Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v46i1.65131

ANIMAL PRODUCTION

Effect of bait nest position on capture of stingless bee species

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ABSTRACT. This study aimed to evaluate the effect of the position of bait nests on the capture of stingless bee species. The experiment was conducted in the rural area of the municipality of Campo Grande, state of Mato Grosso do Sul, from May 2022 to September 2022. Thirty bait nests were distributed in a randomized block design, with two treatments (A: bait nests set up one meter above the ground, and B: bait nests buried in the ground), with 15 replications per treatment. Of treatment B, two baits were nested by jataí (*Tetragonisca angustula*), and two were nested by Mandaguari-preta (*Scaptotrigona postica*). Therefore, the position of the bait nest influenced ($p < 0.05$) the success in capturing stingless bee species, in which treatment B, with baits buried in the ground, was significantly superior to treatment A, with baits set up one meter above the ground.

Keywords: passive capture; swarming; Jataí; Mandaguari-preta; meliponiculture.

Received on September 23, 2022.
Accepted on October 2, 2023.

Introduction

In Brazil, there are approximately 250 species of stingless bees described, which makes it the country with the highest variety of stingless bees in the world (Menezes, 2020). Meliponiculture, the rational breeding of stingless bees, was already practiced by indigenous peoples and is currently carried out for commercial purposes, as it allows the production of honey, pollen, and colonies, among other by-products (Andrade & Silva, 2016). Furthermore, this activity plays an important role in sustainability, as it contributes to the pollination of various crops, contributing to environmental preservation and the maintenance of biodiversity (Witter & Nunes-Silva, 2014; Silva, Pacheco Filho, & Freitas, 2015).

In this way, meliponiculture is a secondary activity option for rural producers as it has low initial cost and good profitability and is considered environmentally correct due to its character of harmony and integration with environmental preservation (Magalhães & Venturieri, 2010). However, stingless bees are wild animals, native to Brazilian territory, and the capture of nests in their natural habitat is not permitted (Villas-Bôas, 2012). According to Conama Resolution 496 of August 19, 2020, among the options for obtaining mother colonies is 'collection in nature using a bait container', a simple alternative that takes advantage of the natural swarming process of bees (Villas-Bôas, 2012; Brasil, 2020).

The swarming process is the way stingless bee colonies reproduce. In this process, the connection between the mother colony and the daughter colony can last weeks or months, in which the workers carry construction materials and food from the mother colony to the new nesting site, and, subsequently, the princess and a large number of workers fly to the new nest, giving rise to daughter colonies (Nogueira-Neto, 1997). In this way, a bait container can simulate the favorable conditions for this nesting to occur in a pre-determined site, and later, these colonies are transferred to rational hives, facilitating management (Koser, Barbiéri, & Francoy, 2020).

Several techniques can be used to produce a bait container, also called a bait bottle or bait nest. Wooden boxes, PET bottles, cardboard packaging, Styrofoam boxes, or other artificial cavities that mimic the natural characteristics of the nest can be used to make it an attractive place for bees to nest (Koser et al., 2020). In nature, bees usually nest in cavities previously used by other bees, which have a characteristic smell resulting from wax and/or batumen remains. Due to this characteristic, an attractant inside the bait nest is commonly used to increase the chances of capturing a swarm.

Research has been carried out to evaluate different methodologies for producing and installing bait nests (Oliveira, Menezes, Silva, Soares, & Imperatriz-Fonseca, 2009). One of the factors that can influence nesting success is the height from the ground. In a literature review, Santos, Barbosa, and Prezoto (2020) analyzed 44 articles and found a variation of 0.09 to 7 meters in height from the ground of *Tetragonisca angustula* nests. *Scaptotrigona* bee nests ranged from 0.38 to 3.32 meters, which may indicate a variation in the ideal height for installing the baits.

Therefore, there are questions to be clarified regarding the most efficient way of making and setting up bait nests, highlighting the importance of further studies on the topic. In this context, this study aimed to evaluate the effect of the position of bait nests on the capture of stingless bee species.

Material and methods

The experiment was conducted in the rural area of the municipality of Campo Grande, state of Mato Grosso do Sul, from May 2022 to September 2022, totaling 120 days. Thirty bait nests were distributed in a randomized block design, with two treatments (A: bait nests set up one meter above the ground, and B: bait nests buried in the ground), with 15 replications per treatment. The bait nests were made using 30 two-liter PET bottles, which were washed with neutral detergent (Figure 1A), rinsed with water (Figure 1B), and, subsequently, had their interior bathed in an attractant made with alcoholic extract of cerumen and propolis from the species *Tetragonisca angustula* (Figure 1C), simulating the odor of a previously inhabited colony. In treatment A, a hole was made in the cap to allow the bees to enter using a Phillips screwdriver 3/16" X 3" (Figure 1D); the bottles were wrapped in two sheets of newspaper (Figure 1E) and plastic canvas (Figure 1F) to waterproof the trap and ensure a dark interior. In treatment B, five holes were made in the bottom of the bottle with a heated nail (1 ¼ X 15; Figure 1G) so that humidity would not be trapped inside.

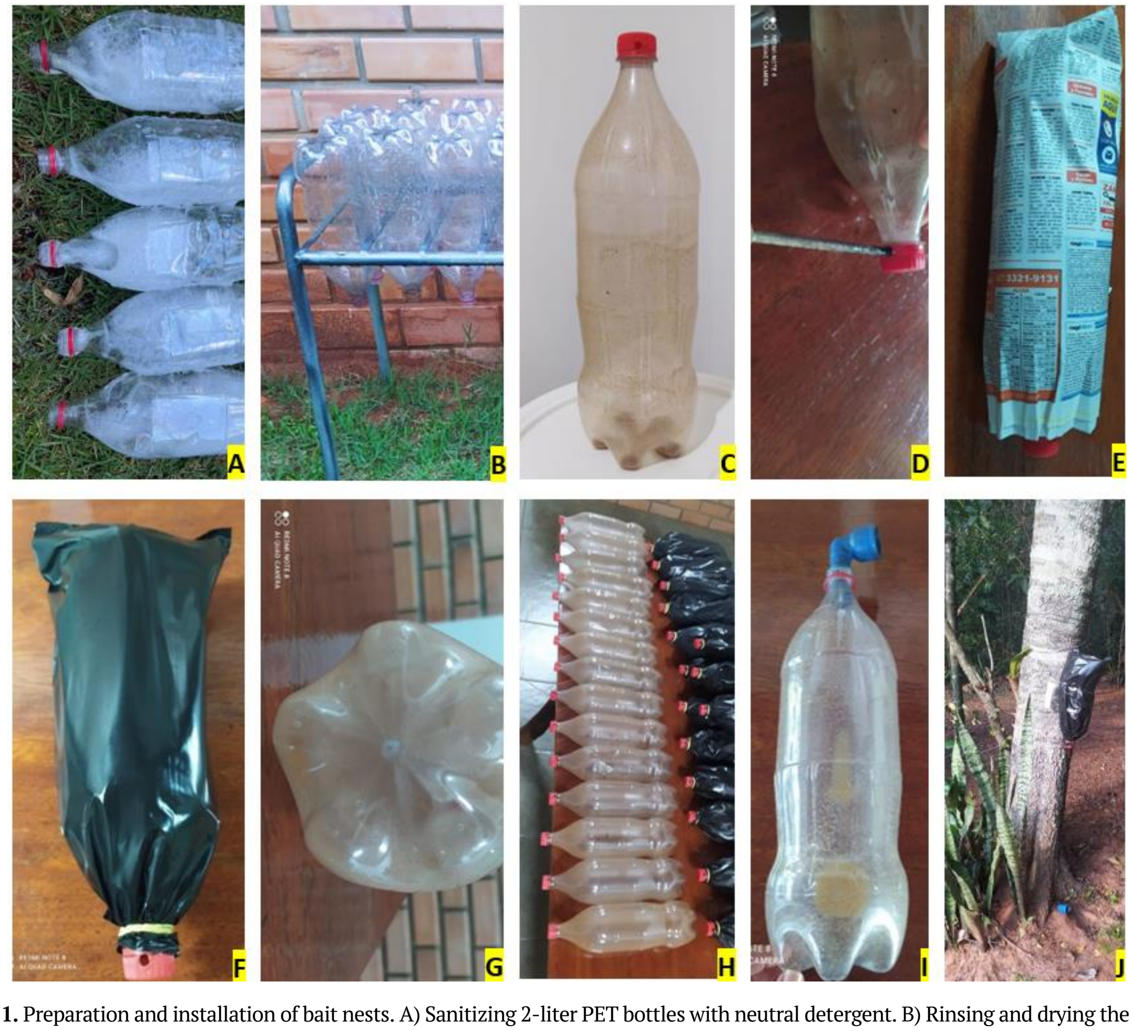

Figure 1. Preparation and installation of bait nests. A) Sanitizing 2-liter PET bottles with neutral detergent. B) Rinsing and drying the bottles. C) Application of the attractant inside the bottle. D) Hole in the cap of treatment A bottles for bees to enter. E) Bottles of treatment A wrapped in newspaper sheet. F) Bottles of treatment A wrapped in black plastic canvas. G) Five holes made in the bottom of the PET bottle for treatment B. H) Baits of treatments A and B. I) Insertion of the 90º blue PVC ¾” connection into the mouth of the bottles of treatment B. J) Bait of treatment A tied to the tree at a height of one meter, and treatment B buried in the ground, in the same location.

After making all the bottles (Figure 1H), the caps of the treatment B bottles were removed, and, in their place, a blue ¾ PVC connection (22 mm, 90º elbow threadable tube) was inserted (Figure 1I) to avoid water inflow during rains. Bait nests of both treatments were positioned in the same location (Figure IJ), with their entrances facing east. Treatment A bottles were tied to trees, and treatment B bottles were buried, with 15 replications.

All bait nests were installed on the edge of the forest so that they were protected from direct sunlight by the shadows of the trees. They were identified with a label wrapped in transparent and waterproof adhesive tape, with the identification of the block, bait nest number, and treatment. The baits were monitored daily in the early morning and late afternoon to check their integrity and the capture of stingless bees, and the information obtained was compiled. Every 30 days, the attractant was sprayed at the entrance of all bait nests to increase attractiveness.

The variable analyzed was the success in capturing stingless bee species according to the position of bait nests. Success was considered when the colonies built the entrance to the colony, had drones standing in the vegetation around the bait, forager bees entering with pollen, and the presence of construction materials (wax, pots, or brood cells) inside the bottle. Data obtained were subjected to Fisher's Exact test, at 5% significance, using the SAS Studio program.

Results and discussion

Following the criteria established for the successful capture of the colony in the bait nest, in treatment B (buried in the ground), two baits were nested by jataí (*Tetragonisca angustula*) 28 and 70 days after the start of the experiment (Figure 2A and B), and two were nested by Mandaguari-preta (*Scaptotrigona postica*) 28 and 42 days after the start of the experiment (Figure 2C and D). In treatment A (bait set up 1 meter above the ground), there was no success in capturing stingless bee colonies. The position of the bait nest influenced ($p < 0.05$) the success in capturing stingless bee species, in which treatment B, with the bait buried in the ground, was significantly superior to treatment A, with the bait set up one meter high.

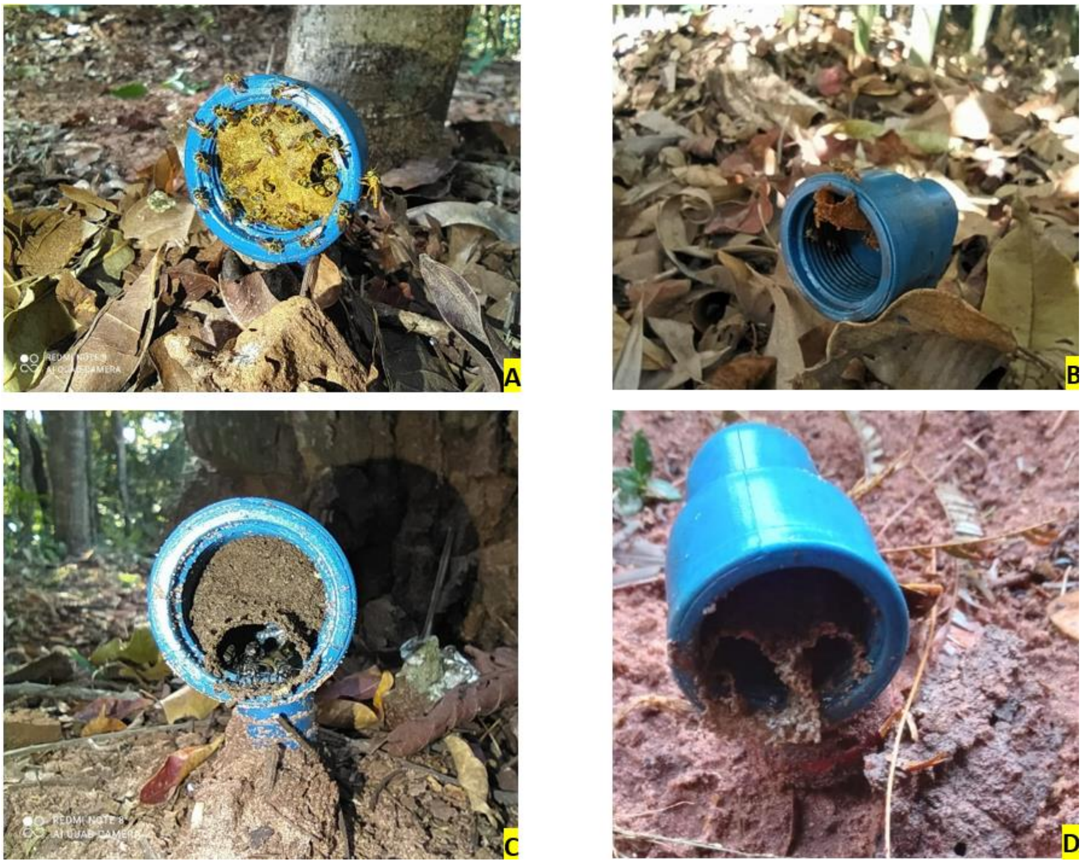

Figure 2. Bait nests of treatment B colonized by stingless bees. A) Bait nest nested by jataí bees, 28 days after the start of the experiment. B) Bait nest nested by jataí bees, 70 days after the start of the experiment. C) Bait nest nested by Mandaguari-preta bees, 28 days after the start of the experiment. D) Bait nest nested by jataí bees, 42 days after the start of the experiment.

The sites chosen for nesting vary according to the species but, in general, stingless bees that build internal nests seek out locations in tree hollows, termite mounds, anthills, crevices in rocks or cavities in the ground (Palumbo, 2015; Pereira, Souza, & Lopes, 2017). In this way, bait nests buried in the ground probably have an ambience closer to what bees prioritize in nature, making this position more attractive for nesting.

Temperature is an important factor for the colony to develop and be able to perform its functions. Due to their small size, the surface/volume ratio of stingless bees is high, and heat exchange with the environment is high. Therefore, at low temperatures, metabolism decreases, preventing flight and other activities. Very high temperatures cause bees to reduce external activities and ventilate the colony (Campos, Gois, & Carneiro, 2010). Furthermore, maintaining the temperature in the nest is essential for brood development, as at improper temperatures, development is hampered or even made impossible (Palumbo, 2015).

The initial mechanism for stingless bees to control the colony temperature is the definition of the nesting site, considering that the structural characteristics of the nest, which must guarantee good thermal insulation, are one of the factors that influence the thermoregulatory ability (Campos et al., 2010). Therefore, one of the hypotheses for successful captures with buried bait nests is that this position inside the ground provides greater thermal comfort. In addition, buried baits are less susceptible to shaking caused by rain and strong winds, which can also influence their nesting site selection.

Mortality of captured colonies

The two baits nested by Mandaguari-preta (*Scaptotrigona postica*) were lost due to attacks by phorid flies. In one of them, there was also excess humidity. The presence of phorid flies was also verified in one of the baits nested by jataí; however, even after the attack, the colony managed to survive.

The colonies captured in the bait nests would be transferred to Inpa model hives 60 days after the swarming process's start, which showed the presence of many males close to the entrance of the daughter colony (Figure 3A and B). This time was defined based on the swarming process of stingless bees, in which, unlike an Apis colony that divides from the mother colony and never returns, the bees stay for days, or even months, transferring food and construction materials from the mother colony to the new nest (Nogueira-Neto, 1997). The change time varies according to the species; however, some authors (Palumbo, 2015; Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina [Epagri], 2017) recommend the permanence of the bait nest for 60 days after capture to allow the colony to complete the swarming process and disconnect from the mother colony. However, due to events such as attacks by the *Lestrimelitta limao* bee, this period had to be brought forward to an average of 30 days after the start of nesting.

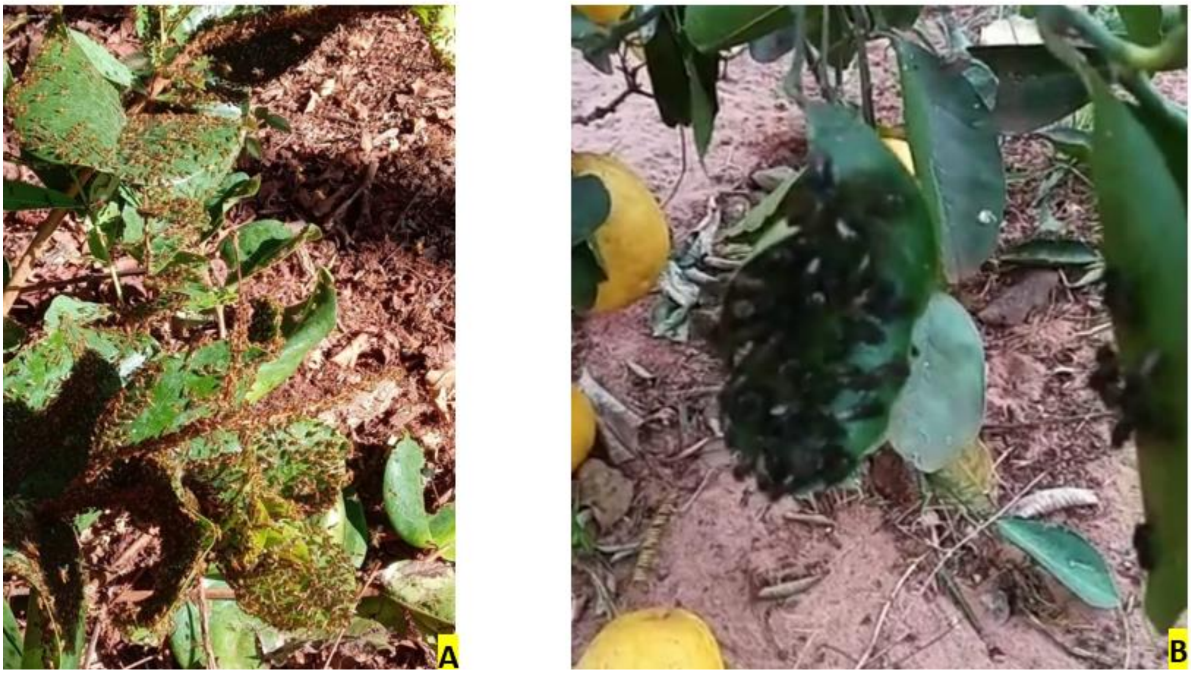

Figure 3. Presence of drones near the entrance to the bait nests. A) Presence of jataí drones. B) Presence of Mandaguari-preta drones.

L. limao do not visit flowers to collect food, they survive exclusively by robbing food from other bee colonies and are considered obligatory kleptoparasites. The attacked colony can react in two different ways, with the bees retreating and remaining motionless until the end of the attack or reacting aggressively by attacking the invading bees. During robbing, *L. limao* plunder honey, pollen, nest materials, and brood food, and in the case of the host colony that reacts to the attack, high mortality occurs (Von Zuben & Nunes, 2014).

The first colony attacked was the Mandaguari-preta, captured after 28 experimental days. The attack occurred 13 days after the start of nesting. After the attack, the colony did not survive. The bait nest was unearthed and showed residues of construction materials (cerumen, brood cells, propolis, food storage pots) and the presence of phorid flies.

Phorid flies are small black flies belonging to the family Phoridae and are one of the main enemies that attack stingless bees. Adults are attracted by the smell of fermented pollen and move quickly, looking for ways to enter the colony. Females lay eggs in open pollen pots, youngest brood with exposed food, destroyed combs, and trash deposit, where eggs transform into larvae that consume the pollen and the youngest brood cells, where there is larval food (Witter & Nunes-Silva, 2014; Pereira et al., 2017). Due to the disorganization and mortality that the *L. limao* attack caused in the Mandaguari-preta colony, the defenses were compromised, and the colony became susceptible to attack by phorid flies, factors that led to its loss.

The second colony attacked was the Jataí colony, captured after 28 experimental days and 51 days after the start of nesting. However, despite the impact on bee mortality, the bait nest was unearthed (Figure 4A) and transferred to the hive (Figure 4B), and the colony managed to recover and show excellent development.

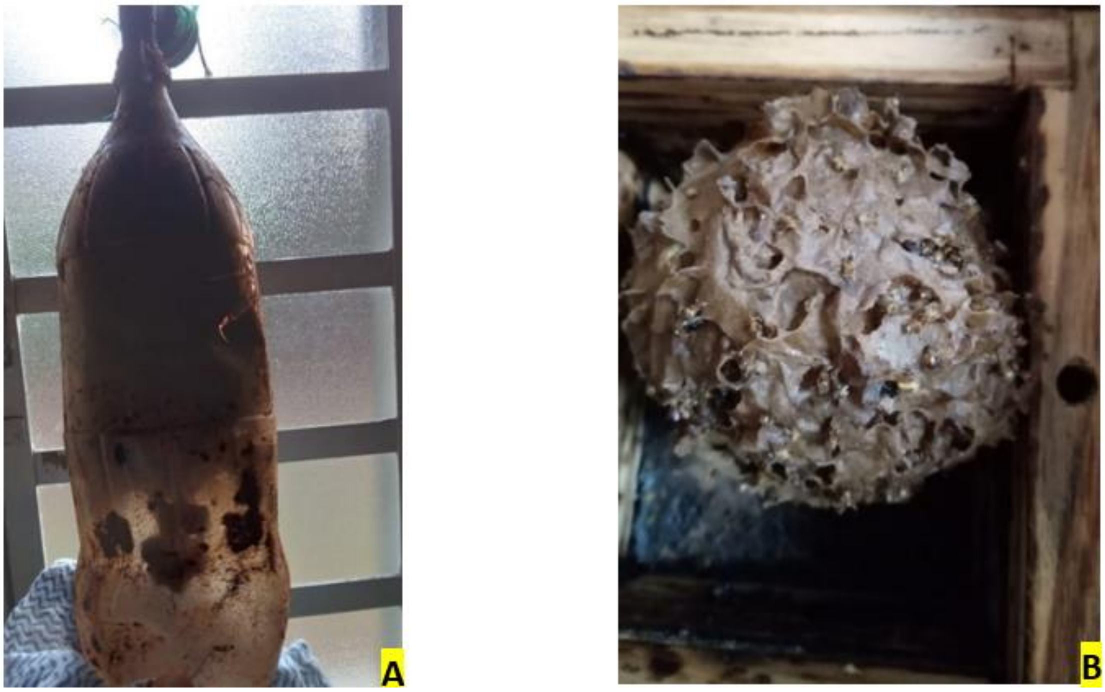

Figure 4. Jataí bait nest attacked by *L. limao*. A) Bait nest unearthed, showing nesting materials inside the bottle. B) Transfer of the colony to the hive, where there was still some *L. limao* in the involucrum.

Another factor that must be taken into consideration when capturing using a buried bait nest is humidity x species. The Mandaguari-preta colony, captured after 42 experimental days, faded away 30 days after the start of nesting, due to the occurrence of two days of intense rain. After these events, the movement of bees ceased, and the bait nest was subsequently unearthed. The bottle had water droplets inside and the bottom had approximately 5 cm of accumulated water, with a large number of dead workers. There was nesting material (Figure 5A), wax, food storage pots, and brood cells, and the only live bee found was the queen transiting in the brood area (Figure 5B). Several phorid flies and their larvae were also observed in the brood area (Figure 5C). An alternative to avoid the accumulation of water and excess humidity inside the bait would be to increase the diameter of the holes at the bottle's bottom and add some material to the bottom of the hole in the ground to allow for water to drain, such as crushed stones, so that the water can drain from the bottle to the ground and not accumulate.

The jataí colony, captured 70 days after the onset of the experiment, which suffered the same rainy weather, was transferred to the hive, 29 days after the start of nesting (Figure 6A, B and C), and did not suffer any negative impact from the rainfall events, which may indicate that *Tetragonisca angustula* is more rustic and adapts better to the conditions of the buried bait nest.

Figure 5. Mandaguari-preta bait nest after two days of rain. A) Bait nest unearthed, after two days of rain, with nesting materials inside. B) Surviving queen in brood area. C) Brood cells with phorid fly larvae.

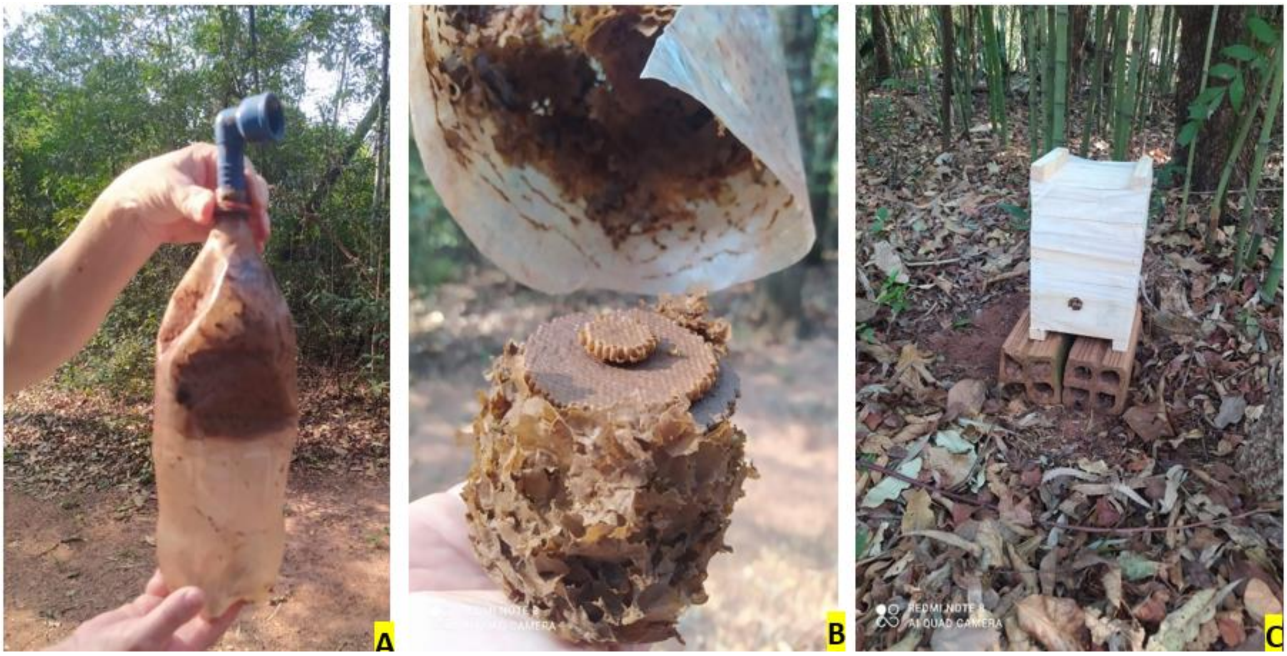

Figure 6. Transfer of the jataí colony, captured 70 days after the onset of the experiment, which was exposed to rain. A) Bait nest unearthed with nesting material inside. B) Opening of the bait nest exposing the brood area. C) Transfer completed, and colony maintained at the capture site.

Transfer of nests into rational hives

The two jataí colonies that succeeded in the nesting process were transferred to Inpa model hives. For the transfer, the bottles were unearthed and delicately cut in half and lengthwise with a knife, taking care not to injure the bees inside. After cutting, the bottle was opened, which allowed the nest and its involucrum to be removed. Only a little pollen and honey were inserted into the colony during the transfer to avoid attack by enemies, and the rest was inserted the following day after the bees' organization.

Thus, the buried bait nest was more successful in capturing stingless bee species than the bait nest set up one meter above the ground. Nevertheless, some factors must be considered when using this type of bait, such as the rusticity of the species captured, the ideal time for the baits to remain in the ground, and water drainage into the ground to increase the chances of successful colony development after transfer to the hive.

Conclusion

The position of the bait nest influenced the success in capturing stingless bee species. The buried position was superior to baits set up one meter high.

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