.55 ± 6.06^{aA}
Autumn	21.60 ± 7.67^{aA}	22.75 ± 12.08^{aA}	1.05 ± 1.43^{bB}	1.35 ± 1.82^{bB}
Winter	20.15 ± 6.88^{aA}	19.40 ± 11.82^{aAB}	1.15 ± 1.14^{bB}	4.30 ± 0.81^{bB}
Spring	11.65 ± 3.10^{aB}	12.45 ± 4.57^{aBC}	1.95 ± 1.27^{bB}	3.95 ± 5.23^{bB}

Degree of freedom = 39.
Averages followed by the same letter, lower case in rows and upper case in columns, do not differ from each other according to the Scott Knott test at 5%.

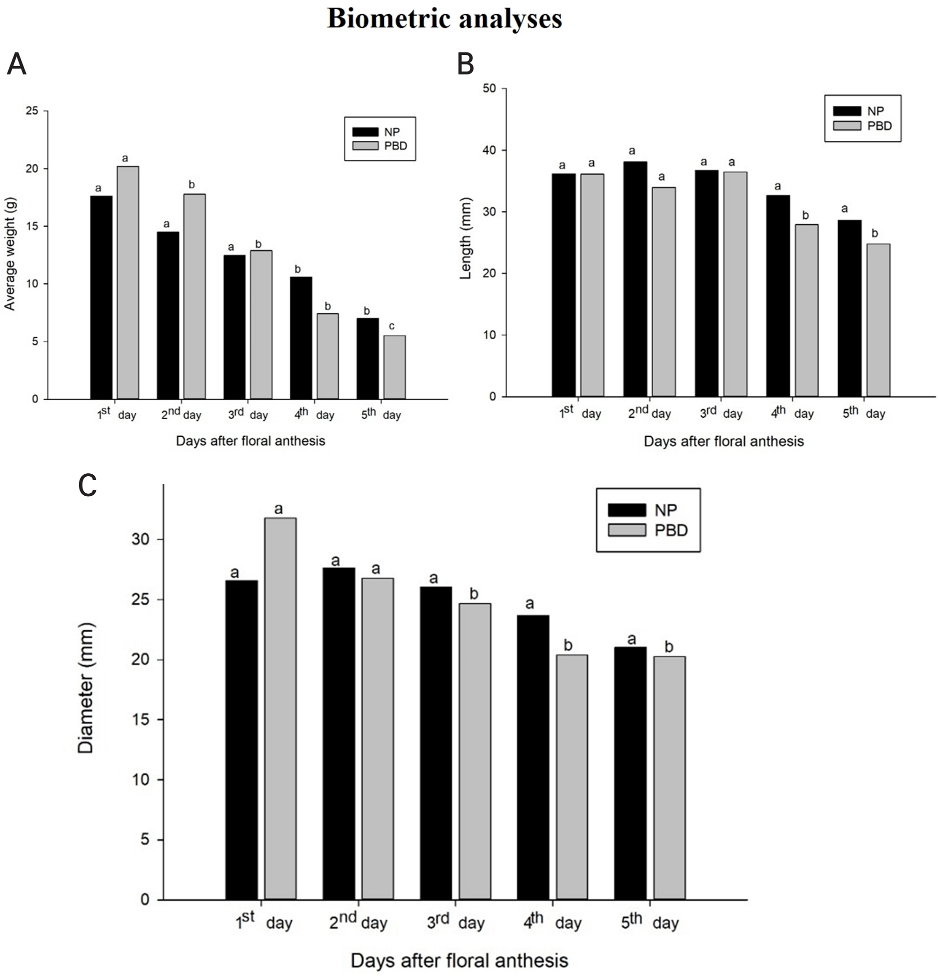

Fig. 2 (A–C) Biometric analyses (weight, length, and diameter) of strawberry fruits 'San Andreas' cultivar resulting from natural pollination (NP) and pollination with bee densification (PBD). Barra do Choça- Ba.

different pollination conditions were exclusively evaluated in this season.

Fruit evaluation

The amount of fruits formed in the different treatments depending on floral anthesis did not differ ($\chi^2 = 6.91^{ns}$).

Considering the physical characteristics of fruits, it was observed that the weight was greater (20.19 g) when fruits were pollinated on the first day after floral anthesis under pollination conditions with bee densification, being higher than the other treatments (Fig. 2A). For pollination under natural conditions, the highest average weights occurred on the first, second, and third day after anthesis, with averages of 17.62 g, 14.48 g, and 12.46 g respectively (Fig. 2A), showing increase of 15% in fruit weight only when pollination occurred on the first day after anthesis with bee densification.

For variables length and diameter, no differences between days under NP conditions were observed, while under densification conditions, fruits from the first, second, and third days after floral anthesis showed higher average length values. For diameter, only fruits from the first and second days were superior.

Regarding the degree of fruit deformity (DFD), there was a difference between treatments ($\chi^2 = 113.57$, $p < 0.0001$). It was observed that free pollination provided fruits with a

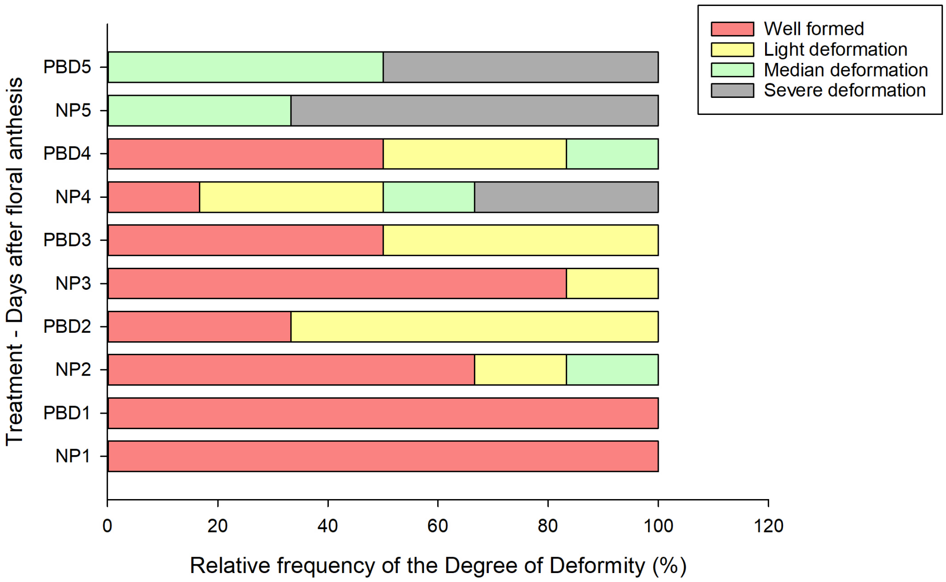

Fig. 3 Relative frequency with different degrees of deformity of strawberry 'San Andreas' cultivar resulting from the five days of flower after anthesis under natural pollination (NP) and pollination with bee densification (PBD). Barra do Choça- Ba.

greater degree of deformation of flowers on the fourth and fifth day after anthesis (Fig. 3). However, when bee density increased, the greatest degree of deformity was observed only in fifth-day fruits.

Percentage of fertilized achenes

The average PFA showed that, under NP conditions, the first and second day of flower presented higher percentages (86% and 85%, respectively), not differing from each other. In PBD, the days with the highest values were the first, second, and third (90%, 90%, and 85%).

Joint analysis

The joint analysis of data using PCA showed that bees and flowers were responsible for the variation along principal component 1 (PC1) and explained 72.57% of the dissimilarity (Fig. 4). Variables weight, diameter, length, DFD, and PFA were the attributes most associated with main component 2 (PC2), which explained 19.80% of the variation between treatments.

The graphic dispersion provided by PCA was presented in two dimensions and showed that the biometric parameters of fruits are associated with days of pollination after anthesis, being more influenced on days 1, 2, and 3 (NP1, NP2, NP3, PBD1, PBD2, PBD3). The degree of deformation is more correlated to the fifth day after anthesis, and PFA is correlated to the first day of pollination after anthesis with densification (PBD1). The number of bees is correlated with bee density.

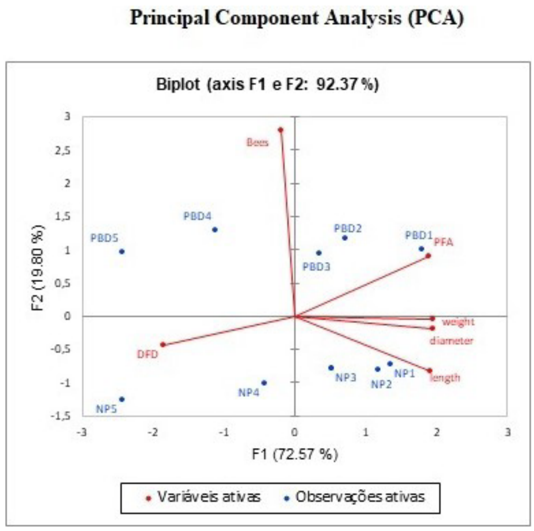

Fig. 4 Ordination diagrams produced by the analysis of the main components (bees, weight, length, diameter, percentage of fertilized achenes [PFA], and degree of fruit deformity [DFD]) of treatments with natural pollination (NP) and pollination with bee density (PBD) in strawberries and characteristics of the fruits obtained.

Discussion

Strawberry cultivation showed variations in the supply of flowers in the year of evaluation, with more flowers in autumn and winter, months where the lowest temperatures occurred under the study conditions. The 'San Andreas' cultivar is considered a photoperiod-neutral cultivar that flowers at temperatures varying between temperatures equal to or below 10°C and above 28°C (Costa et al. 2014). However, as observed by Taylor (2002), strawberry crops behave better at daytime temperatures around 18°C, corroborating our results.

The density of bees in flowers was influenced by seasons, marked by the semiarid climate characteristic of the region, assuming an effect of temperature, sunshine, and precipitation on the foraging of individuals. The influence of climatic factors on the flow of external activity of bees has been addressed in different species, and there is evidence of positive modulation in foraging intensity with temperature and light intensity (Alves et al. 2015; De Bruijn and Sommeijer 1997; Palma et al. 2008; Silva and Gimenes 2014). Under the conditions of this study, there was bee activity in the different seasons, but with a significant reduction in the colder seasons.

To ensure the ecosystem service of pollination, it is essential to maintain colonies/boxes close to crops and with good density. Some crops are especially attractive to bees, such as soybeans, sunflowers, coffee, cotton, strawberries, citrus, peaches, pumpkins, melons, and many others. To achieve good pollination, the number of colonies/boxes in the cultivation area must be high, ranging from 4 or 6 colonies/boxes per hectare of *Apis mellifera*, according to the needs of the target crop and climatic conditions (Wolff et al. 2022). In the present study, it was decided to add eight colonies in order to guarantee greater success in pollination and because they are two different species (Jataí and Iraí) and smaller in size (one of each).

In the state of Espírito Santo, Ferreira et al. (2020) observed that in all seasons of the year, Jataí bees foraged on plant species in diversified vegetable crops, various species of fruit trees, and medicinal plants, among other species, which flowered in the climatic season of autumn, followed by winter, spring, and summer.

In the same sense, Alves et al. (2015) found that the Iraí species forages even on cold days, but they return from the field earlier and quickly close the nest entrance, while in hot seasons of the year, characterized by high temperatures and long light periods, they forage for longer, delaying their return to the nest until after sunset.

Pollination services associated with bee behavior in flowers are important in strawberry cultivation, especially when pollination is favored on the first day of floral anthesis. There was not only an increase in fruit weight, but also in the proportion of fruits with a lower degree of deformation and greater fertilization of achenes. The behavior of visits by bees of different species to flowers results in a uniform distribution of pollen on pistils, producing well-formed and heavier fruits (Scheid et al. 2020). The pollination service by Jataí and Iraí bees provides a 15% increase in weight, which contributes to the productive potential of the cultivar. In cultivars such as 'Oso Grande' and 'Sweet Charlie', Jataí bees (*T. angustula*) demonstrated pollination effectiveness, in addition to reducing the percentage of deformed fruits, showing an increase in fruit weight (Malagodi-Braga 2018). Thus, like Iraí bees (*N. testceicornis*), which in the study by Silva et al. (2020), visits of bees also provided an increase in weight for the same cultivar as in the present work.

It is noted that increasing the mass of the strawberry receptacle does not necessarily result in a proportional increase in the diameter and length of the fruit. This suggests that fruit development can occur independently in different aspects, such as size and weight. This discovery could have important implications for understanding the growth and development processes of strawberries, as well as influencing cultivation practices and variety selection.

This relationship can vary depending on several factors, such as the variety of strawberry, the growing conditions, the environment in which they are grown and the type of pollination that occurred. In a similar study evaluating the performance of strawberry cultivars subjected to different types of pollination in protected cultivation, Witter et al. (2012) also found no correlation between fruit weight and diameter, where the fruit with the greatest weight was not always the one with the largest diameter.

The different analyses of the results show that the greater or lesser degree of deformation of fruits is associated with the period of exposure of the flower after anthesis, and the later the flower is pollinated, the greater the degree of deformation. Deformations in strawberry fruits are due to poor pollination through unfertilized stigmas in the basal, lateral, and apical regions of the floral receptacle (Ariza et al. 2011). Thus, it could be considered that pollination in the first days of floral anthesis provides uniform fertilization of stigmas, causing homogeneous growth of the floral receptacle, resulting in well-formed fruits.

It was observed that bee densification in the area extended until the fourth day of flower opening for pollination to occur, when fruits were 83.33% suitable for the fresh consumption market, as they were well formed.

On the fifth day, both under NP and under PBD, flowers showed higher rates of serious deformation, being considered unsuitable for the fresh consumption market (66.66% and 50%, respectively). This was probably due to the flower opening time and the fact that they were bagged, when self-pollination may have occurred, resulting in a high frequency of fruits with serious deformations. Corroborating results were found by Zapata et al. (2014), in which, even though the strawberry flower was considered self-fertile, self-pollination was not able to distribute the pollen evenly among stigmas, a fact that gave rise to deformed fruits.

Deformities occur when the achenes are not fully fertilized, the receptacle does not develop completely and presents different degrees/levels of deformation. Therefore, the complete development of the floral receptacle is associated with the fertilization of all pistils (Malagodi-Braga 2018). Scheid et al. (2020), in a study on the effectiveness of bees as strawberry pollinators, reported that the 'San Andreas' cultivar was dependent on the biotic pollination of bees, showing a significant increase in the fertilization of achenes with visits carried out with different species. In the present study, the parameter PFA indicated that on the first and second days, there was a higher rate in both pollination conditions (NP and PBD), and on the third day with bee densification, the increase of the first days was maintained, assuming that the presence of bees favors the fertilization of achenes for another day of open flower.

The results obtained by the graphic dispersion given by

PCA suggest that variations in biometric characteristics are associated with the day of pollination after anthesis, and the bee densification promoted a greater presence of bees in the field. Bees present in natural conditions in the environment may not be sufficient for an ecosystem pollination service, and the introduction of stingless bees can provide great benefits. Albano et al. (2009) recommend that, to ensure year-round strawberry pollination, strawberry growers should take advantage of various pollinators, such as native bees, honey bees, and flies, to prolong pollination activity and increase the resilience of the pollinator community against environmental changes. Although in Brazil renting beehives (migratory beekeeping) is not a common practice, considering that in the tropical climate there is a greater number of pollinators, such as coleopterans, dipterans, and others, there has been a growing interest among rural producers in using bees to increase productivity (Salomé and Orth 2014; Vieira et al. 2010).

In general, the results confirm that the bee pollination service provides quality improvements in terms of fruit formation, reducing the degree of deformation of pollinated strawberry fruits up to the fourth day of the summer season, being considered viable for the fresh consumption market.

For the other seasons (autumn, winter, and spring), complementation with other species of pollinators with different body sizes and foraging behaviors is suggested, which can contribute to guaranteeing production, minimizing pollination deficits, and thus, increasing crop yield.

The use of stingless bees in family farming offers a series of significant advantages. In addition to the low initial investment requirement, these bees are less aggressive compared to traditional honey bees, which reduces the need for protective equipment. This makes them especially accessible to small farmers, who often have limited resources. The honey produced by these bees generally has a high added value due to its quality and its appeal as a natural and sustainable product. This can represent an additional source of income for family farmers, whether through the direct sale of honey or derived products such as propolis, royal jelly, and wax (Magalhães and Venturieri 2010). There are a growing number of studies showing the benefits of using stingless bees in agriculture (Jalil and Roubik, 2017; Quezada-Euán 2018). However, it is necessary to the practices and management of the activity to transform it into a development tool, making it more productive and increasing the income of creators (Jaffé et al. 2013).

In short, the use of stingless bees in family farming is a promising strategy that combines economic, ecological, and social benefits, contributing to the sustainability and development of rural communities. It is an easy-to-manage and low-cost activity that can be found in different parts of the world (Cortopassi-Laurino 2017).

Conclusions

Density with stingless bees provided a 50% increase in the number of bees present on strawberry plant in the summer, with an improvement in fruit quality and aggregate commercial value, providing a 15% increase in fruit weight and a lower degree of deformity under the conditions of densification of Iraí (*N. testceicornis*) and Jataí (*T. angustula*) bees.

Under the evaluated conditions, the introduction of stingless bee boxes in the summer is recommended.

Abbreviations

NP: Natural pollination
PBD: Pollination with bee densification
PFA: Percentage of fertilized achenes
PCA: Principal component analysis
DFD: Degree of fruit deformity

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Author's contributions

PSM and RPM conceived the idea designed in the study. PSM, ZCML, and RPM contributed to preparing, quantifying the samples, and conducting field experiments. AAM, PHMM, and RPM proofread the manuscript. All authors wrote the article and approved the final version of the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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