



## RESEARCH ARTICLE - BEES

## The use of different stingless bee species to pollinate cherry tomatoes under protected cultivation

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### Abstract

Under standard greenhouse conditions, the tomato fruits resulting from spontaneous self-pollination are expected to be of lower quality than those from bee pollination. In addition, the use of species with different behaviors is expected to change productivity differently. To test these hypotheses, we evaluated the pollination services from the use of three native stingless bee species with distinct foraging behaviors, *Melipona bicolor* Lepeletier, 1836, *Nannotrigona testaceicornis* (Lepeletier, 1836), and *Partamona helleri* (Friese, 1900) during the blooming period of cherry tomatoes in greenhouses. Fruit quality parameters resulting from pollination experiments were measured and the acclimatization of the analyzed bee species was evaluated. Visits of *M. bicolor* and *N. testaceicornis* to the tomato flowers contributed significantly to increases in the average weight, seed number, and thickness of the pericarp (only for *N. testaceicornis*) of the fruits, compared to the spontaneous self-pollination treatment. *Partamona helleri*, however, did not show any pollen collection behavior in the experimental conditions. Although *N. testaceicornis* did not perform the buzzing behavior, fruits from its pollination were equivalent to fruits from pollination by *M. bicolor*. The use of bee species with different flower-visiting behaviors can optimize tomato pollination in greenhouses and help to standardize fruit weights, contributing significantly to the quality of the fruits and increasing productivity, with consequent increases in commercial value.

### Introduction

Between 15,000 and 20,000 plant species, including agricultural species, have flowers with poricidal dehiscence anthers (e.g. De Luca & Vallejo-Marín, 2013) and foraging insects usually release the plant pollen through thorax vibration in a behavior known as buzzing (Buchmann, 1983; Thorp, 2000; Portman et al., 2019). Alternatively, some bee species “scrape” pollen (Wille, 1963; Thorp, 2000), using their mouthpieces in a “milking” movement to collect pollen from the poricidal flowers (Portman et al., 2019). These behaviors

performed by a high diversity of pollinators is a key factor for quality enhancement in crop production (Westerkamp & Gottsberger, 2000), including tomatoes (Depra et al., 2014; Gaglianone et al., 2018).

The tomato (*Solanum lycopersicum* L.) is an autogamous Solanaceae with functionally poricidal anthers that must be vibrated to release the pollen grains through the apical pores (McGregor, 1976). In open-field tomato crops, the wind action and bee visitation can promote the necessary vibration and pollen deposition on the flower stigma (Free, 1993; Depra et al., 2014; Gaglianone et al., 2018). On the



other hand, greenhouse crops have a scarcity of wind and animal pollination conditions that are usually counterbalanced by mechanically induced vibration and airflow (Bell et al., 2006). These pollination techniques increase production costs and represent losses to the commercial value (Picken, 1984; Westerkamp & Gottsberger, 2000; Cruz & Campos, 2009).

Therefore, in recent years, the management of tomato pollinators in greenhouse crops has been tested through the introduction of bees including *Amegilla* (*Notomegilla*) *chlorocyanea* Cockerell (Hogendoorn et al., 2006); *Apis mellifera* L. (Bispo dos Santos et al., 2009); *Xylocopa* species (reviewed in Velthuis & van Doorn, 2006); and the stingless bees *Nannotrigona perilampoides* (Cresson) (Cauich et al., 2004; Palma et al., 2008) and *Melipona quadrifasciata* Lepeletier (Del Sarto et al., 2005; Bispo dos Santos et al., 2009; Hikawa & Miyanaga, 2009; Bartelli & Nogueira-Ferreira, 2014; Silva-Neto et al., 2018). The stingless bees *Partamona helleri* (Friese), *Melipona bicolor* Lepeletier, and *Nannotrigona testaceicornis* Lepeletier display different behavior during visits to poricidal anthers, buzzing (*P. helleri* and *M. bicolor*) and scraping (*N. testaceicornis*), and there is no information about these species on greenhouse crops. Although *N. testaceicornis* has been surveyed in some open-field crops (Dos Santos et al., 2008; Cruz & Campos, 2009) and *P. helleri* in wild plants in Brazil (Lopes De Carvalho et al., 1999, Ramalho, 2004, Taura & Laroca, 2004, Azevedo et al., 2007, Carvalho, 2007), we did not find any data about their interaction with plants in greenhouses. Interest in managing stingless bees for pollination of agricultural crops has increased considerably in recent years. This is due to the high richness of species, their little aggressive behavior with easy and known handling and multiplication of colonies (Nogueira-Neto, 1997).

In the current study, we evaluated the viability of the stingless bees *Melipona bicolor*, *Nannotrigona testaceicornis*, and *Partamona helleri* for pollination of cherry tomatoes (*Solanum lycopersicum*) in greenhouses, as well as their effectiveness in simultaneous use. Our expectation was that pollination by more than one bee species would enhance the fruit set and quality of the fruits produced.

## Material and methods

### Crop implementation

The study was conducted in two greenhouses at the Federal University of Viçosa (20°45'14"S e 42°52'53"W), municipality of Viçosa, MG (Brazil), implementing crops between April and July 2014. Cherry tomato seedlings (hybrid "Chipano®" - red) were obtained by sowing and after 30 days, 200 seedlings were transplanted to 8 kg pots filled with commercial vegetable substrate, individually fertilized, and equally distributed in two vegetation greenhouses (100 in each). The tomato plants were carried with only one stem, eliminating all lateral shoots. The anthesis of the flowers started 30 days after transplanting the seedlings. We used arch-shaped greenhouses 3.2 m high at the sides and 5.2 m at

the top (total area of 108 m<sup>2</sup>), closed at the sides with 50 mesh antivirus screen, covered with 150 micron light diffuser anti-UV agricultural film.

An automated irrigation system was programmed to irrigate plants twice a day with about 600 ml of water to each pot. The cover fertilization was applied to each pot every 20 days with 40 g of ammonium sulfate. Temperature and relative humidity data were obtained over 24 hours using Data Logger (HOBO® U10). The experiment lasted 120 days.

### Beehives placement

We selected strong nests (with large numbers of individuals) of *Melipona bicolor*, *Nannotrigona testaceicornis*, and *Partamona helleri*, from the Central Apiary of the Federal University of Viçosa. The number of individuals was estimated indirectly from the observation of a large number of brood cells and food storage pots. We opted not to use colonies with reduced food reserves and few brood cells due to the risk of loss during the experiment, as it was conducted during a period considered as low flowering when colonies generally decrease the production and storage of resources. The nests were transported from the Central Apiary and installed in the greenhouses seven days before the anthesis of the first flowers.

In greenhouse A, we installed one nest of *M. bicolor* and two nests of *N. testaceicornis*, and in greenhouse B we installed two nests of *N. testaceicornis* and one of *P. helleri*. The nests were kept closed for 07 days before being opened to provide access to the interior of the greenhouses. The access was opened at night.

In order to minimize the effects of temperature on nest development and bee behavior, the nests were installed on 1.5 m high supports positioned outside the greenhouse and their entrances were connected to the insides through a PVC pipe. Only *P. helleri* was kept inside the greenhouse in order to preserve the nest entrance architecture and facilitate the return of these bees to the nest.

Throughout the study, artificial feeders containing aqueous honey solution (70%) and water were placed inside the greenhouse to supply the nests with energetic food (tomato flowers do not produce nectar). We randomly distributed geometric shapes (square, triangle, and round) of different colors (yellow, green, and black) throughout the greenhouse in order to assist the flight orientation of the bees. Bees learn and memorize visual features such as shapes, patterns, direction, depth, contrast, intensity, and movement of light that are important in flight control and use visual coordinates to explore places of interest such as nest and flower locations (Srinivasan, 1994; Giurfa & Lehrer, 2001).

### Pollination tests and behavior of the bees

During the pollination experiments, we also recorded the pollination behavior and average time spent on each single visit by *M. bicolor* and *N. testaceicornis* workers between 08:00 and 11:00 on two non-consecutive days.

The flowers of the fourth branch of both plantations were subjected to the following treatments: (i) spontaneous self-pollination (SP): 20 branches with flowers in pre-anthesis phase were marked and protected with organza bags (16x30 cm) to avoid any eventual visit; (ii) induced self-pollination (ISP): 20 branches were protected with organza bags and manually vibrated using cuttings during anthesis phase (Higuti et al., 2010); (iii) hand cross-pollination (HCP): the pre-anthesis flowers in 20 branches were covered and then manually pollinated 24 hours after anthesis, touching the stigmatic surface with a pollen mixture extracted from anthers of 20 flowers from different plants; (iv) pollination by *M. bicolor* and *N. testaceicornis* (MBNT): open flowers from 20 branches were available for visits of the two bee species during three consecutive days; (v) pollination by *M. bicolor* (MB): same as in the MBNT treatment, but only visited by *M. bicolor*; (vi) pollination by *N. testaceicornis* (NT): performed as MB treatment, but preventing foraging of *M. bicolor* by closing its nest; (vii) pollination by any bee (BP): visits inferred through necrotic marks on the anthers, regardless of the visitor species; (viii) single visit by *M. bicolor* (SMB): 60 flowers monitored and bagged after the single visit; (ix) single visit by *N. testaceicornis* (SNT): 60 flowers monitored and bagged after the single visit; and (x) single visit by *P. helleri* (SPH): 60 flowers monitored and bagged after the single visit. The flowers or inflorescences were protected with an organza bag immediately after the completion of each of the treatments mentioned above.

To perform the hand cross-pollination test (HCP), stigma receptivity was tested on 10 pre-harvest flowers and 10 open flowers after 24 h using 3% hydrogen peroxide. The receptivity was confirmed by detecting the air bubble formation on the surface of the stigma due to the activity of the peroxidase enzyme, indicating that the stigma was receptive (Kearns & Inouye, 1993). In all treatments, the bags were removed after the beginning of fruit formation. The complementarity of the pollination services of the three bee species was analyzed through comparisons of the fruit quality parameters resulting from the experiments.

To determine the influence of pollination tests on tomato production and fruit quality, the first three fruits of each branch were harvested, and five attributes were verified: (1) weight, (2) number of seeds, (3) transverse diameter, (4) longitudinal diameter, and (5) pericarp thickness. Fruit weight was determined with the aid of a precision scale. Transverse diameter, longitudinal diameter, and pericarp thickness were measured using a digital caliper. The number of seeds was manually counted by opening the fruit and removing its pulp to access the seeds.

For the data analysis, we tested data normality using the Kolmogorov-Smirnov test. To evaluate the independence of the tested variables we used the Pearson's correlation coefficient. We assumed that width and height would be previously correlated, then we expressed this relationship

resuming the values as volume in  $\text{cm}^3$  considering an irregular sphere:  $v = \frac{4}{3}\pi r_1 r_2 r_3$ , where  $r$  is the radius for each sphere axis. We used the Kruskal-Wallis and the pairwise Dunn post hoc tests with the sequential Bonferroni correction ( $p < 0.05$ ) to assess the differences among fruit parameters from the 4th branch in the different pollination treatments (SP, ISP, HCP, MBNT, MB, NT, BP, SMB, and SNT) (Zar, 1999). In addition, we evaluated the median differences from the SP treatment through the box-plot method, since sensitive median differences may be important when evaluating production increases. All tests were performed at a significance level of 5%, using PAST v 3.26 (Hammer et al., 2001).

## Results

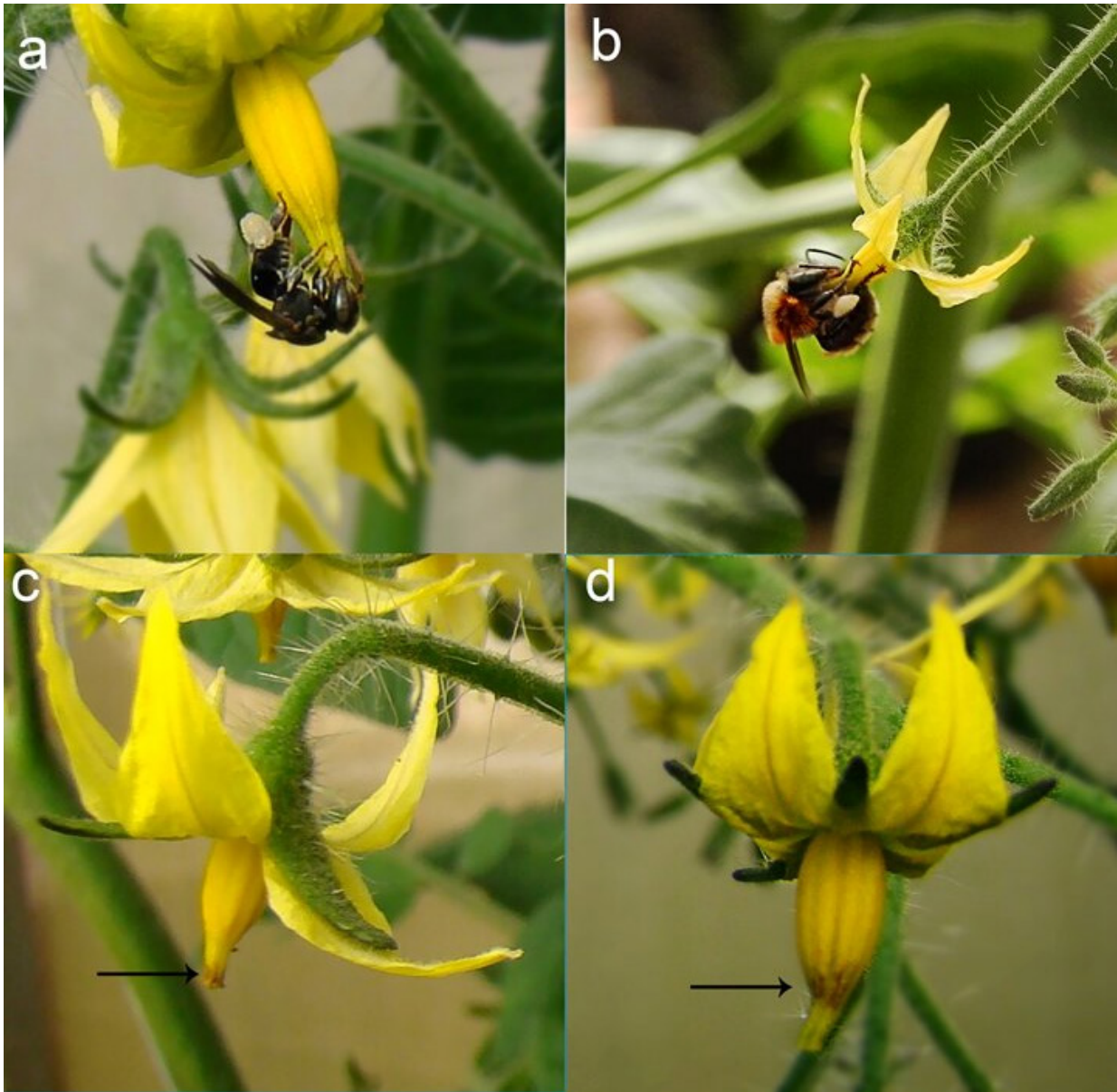
### *Stingless bee behavior*

Regarding the species behavior, after opening the entrances of the *M. bicolor*, *N. testaceicornis*, and *P. helleri* nests to access the greenhouse, foragers of the three species displayed the behavior of flying towards sunlight and colliding with the screen, where they remained and consequently died of exhaustion. Flight behavior toward sunlight ceased after three days of confinement. After this period, outside activities were restricted to short flights to remove trash from the nests.

Sixteen days after the opening of the *P. helleri* nest entrance, some foragers were observed to be under the petals, branches, and leaves. However, the pollination tests for *P. helleri* (SPH) were not possible, as this species did not show any pollen collection behavior under the experimental conditions. During the experiment period in which *P. helleri* was confined, the average temperature in the greenhouse was 19.28°C (max 32.09°C and min. 10.05°C) and average relative air humidity was 85% (max. 98.53% and min 62.44%).

Twenty-four days after the opening of the nest entrances to the interior of greenhouse A, foragers of the species *M. bicolor* and *N. testaceicornis* began the visits to the flowers (Fig 1). The average temperature during the period in which the bees were confined in this greenhouse was 19.83°C (max 35.04°C and min. 9.68°C) and average relative air humidity was 82.21% (max. 97.14% and min 47.86%). In greenhouse B, the foragers of *N. testaceicornis* began foraging in tomato flowers 27 days after the opening of the nest entrance to the greenhouse.

Foragers of *N. testaceicornis* and *M. bicolor* started foraging activities in both greenhouses at around 8:00 am and ceased at 11:00 am, with the highest activity recorded at 09:30 am. During the period in which both species had free access, foragers of *N. testaceicornis* always drove away *M. bicolor* foragers from the flowers and then remained collecting the pollen grains exposed after the *M. bicolor* visit, as well as the pollen grains inside the anthers. No more interactions were observed between the two species on flowers. It was very common to observe two foragers of *N. testaceicornis* collecting pollen from the same flower.



**Fig 1.** Visitation and necrotic lesions in flowers of cherry tomatoes (*Solanum lycopersicum* L). (a) visitation by *N. testaceicornis* buzzing behaviour; (b) visitation by *M. bicolor* pollination scraping behavior; (c) necrotic lesions left by *N. testaceicornis*; (d) necrotic lesions left by *M. bicolor* visitation. Black arrows indicate the exact point of the lesions in both flowers.

Bees of *N. testaceicornis* showed a high floral constancy, randomly foraging flowers in either the same or distinct plants. When landing on the anthers, the foragers of *N. testaceicornis* went to the apical portion of the anthers and circled around it, introducing their glossa into the anther's pore in a behavior of scraping the extremities. Pollen grains accumulated in the ventral portion of the thorax during visits were transferred to the corbiculae. When foraging the flowers, *M. bicolor* vibrated the flowers of the tomatoes by buzzing. The individuals of *M. bicolor* explored several flowers of the same plant before moving to the next plant, almost always in the same line.

Flowers on the first day of anthesis were not visited by *M. bicolor* or *N. testaceicornis* foragers. On the 2nd day of anthesis, *M. bicolor* and *N. testaceicornis* performed longer visits on flowers not yet visited, compared to flowers that had already been visited by any bee. The visits of *M.*

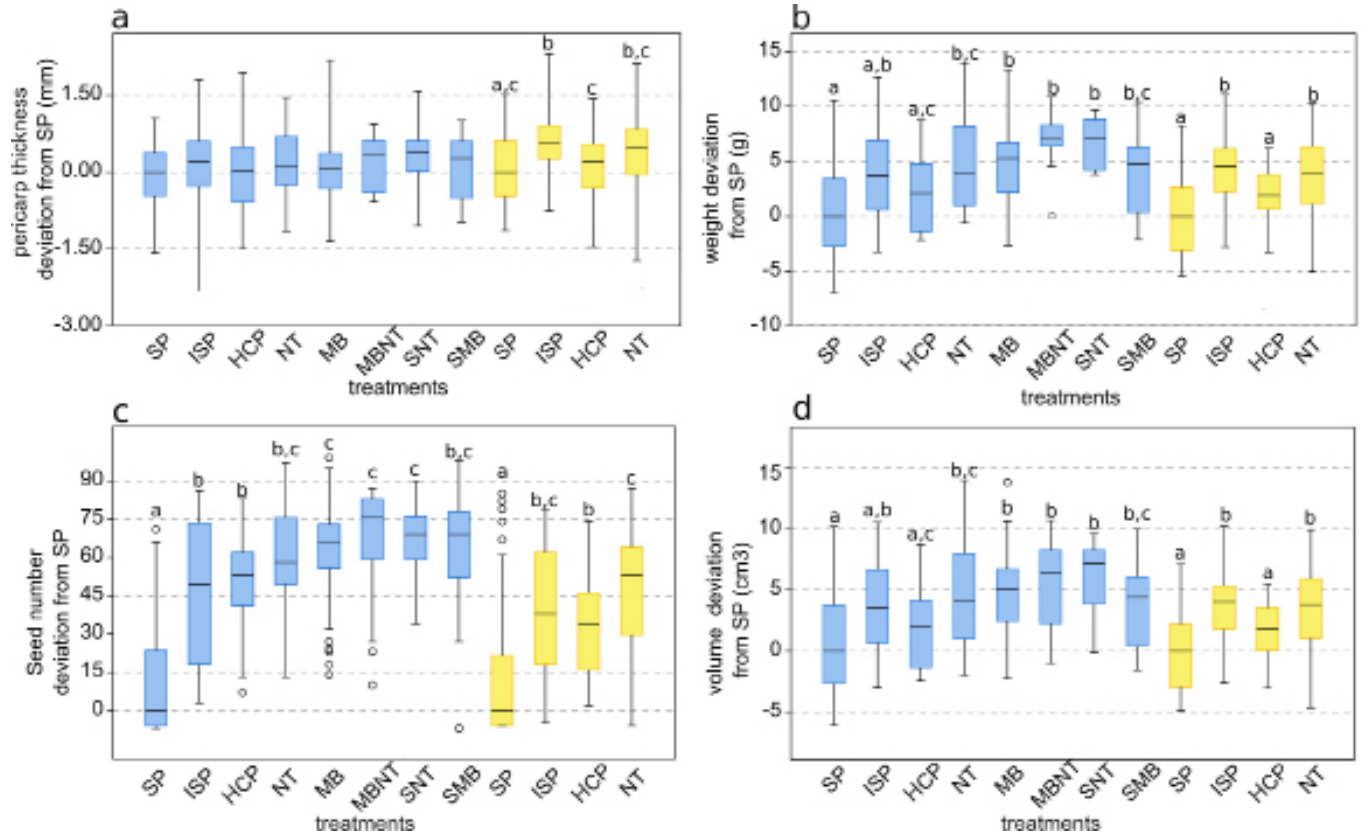
*bicolor* lasted on average 12 s ( $22 \pm 7$  s) on unvisited flowers and 3.8 s ( $6 \pm 2$ ) on flowers already visited on the same day. Similarly, *N. testaceicornis* spent an average of 91 s ( $140 \pm 50$ ) on unvisited flowers and 29.5 s ( $42 \pm 11$ ) on already visited flowers. Visited flowers could be recognized by necrosis marks on the anthers (Fig 1).

#### Pollination experiments

The results from the greenhouse A experiment showed significant differences in all pollination tests and attributes evaluated, except for pericarp thickness (Fig 2a), when compared to fruits from spontaneous self-pollination treatment (SP) (Table 1). Fruits from bee pollination treatments were an average of  $4.69 \pm 0.71$  g heavier and had  $52.44 \pm 5.3$  more seeds than those from SP treatment. In addition, pericarp thickness was larger in the treatment with pollination by *N. testaceicornis* (NT)

(3.41 ± 0.16 cm) compared to SP (2.93 ± 0.65) in greenhouse B. On the other hand, we did not find consistent significant differences between treatments involving induced self-pollination (ISP) or hand cross-pollination (HCP) compared to the bee treatments. Fruits from SNT, MB, and MBNT showed significant differences ( $p < 0.05$ ) only for weight (Fig 2b) and

number of seeds (Fig 2c), compared to HCP in greenhouse A, and NT compared to HCP in greenhouse B. Transverse and longitudinal diameters, and therefore the volume were highly correlated with the weight (Person's  $r > 0.96$ ,  $p < 0.001$ ), and the statistical tests followed the same pattern throughout the different comparisons.



**Fig 2.** Box-plot and statistic differences among four parameters of cherry tomatoes measured in the present study in greenhouses A (blue) and B (yellow). (a) Pericarp thickness; (b) Weight; (c) number of seeds; (d) volume derived from transverse and longitudinal diameters. Boxplot bars depicts quartile intervals, inner line the median position, out lines the boundaries of the distribution and circle the outliers. Letters over the box-plots indicate the statistical differences ( $p < 0.05$ ) according to the Kruskal-Wallis and Dunn's post hoc tests. Boxplot bars depicts quartile intervals, Spontaneous self-pollination (SP); induced self-pollination (ISP); hand cross pollination (HCP); pollination by *M. bicolor* and *N. testaceicornis* (MBNT); pollination by *M. bicolor* (MB); pollination by *N. testaceicornis* (NT); pollination by bees (BP); single visit pollination by *M. bicolor* (SMB); and single visit pollination by *N. testaceicornis* (SNT).

**Table 1.** Means followed by different letters (a-d) represent statistical differences ( $p < 0.05$ ) for the same attribute by the Kruskal-Wallis test and Dunn's posterior test.

Greenhouse	Experiment	N	Weight (g)	Number of seeds	Transverse diameter (mm)	Longitudinal diameter (mm)	Pericarp thickness (mm)
A	SP	60	9.00 ± 0.53 <sup>a</sup>	18.41 ± 2.95 <sup>a</sup>	23.11 ± 0.50 <sup>a</sup>	24.83 ± 0.58 <sup>a</sup>	4.00 ± 0.56 <sup>a</sup>
	ISP	64	12.70 ± 0.50 <sup>b,c</sup>	52.98 ± 3.43 <sup>b</sup>	26.62 ± 0.35 <sup>b,c</sup>	28.28 ± 0.41 <sup>b,d</sup>	3.71 ± 0.08 <sup>a</sup>
	HCP	39	11.07 ± 0.56 <sup>a,c</sup>	56.74 ± 2.71 <sup>b</sup>	25.32 ± 0.45 <sup>a,b</sup>	26.76 ± 0.50 <sup>a,b,c</sup>	3.50 ± 0.12 <sup>a</sup>
	BP	56	13.34 ± 0.57 <sup>b,c</sup>	66.71 ± 2.57 <sup>b,c</sup>	26.95 ± 0.43 <sup>b,c</sup>	29.11 ± 0.47 <sup>d</sup>	3.72 ± 0.07 <sup>a</sup>
	MB	122	13.39 ± 0.27 <sup>b</sup>	70.11 ± 1.37 <sup>c</sup>	27.27 ± 0.20 <sup>c</sup>	29.13 ± 0.23 <sup>d</sup>	3.57 ± 0.04 <sup>a</sup>
	MBNT	20	14.64 ± 0.79 <sup>b</sup>	73.65 ± 4.97 <sup>c</sup>	27.96 ± 0.62 <sup>c</sup>	29.98 ± 0.64 <sup>d</sup>	3.70 ± 0.12 <sup>a</sup>
	SNT	17	14.37 ± 0.89 <sup>b</sup>	72.58 ± 3.87 <sup>b,c</sup>	27.70 ± 0.84 <sup>c</sup>	30.13 ± 0.64 <sup>d</sup>	3.84 ± 0.15 <sup>a</sup>
	SMB	30	12.71 ± 0.62 <sup>b,c</sup>	71.20 ± 4.00 <sup>b,c</sup>	26.76 ± 0.44 <sup>b,c</sup>	28.46 ± 0.52 <sup>c,d</sup>	3.63 ± 0.10 <sup>a</sup>
B	SP	60	7.03 ± 0.45 <sup>a</sup>	20.45 ± 3.48 <sup>a</sup>	21.52 ± 0.08 <sup>a</sup>	22.43 ± 0.52 <sup>a</sup>	2.93 ± 3.48 <sup>a</sup>
	ISP	63	11.21 ± 0.38 <sup>b</sup>	45.86 ± 3.21 <sup>b</sup>	25.68 ± 0.31 <sup>b</sup>	26.94 ± 0.34 <sup>b</sup>	3.48 ± 0.07 <sup>b</sup>
	HCP	31	8.74 ± 0.47 <sup>a,c</sup>	39.06 ± 3.56 <sup>b,c</sup>	23.92 ± 0.53 <sup>a,b</sup>	24.54 ± 0.48 <sup>a</sup>	3.00 ± 0.12 <sup>a,c</sup>
	NT	102	10.42 ± 0.34 <sup>b,c</sup>	52.48 ± 2.38 <sup>c</sup>	24.96 ± 0.31 <sup>b</sup>	26.43 ± 0.35 <sup>b</sup>	3.41 ± 0.16 <sup>b,c</sup>

## Discussion

The behavior on the first days after the transfer of *M. bicolor*, *N. testaceicornis*, and *P. helleri* nests into the greenhouse was similar to that reported in studies with other stingless bee species (Cauich et al., 2004; Cruz et al., 2004; Bomfim et al., 2014). The limited size of an artificial environment such as a greenhouse imposes physical barriers to flying activities, with collisions against the greenhouse cover being very common in the first days (Slaa, 2006). Older foragers already have certain established habits and experiences from the environment in which they were previously maintained, so they have greater difficulty acclimating to the protected environment (Free, 1993). This explains the high mortality of foragers of the three species studied in the first days of introduction of the nests in the greenhouse.

Acclimatization of stingless bees to greenhouse conditions can vary greatly between species and between colonies of the same species (Cauich et al., 2004; Cruz et al., 2004; Bartelli et al., 2014; Bartelli & Nogueira-Ferreira, 2014; Bomfim et al., 2014; Da Silva et al., 2016; Silva-Neto et al., 2018). For instance, foragers of *Nannotrigona perilampoides* (Cresson, 1878) may take from five to nine days to consistently start their activities in tomato greenhouse crops (Cauich et al., 2004). In different crops, such as small watermelons, colonies of *Scaptotrigona* sp. started foraging after the second day and *M. subnitida* started to explore resources on bell pepper flowers after seven days in the greenhouse (Cruz et al., 2004; Bomfim et al. 2014). The acclimatization time of *Melipona* species seems to be variable; Bartelli et al. (2014) observed that it took 22 days for *M. quadrifasciata* workers to start foraging tomato flowers. Bumblebee species on the other hand might expend 9 to 10 days for acclimatation (Morandin et al., 2001a).

*Partamona helleri* was not considered a pollinator of cherry tomatoes under the conditions of this study. Several factors may be involved in this outcome, such as lack of interest in the floral resources or inability to acclimate to greenhouse conditions (Bomfim et al., 2014). A determining factor for bees to use their functions is a temperature not exceeding 30 °C (Brand, 2005; Kiss, 2006). In the greenhouses, the maximum temperature recorded during the day was 32.09 °C and the relative maximum humidity was 98.53%. The temperature is the factor that most influences the internal and external activities of the colony; when very high, foraging decreases and ventilatory behavior increases in order to stabilize the internal temperature of the colony (Michener, 2000; Roubik, 1989; Teixeira & Campos, 2005). According to Kleinert et al. (2009) stingless bees have excellent foraging activity when the temperature remains between 20 and 30°C, and the relative humidity values are between 30% and 70%.

Foraging activities were concentrated in the early hours of the day, when the temperature in the greenhouse was mild, which agrees with observations from other studies for different stingless bee species (Cruz et al., 2004; Teixeira

& Campos, 2005; Nunes-Silva et al., 2013; Bomfim et al., 2014; Silva-Neto et al., 2018). However, one factor that may have caused the absence of foraging activity observed in the afternoon is the high number of visits per flower in the morning, leading to pollen depletion. Some bee species are able to evaluate the amount of pollen available during the visit (Buchmann & Cane, 1989; Shelly et al., 2000; Nunes-Silva et al., 2013), and the perception that flowers would have a low amount of pollen may lead to the bees no longer visiting these flowers. Some studies reported that two visits per flower are enough for pollination of tomatoes in greenhouses and that higher levels of necrotic markings on the anthers lead to a decrease in return visits (Morandin et al., 2001b). The foraging behavior of *M. bicolor* exploring several flowers on one plant is expected and often described for species from the genus *Melipona* (Camargo, 1972; Cruz et al., 2004).

Fruits from flowers visited only by *N. testaceicornis* in both single and multiple visits, showed higher average values in all analyzed parameters when compared to fruits from flowers visited by *M. bicolor*. Previous studies reported that bee species from the genus *Melipona* were efficient pollinators of tomatoes (Del Sarto et al., 2005; Bispo dos Santos et al., 2009; Hikawa & Miyanaga, 2009; Bartelli & Nogueira-Ferreira, 2014; Silva-Neto et al., 2018). Although we did not measure the pollen grain removal rate, the fact that all evaluated attributes present values similar to the fruits of the Induced self-pollination tests (ISP) and higher than those found in self-pollinated fruits (SP) corroborates the effective pollination in the different types of foraging, scraping (*N. testaceicornis*) and buzzing (*M. bicolor*) (Cauich et al., 2004; Del Sarto et al., 2005; Dos Santos et al., 2008; Palma et al., 2008; Bispo dos Santos et al., 2009; Hikawa & Miyanaga, 2009; Roselino et al., 2009; Bartelli & Nogueira-Ferreira, 2014; Silva-Neto et al., 2018). In addition to foraging behavior, the time spent visiting flowers seems to maximize the distribution of pollen grains on stigma, essential for fruit development (Mann, 1943). The differences in visit time between *M. bicolor* and *N. testaceicornis* species may be related to different visitation behaviors. The buzzing behavior of *M. bicolor* can be considered more efficient for pollen extraction and lower energy expenditure, when compared to the time spent in each visit of *N. testaceicornis*. The time spent by *N. testaceicornis* may be the main factor in maintaining a greater floral constancy in flowers of the same and nearby plants, which could contribute to higher mean values of the analyzed tomato parameters. Even without statistical significance, we can highlight the higher medians and quartiles distribution found in the weight and number of seeds of the fruits produced from the visit of the two bees, corroborating the expectations of the simultaneous use of these species for the pollination of cherry tomatoes.

Based on the observed results, we conclude that stingless bees *M. bicolor* and *N. testaceicornis* can be used simultaneously to pollinate cherry tomatoes grown in greenhouses. The fruit set resulting from non-buzzing *N.*

*testaceicornis* visits is similar to the fruit set after visits from *M. bicolor*, a buzzing species. In future work perspectives, we suggest testing the effectivity of simultaneous use of stingless bees in other tomato varieties.

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