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ANIMAL PRODUCTION

Stingless bee *Trigona spinipes* (Hymenoptera: Apidae) behavior on chayote flowers (*Sechium edule*)

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ABSTRACT. This experiment aimed to evaluate the forage species and their effect on fruit production of the chayote crop. For this, the culture was under observation in the first ten minutes at each time, from 7:00 am to 6:00 pm, with three repetitions, in each year studied (1994, 2001, 2009 and 2016). With the exception of 2009, *Trigona spinipes* stingless bees were frequent and constant insects in these flowers between 8:00 am and 1:00 pm, visiting mainly male flowers for nectar collection and due to foraging behavior this stingless bee may be considered a pollinator of the chayote.

Keywords: chayote; forage behavior; irapuá; pollination.

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Introduction

Bees are essential for the maintenance of native plant populations and for the production of human and animal food, as they provide the relevant ecosystem service resulting from pollination (Imperatriz-Fonseca & Nunes-Silva, 2010). The intimate bee-flower association began more than 50 million years ago and, since then, bees have depended on flowers to obtain substances used in their food, in the construction of their nests and other purposes; plants benefit when pollinated, or are sometimes harmed when bees plunder resources without pollination (Imperatriz-Fonseca, 2010, Nascimento, Gomes, Batista, & Freitas, 2012).

Reilly et al. (2020) reported that most of the world's crops depend on pollinators, therefore declines in honey bee *Apis mellifera* and wild bees raise concerns about food security, and assessed the extent of pollinator limitation in seven plantations in 131 locations, located in the main areas of production in the United States, including: apples, cherries, watermelon and pumpkins. They found that five out of seven plantations showed evidence of pollinator limitation and even estimated the annual pollinator production value for those plantations at $1.5 billion. According to the authors, the value of pollination by bees native to all cultures dependent on pollinators would be much greater.

Around 300 species of meliponines are known with a diversity of shapes, size and nesting habits in Brazil (Pedro, 2014). *Trigona spinipes* (Hymenoptera: Apidae, Meliponini) known as irapuá or arapuá are social stingless bees that form perennial colonies with hundreds to thousands of workers. These bees are considered abundant and found in most parts of Neotropical America, from the Brazilian state of Rio Grande do Sul to Mexico, as well as Australia, Indonesia, Malaysia, India and Africa. The wide distribution and large population of nests are reflected in their presence in many plants including cultivated ones (Silveira, Melo, & Almeida, 2002, Costa, Menezes, & Soares, 2012).

They are often considered pests as they have the habit of destroying parts of cultivated plants, such as flowers, leaves, stems and fruits with their jaws (Boiça Junior, Santos, & Passilongo, 2004; Azeredo, Lima, & Cassino, 2006). They can act as plunderers of resources stealing pollen or nectar without pollinating the plants (Kill & Siqueira, 2006).

The *T. spinipes* stingless bees uses resinous substances extracted from passion fruit flowers (*Passiflora edulis f. flavicarpa*) and sometimes from the stem in the construction of its nest and perforates the nectariferous chamber removing the nectar before opening the flowers, making them less attractive to carpenter bees. For this reason, their nests have been destroyed by farmers who consider them harmful to their crops (Malerbo-Souza, Nogueira-Couto, & Toledo, 2002). However, stingless bees species including *T. spinipes* are protected in Brazil by the environmental crimes law (Law no. 9,605, February 12, 1998), making no chemical control or nest destruction possible.

On the other hand, Cardoso et al. (2007) reported that the behavior of stealing nectar from these bees could indirectly contribute to increased visitation of effective pollinators in cotton (*Gossypium hirsutum latifolium*) suggesting that other floral visitors (and pollinators) would have to visit more flowers to get the same volume of nectar. In addition, experiments carried out to evaluate the effect of perforation on flower buds carried out by *T. spinipes* stingless bees showed that, despite being perforated, the flower buds turned into fruits, in the same proportion as unperforated buds, both in passion fruit flowers (Malerbo-Souza et al., 2002) and Orange flowers (*Citrus sinensis*) (Malerbo-Souza & Halak, 2013) not interfering in fruit production.

T. spinipes stingless bees have been observed in many agricultural crops collecting nectar and/or pollen without causing harm and were considered an important pollinator and can even be used as a commercial pollinator (Hickel & Ducroquet, 2000). *T. spinipes* were important in the pollination of free-range pumpkin (*Curcubita mixta*) (Lattaro & Malerbo-Souza, 2006) and Italian pumpkin (*C. pepo*) (Malerbo-Souza, Andrade, Medeiros, Silva, & Siqueira, 2019). No studies were found that evaluated the foraging behavior of *T. spinipes* in the pollination of the chayote, thus, we hypothesize that these bees are important in the pollination of this cucurbit crop.

Therefore, the objective of this work was to evaluate the foraging behavior of *T. spinipes* stingless bees, its interaction in the presence of other pollinating species and effect on fruit production in chayote culture in Jaboticabal and Ribeirão Preto, São Paulo state, Brazil.

Material and methods

The present experiment was carried out in different locations and different years. The first, in 1994, was conducted in chayote (*Sechium edule*) crop located in an experimental area, in the Beekeeping Sector of the Faculty of Agrarian and Veterinary Sciences, Campus de Jaboticabal/UNESP, whose altitude is 595 meters, with the following coordinates areas: 21°15'22" south latitude and 48°18'68" west longitude, with temperate subtropical climate and average annual temperature around 21°C. The average annual rainfall is 1,451.2 mm^3. In 2001, 2009 and 2016, the experiments were conducted in an experimental area of the Department of Agrarian Sciences, of the Moura Lacerda University Center (CUML), in Ribeirão Preto, SP, whose altitude is 620 meters, with the following geographic coordinates: 21°10'04” south latitude and 47°46'23” west longitude, with temperate subtropical climate and average annual temperature around 21°C and average annual rainfall of 1500 mm^3. These cities belong to a region where large areas of sugar cane plantations (*Saccharum officinarum*) are concentrated.

All crops were planted on vines and the cultures were under observation throughout the flowering period and, in order to observe the development of the flowers, they were marked with colored lines, and 100 flowers (50 male and 50 female) were monitored, from bud to wilting. The relationship between male and female flowers was obtained by counting the open flowers at 12:00 with four repetitions (different days) in each year.

Three specimens of each of the species of insects visiting the culture were collected. These insects were preserved in 70% alcohol, properly labeled, and identified by comparison in an entomological collection. In each studied year, the frequency of visits and the type of collection (nectar and/or pollen) of these insects during the day were evaluated. These data were obtained through counting, in the first 10 minutes of each time, from 7:00 am to 6:00 pm, with three repetitions (three different days). This counting was performed by walking under the vine for 10 minutes at each time, noting the insects present in the flowers and what they were collecting, according to the methodology of Nogueira-Couto, Pereira, and Couto (1990). The foraging behavior of each insect species and the interaction between these species were evaluated through visual observations during the day in the experimental period.

The number of observed insects was used to estimate the number of insects in flowers in one hour. The constancy (C) of these insects was obtained using the formula: $C = (P \times 100) N^{-1}$, where P is the number of collections containing the studied species and N is the total number of collections carried out (Silveira-Neto, 1976).

In 1994 and 2001, the percentage of fertilization and fructification was evaluated, marking 30 female flower buds, with three repetitions in each year, which were followed until fruit formation.

All data were statistically analyzed using the BIOSTAT program. For the comparison of means, when necessary, the Tukey test was used at the level of 5% probability. To analyze the frequency of insect visitation to flowers during the day, regression analysis by orthogonal polynomials was used, thus obtaining adequate equations for the observed patterns, under the conditions of the experiment.

Results and discussion

In both experiments, the female and male flowers (Figure 1) lasted, on average, 2 ± 0.5 days, from opening until wilting, and consequent or not, fruiting. At the end of the 2nd day, the male flowers were withered and beginning to fall and the female ones with the withered flowers and the fruit starting to grow or withering too.

Figure 1. Female and male flower, respectively, of the chayote crop (*Sechium edule*), in Jaboticabal, SP (Photos: personal archive).

The occurrence of a greater number of female flowers (84.2%) compared to male flowers (15.8%) was observed, and the ratio was 5.33:1, in 1994, in Jaboticabal, SP. Many *Trigona spinipes* stingless bees were observed in the chayote sprouts collecting resins and causing injuries. Many flower buds fallen to the ground were also observed, however, these bees were not observed piercing or cutting the flower buds. In 2001, 2009 and 2016 the relationship was inverse, there were in the experimental areas, on average, 11.9 male flowers for each female flower. Nicodemo, Couto, Braga, and De Jong (2007) observed 2.2 male flowers for each female flower in a pumpkin crop (*Cucurbita maxima*). Malerbo-Souza et al. (2019) studying the Italian pumpkin (*C. pepo*) for three years (2013, 2014 and 2015) found a ratio of 3.2:1, on average.

These data demonstrate the diversity of this relationship between species, which can be attributed to various factors such as edaphological conditions, climatic conditions and morphological differences between plant species. In addition, the difference observed in the four years studied (1994, 2001, 2009 and 2016), was that in 1994, there were more female flowers and, in other years, more male flowers, which is more common in cucurbits, which may be explained by the nutritional status of the vine. In 1994, the vine had received an application of foliar fertilizer and fungicide, as it was not supporting the fruit load, where many male flower buds had fallen to the ground. However, this application of fertilizer and fungicide was carried out in the late afternoon, not affecting insect visitation.

Regarding visiting insects only *T. spinipes* stingless bees were observed in the flowers of the chayote collecting nectar in 1994, in Jaboticabal, SP (Figure 2). It was noted that these bees preferred to collect nectar from female flowers (73.1%) to male flowers (26.9%). And, despite the male flower providing nectar and pollen, only nectar collection was observed. It was observed that *T. spinipes* stingless bees collected resin from the chayote shoots, that is, at the ends of the branches, placing it in the corbicles, and up to 15 bees were observed in the same shoot.

T. spinipes stingles bees frequented the flowers until 14:00, although these flowers were open all day, and remained in the flower for a long time (on average, 5.3 ± 1.2 minutes), collecting nectar. Despite the crop being planted very close to the hives of Africanized honey bees *Apis mellifera*, at around 50 meters, their presence was not observed in chayote flowers. Chayote flowers are probably not attractive to these honey bees, in this case, and the presence of other food sources in the surroundings was observed, for example, love clawed or meddler (*Antigonum leptopus*) and cosmos (*Cosmos sulphureus*), in addition to the small number of female flowers open, in the chayote crop, due to the nutritional status of the plant.

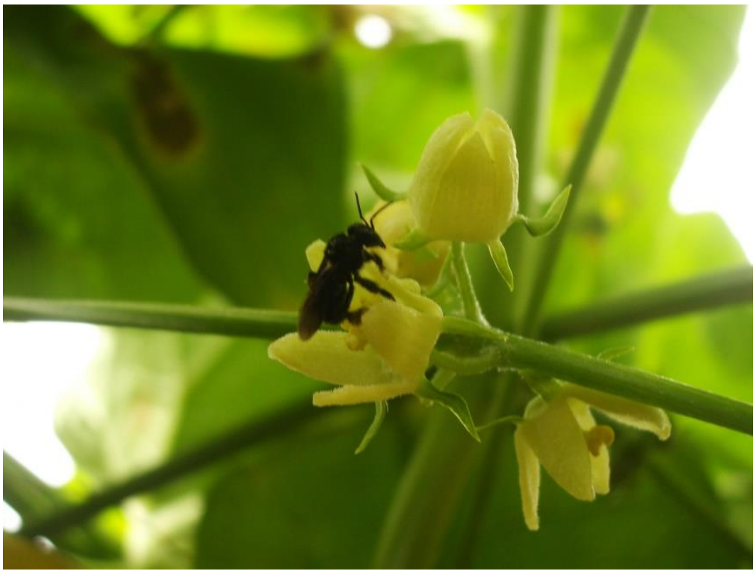

Figure 2. Stingless bee *Trigona spinipes* in a male chayote flower (*Sechium edule*) in Ribeirão Preto, SP (Photo: personal archive).

In 2001, in Ribeirão Preto, SP, *T. spinipes* stingless bees (86.8%), Africanized honey bees *A. mellifera* (6.4%), vespids (4.5%), and *Cyclonela sanguinea* coleopteran (ladybug) (0.8%), *Diabrotica speciosa* (0.5%), *Chloralictus sp* stingless bees (0.5%), *Xylocopa* sp carpenter bees (0.3%) and *Leptoglossus* sp (bedbug) (0.2%) were observed. Insects collected mainly nectar and, from 4:00 pm onwards, they were no longer observed in the culture. *T. spinipes* stingless bees were more frequent between 7:00 am and 1:00 pm visiting mainly the male flowers to collect nectar taking on average, 51.6 ± 3.8 seconds. *T. spinipes* stingless bees visited chayote flowers from 7:00 am to 2:00 pm, increasing their frequency until 10:00 am, then decreasing, according the second- degree equation: $Y = -0.5184x^2 + 3.5355x - 26.947$ (R^2=0.8529) (Figure 3).

In 2009, in Ribeirão Preto, SP, the only insect observed in the chayote flowers were ants, with 60% of the visits being in the male flowers and 40% in the female flowers. The ants visited the male flowers from 8:00 am to 5:00 pm, increasing their frequency until 2:00 pm, then decreasing it, according thesecond-degree equation: $Y = -0.5606x^2 + 57.3242x - 5.8$ (R^2=0.8951) (Figure 3). For female flowers, the ants visited the flowers from 8:00 am to 4:00 pm, showing no pattern of frequency, the number of visits varying throughout the day. Possibly, *T. spinipes* stingless bees were in more attractive food sources than chayote flowers, or their nests may have been destroyed by producers, a common practice, as many consider these bees as crop pests, without knowing their potential as a pollinator. In addition, there may have been visits by bees at times that were not being measured.

In 2016, ants (50.0%), *T. spinipes* stingless bees (29.1%), *Tetragonisca angustula* stingless bees (29.10%), *Plebeia* sp. stingless bees (10.3%), Africanized honey bees *A. mellifera* (0.6%) and bed bugs (0.6%) were observed. The *T. spinipes* stingless bees preferred to visit the male chayote flowers between 7:00 and 11:00 and the female flowers between 12:00 and 14:00, and were no longer observed in the culture after that time (Figure 3). The *T. spinipes* visited the male chayote flowers from 7:00 am to 2:00 pm increasing their frequency until 10:00 am then decreasing, according the second-degree equation: $Y = -0.1012x^2 + 0.3135x + 3.2857$ (R^2=0.7767). The frequency of these bees in female flowers followed another pattern, decreasing until 9:00 increasing until 12:00 then decreasing again ($Y = -0.0455x3 + 0.6374x^2 - 2.3528x + 2.5711$, R^2 = 0.5179).

In cucurbits, Malerbo-Souza, Tadeu, Bettini, and Toledo (1999) observed *T. spinipes* stingless bees collecting only pollen between 8:00 am and 10:00 am in the watermelon crop (*Citrulus lanatus*), being considered an important pollinating agent of the crop. According to Sousa and Malerbo-Souza (2005), *T. spinipes* stingless bees did not damage watermelon flowers preferring male flowers (82.9%) compared to female ones (17.1%). This stingless bee was more frequent collecting pollen (46.7% of the visits) and, for collecting nectar, preferred the male flowers (36.2%) to the female ones (17.1%).

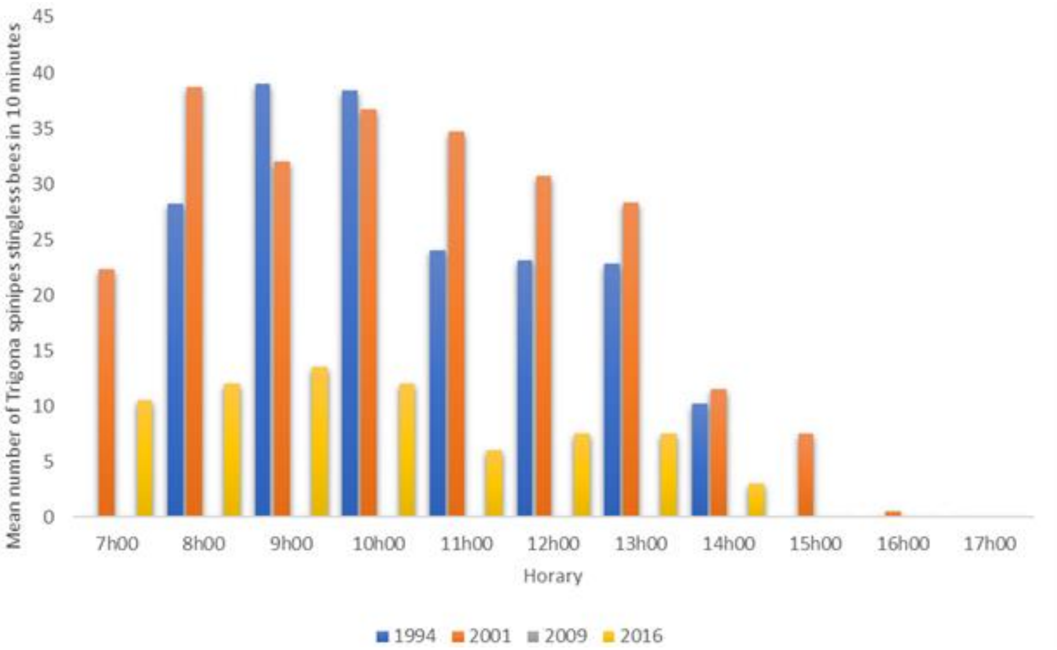

Figure 3. Mean frequency of *Trigona spinipes* stingless bees collecting nectar in female and male chayote flowers *(Sechium edule*), in 1994, 2001, 2009 and 2016.

In Italian pumpkin culture (*C. pepo*), Malerbo-Souza et al. (2019) observed several species of bees collecting nectar and pollen from flowers: Africanized honey bees *A. mellifera*, *T. spinipes* stingless bees, *Peponapis fervens* bees and bees of the Halictidae family. The most frequent insect in the flowers was the Africanized honey bee *A. mellifera* (79.25%) followed by the *T. spinipes* (20.75%). According to these authors, the *T. spinipes* stingless bees were considered to be pollinators of the culture.

There was a difference between the years of studies regarding the frequency of insects, sometimes several insects were observed, in others only one species of insect, bees or ants. Table 1 and Figure 4 show the average number of *T. spinipes* stingless bees, in different years, which were significantly more frequent in 1994 and 2001, and these bees preferred to visit the chayote flowers between 8:00 and 13:00 with peak visitation at 10:00 am.

Due to the fact that the chayote crops need a pollinating agent, it is clear that this plant species is dependent on the entomofauna existing in the region, as well as dependent on the competitive sources of food offered to these insects. It is essential to raise awareness among farmers for the preservation of forests, a nesting place for several species of insects, especially native bees. *A. mellifera* honey bees are not very attracted to the chayote flowers, but can be an alternative management when the natural insect population is low in the vicinity of the culture.

Light plays an important role in the activity of *T. spinipes* stingless bees indicating that the foraging time of these bees probably occurs between 10:00 am and 3:00 pm (Macieira & Proni, 2004), and these bees can change their behavior due to the modification of the meteorological conditions due to daily or seasonal variation in the flow of existing food resources (Hilário, Imperatriz-Fonseca, & Kleinert, 2000).

Table 1. Total number of *Trigona spinipes* stingless bees on chayote flowers (*Sechium edulis*) in 1994, 2001, 2009 and 2016.

Time/Years	1994	2001	2009	2016
7h00	0	22.3	0	10.5
8h00	28.2	38.7	0	12
9h00	39	32	0	13.5
10h00	38.4	36.7	0	12
11h00	24	34.7	0	6
12h00	23.1	30.7	0	7.5
13h00	22.8	28.3	0	7.5
14h00	10.2	11.5	0	3
15h00	0	7,5	0	0
16h00	0	0,5	0	0
17h00	0	0	0	0
Total	185.7a	242.9a	0	72b

Means followed by different lowercase letters, on the same line, differ significantly from each other, by the Tukey Test, at the 5% level.

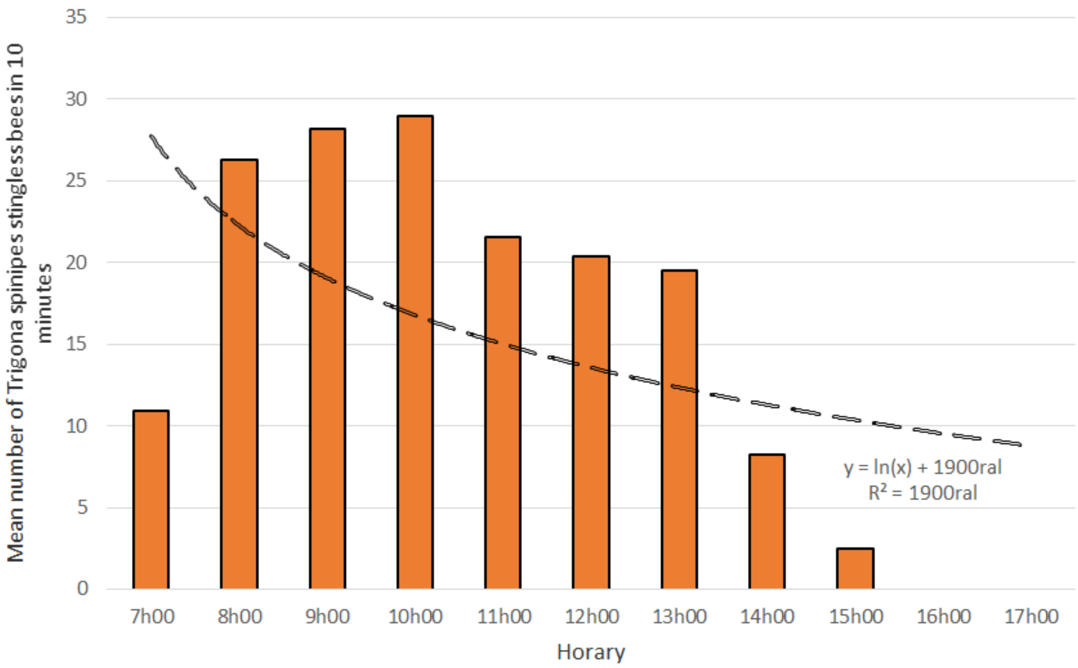

Figure 4. Total average number of *Trigona spinipes* stingless bees in chayote flowers on different years (1994, 2001, 2009 and 2016).

Regarding the constancy of insects, in 1994, the *T. spinipes* stingless bee was a constant species, that is, it was present in more than 50% of the observations, presenting an index of 70.0% for female flowers, however, it was considered an accessory species (present between 25 and 50% of observations) in male flowers, with a constancy rate of 27.5%. In 2001, the *T. spinipes* stingless bee was constant (77.8%), the Africanized honey bee *A. mellifera* was considered an accessory species and all other insects were accidental species, being present in less than 25% of the observations. In 2009, ants were constant in male flowers (93.33%), female flowers (73.33%) and chayote culture (83.33%). In 2016, the *T. spinipes* stingless bee was considered an accessory species in chayote flowers (47.22%).

The *T. spinipes* stingless bee, in addition to being frequent, was also a constant species in the chayote flowers, with the exception of 2009. This bee, when collecting nectar from male flowers, touched the stamens and when collecting nectar in female flowers touched the stigma, visibly carrying pollen in its body. This foraging behavior can characterize as a pollinator of the chayote, a culture where the presence of pollinating agents is essential due to the characteristic of producing separate female and male flowers. This behavior contradicts the concept that this bee is harmful to crops and is even considered a pest of crops. According to Silva et al. (2012), the richness and occurrence of Meliponini species in anthropic environments can be influenced by several ecological factors, such as the quality and types of nesting substrates available, the degree of sociality and the degree of generalist behavior of these bees.

The *T. spinipes* stingless bee can be considered an effective pollinator of different agricultural crops, such as coffee (*Coffea arabica*) (Malerbo-Souza & Halak, 2012), soybean (*Glicine max*) (Toledo et al., 2011), cotton (Sanchez Junior & Malerbo-Souza, 2004), pigeon pea (Parisi & Malerbo-Souza, 2006) and onion (*Allium cepa* L., hybrid) (Lorenzon et al., 1993). They were also observed in different fruit trees, such as jabuticaba (*Myrciaria cauliflora*) (Malerbo-Souza, Nogueira-Couto, & Toledo, 2004), avocado (*Persea americana*) (Malerbo-Souza, Toledo, Silva, & Sousa, 2000), peach (*Prunus persica*) (Mota & Nogueira-Couto, 2002), orange (*Citrus sinensis*), (Malerbo-Souza & Halak, 2013), strawberry (Malagodi-Braga & Kleinert, 2007), lemon (Malerbo-Souza & Halak, 2010), pomegranate (*Punica granatum*) (Malerbo-Souza et al., 2011), among others.

Trigona family have a wider range of niches and greater uniformity in the exploitation of food resources, including the species of honey bees *A. mellifera*, *Tetragonisca angustula*, *Paratrigona lineata* and *Scaptotrigona postiça*, being considered a polytrophic species due to the diversity of botanical species visited (Nogueira-Ferreira & Augusto, 2007). The *T. spinipes* stingless bees visited representatives of the Anacardiaceae, Combretaceae, Euphorbiaceae, Mimosaceae and Fabaceae families, among others, and pollen diversity was recorded in their nests showing their polylectic habit and their potential as a pollinator (Oliveira et al., 2008).

Giannini et al (2015a) showed that the *T. spinipes* stingless bee was the effective pollinator of ten agricultural species: acerola, carrot, chayote, sunflower, orange, mango, strawberry, pumpkin, pepper and pomegranate. Giannini, Cordeiro, Freitas, Saraiva, and Imperatriz-Fonseca (2015b) reported that *T. spinipes* represents an important pollinator for agriculture, especially considering its wide geographic distribution.

Giannini et al. (2015c) classified the *T. spinipes* stingless bees and honey bees *A. mellifera* as 'supergeneralists' because they both visit and collect food (nectar and pollen) in large amounts of different flower species. The authors evaluated the role of *T. spinipes* and *A. mellifera* in networks of interactions between bees and plants in 21 locations in Brazil, from the north to the south, and in different types of habitats. Despite the similarities, the two bees have different roles in the networks, with *T. spinipes* having a greater effect on the interaction pattern of other bees. Environments without vegetation (degraded environments), such as cities and agricultural environments, have a positive effect on *T. spinipes* stingless bees and did not affect this species, which apparently manage to adapt to them and survive in conditions that other bees could not, being dominant in networks interaction with local plants.

Jaffé et al. (2015) showed that *T. spinipes* stingless bees are able to colonize in degraded environments and are able to disperse over long distances, with no significant genetic difference being found, even among bees collected from very distant locations. In addition, no barriers prevented the exchange of genes between individuals, since the same genetic sequences were found in individuals whose populations were separated by different vegetation covers (natural vegetation, cities, abandoned areas, agricultural areas, pastures and areas with different altitudes), indicating they can intersect and disperse between very heterogeneous areas. *T. spinipes* is very well adapted to degraded environments making an important 'rescue pollinator', that is, a species capable of carrying out pollination, even in places where many species are no longer able to survive.

T. spinipes stingless bees were very frequent in flowers, regardless of location, but depending on their need for food and material collection to build their nests. At such times, this bee uses resins from the buds, flowers, leaves and, sometimes, from the fruit. However, most of the time, it visits the flowers to collect only nectar, other times, just pollen. Therefore, the foraging behavior of this species will depend on the needs of their colony, which may have honey and pollen stored in abundance or may be multiplying the swarm.

In the experiments we have already carried out, *T. spinipes* has been observed vigorously pushing away large bees such as *Xylocopa frontalis* and also honey bees, in the flowers of the yellow passion fruit (*P. edulis f. flavicarpa*) (Malerbo-Souza et al, 2002). They were observed cutting parts of wild passion fruit flowers (*P. coccinea*). However, when this bee pierced the flower bud in the orange, to 'pickle' the nectar, the flower developed normally, not affecting production (Malerbo-Souza and Halak, 2013)

Likewise, if beekeepers are encouraged to form an apicultural pasture, farmers must be made aware that they maintain preservation areas or recover degraded areas, introducing plant species with different flowering times, preventing *T. spinipes* stingless bees from using agricultural crops such as nest building material and carry out only the pollination of flowers, which is the main contribution of these bees, including the *Trigona* family.

Regarding fruiting, as expected, of marked female floral buds none produced fruit, precisely because they had separate male and female flowers, requiring visits from pollinators. As there were no visits to the covered flowers, no fruit was produced. Therefore, as pollinators are needed in the vicinity of the planting, it is desirable that horticulturists maintain the nests of *T. spinipes* stingless bees and promote the planting of native species that bloom in different months of the year, so that the native bees can remain in the area.

Conclusion

The *Trigona spinipes* stingless bee can be used as a pollinator of the chayote flowers, as long as there are nests close to the crop. These flowers are not attractive to Africanized honeybees *Apis mellifera*, the culture being more dependent on native bee species for the production of its fruits.

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