Impact of the introduction of stingless bee colonies (Scaptotrigona aff. postica) on the productivity of acai (Euterpe oleracea)

Nilton Akio Muto1; Renan Oliveira de Sousa Leite2; Daniel Santiago Pereira3; Hervé Louis Ghislain Rogez4; Giorgio Cristino Venturieri5

1Biomedicine Doctor; Professor, Federal University of Pará, Center for Valorization of Amazonian Bioactive Compounds; Belém, Pará, Brazil. E-mail: niltonmuto@ufpa.br; 2Biotechnologist; Master in Agro Environmental Biotechnology - Federal University of Pará; Center for Valorization of Amazonian Bioactive Compounds; Belém, Pará, Brazil. E-mail: renan.leite92@outlook.com; 3Agriculture and Livestock technician, Agronomist, Doctor of Animal Sciences; Researcher in Sustainable Systems: Beekeeping - Embrapa Eastern Amazon; Belém, Pará, Brazil. E-mail: daniel.pereira@embrapa.br; 4Agroindustrial Chemical Engineer, PhD in Agricultural Sciences and Biological Engineering; Professor - Federal University of Pará; Center for Valorization of Amazonian Bioactive Compounds; Belém, Pará, Brazil. E-mail: herveroez@gmail.com; 5Agronomist, PhD in Ecology; Pollination Ecology, Meliponiculture and Beekeeping, NATIVO Company; Brisbane, Queensland, Australia. E-mail: giorgio@venturieri.com

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ABSTRACT
The acai palm is a typical palm tree of the Amazonian estuary, especially important in the diet and extractive agriculture economy of the Eastern Amazonia. Its fruit productivity is highly dependent on cross-pollination. Meliponiculture has greatly advanced during the last decades, however, the use of meliponines for the pollination of tropical fruit trees is scarce. In this work, in an irrigated crop of Euterpe oleracea, the fruit yield was evaluated after the introduction of colonies of Scaptotrigona aff. postica (Latreille, 1807). Three gradients, 0-50, 65-115 and 130-180 meters, was used as a methodology to evaluate the productivity increase, from the central point where 30 colonies were installed. The productivity rate was evaluated by fruit weight per bunch, number of fruits per rachilla, average fruit diameter and weight of one hundred sample fruits. A significant increase in the productivity could be observed, reaching 2.5 times more, depending on the distance of the plant to the colonies of bees. The highest productivity interval was the closest, from 0-50 meters.

RESUMO

INTRODUCTION
Palm trees are predominantly pollinated by insects, 29% by beetles, 26% by bees, 8% by flies and 7% by wind, and there are many other pollinators ranging from mammals to crabs (BARFOD et al., 2011). The Euterpe genus fits in the group of palm trees pollinated by the group “bees-flies-wasps”, as reported by Henderson et al. (2000).

Acai palm (Euterpe oleracea Mart.) is native to Amazonian forests and has recently been labeled as a super food, because of the high levels of anthocyanins and other non-anthocyanin flavonoids in its fruits (RUFINO et al., 2010).

The acai culture is mainly concentrated in the state of Pará (Brazil) in Eastern Amazonia, where 1 million tons of the fruit is produced annually, generating approximately US $ 149 million for the economy, representing about 31% of the
national production of non-timber products (IBGE, 2015). Despite its high productivity, the monoecious species is self-incompatible and mainly pollinated by bees and flies (VENTURIERI et al., 2014; VENTURIERI et al., 2016; CAMPBELL et al., 2018).

In addition, the state of Pará has around 50 companies that sell the fruit to other states, whereas 60% of the total production is consumed locally, 35% goes to other regions of Brazil and 5% goes abroad, this amount injects into the state’s economy something around US $1.5 billion, however, this value corresponds only 3% of the Gross Domestic Product (GDP) of the state (CONAB, 2019).

The acai palm flowering period occurs along the year in the Amazon region, with a peak in the months from January to May, coinciding with the period of greatest rainfall. The period in which the harvest of the acai fruit decreases, followed by the harvest period six months after the fertilization of the acai flowers (OLIVEIRA, 2002).

Acai palm flowers are brightly colored, sessile, diclinous, often distributed in rachis in triads composed of two male flowers and one female, except for the terminal part of the racillhas, where male flowers predominate (HENDERSON; GALEANO, 1996). Male ones begin their anthesis by offering more abundant and concentrated nectar than that offered by female ones, which can be interpreted as an stimulus to keep pollinators over the inflorescences and to make them to visit male flowers first and then go to female flowers, whose climax of their offering nectar, in its highest concentration of sugar happens just after the decline in the production of nectar in male flowers, a period coinciding with the greatest receptivity of stigma (VENTURIERI et al., 2014).

In order to generate fruit, the acai palm, being a monoecious and protandrous species (dichogamy in which the male sexual organs develop before the female ones) with a floral characteristic that favors allogamy, (OLIVEIRA, 2002) thus needs cross-pollination, mediated by bees (DORNELES et al., 2013; VENTURIERI, 2014; BEZERRA et al., 2020).

The importance of pollinators in agriculture is well recognized, but the increase in crop yield by using of cultivated tropical pollinators is still a technology poorly used (POTTs et al., 2010; GIANNINI et al., 2015; QUEZADA-EÚAN et al., 2018; RAMÍREZ et al. 2018) and concentrated in a few genera, in particular, *Apis mellifera* to saturate crop flowers with insect visitors (VIANA et al., 2014; GARIBALDI et al., 2017; ISAACS et al., 2017).

Later and Venturieri (2015) reported a stingless bee species of the genus *Scaptotrigona* sp. with high potential for directed pollination of the acai tree. However, *Scaptotrigonas* represent only one species group among several existing bee species. The pollination efficiency of this bee, in particular, would be enhanced by the following factors: 1) compatible size with the reproductive structures of the flower; 2) compatible size with floral rewards and 3) short flight range, forcing this bee species to be contained among the flowers of the cultivated species.

Ne’eman et al. (2010) and Stavert et al. (2016) reported that the key in pollination ecology is that how like flower visitors vary in their performance as pollinators as a product of both their interaction frequency and functional traits that mediate the quantity and quality of pollen deposited during single flower visits.

Several bee species, mostly of the genus *Melipona* Illiger, are used in meliponiculture in Brazil (CONTRERA; MENEZES; VENTURIERI) and Mesoamerica (VILLANUEVA-GUTIERREZ et al., 2013). The genus *Scaptotrigona* Moure is also an important genus for stingless bee keeping with many species, well known for its populous colonies, and production of good quality honey in large quantities, in comparison to other meliponines (REYEZ-GONZÁLEZ et al., 2014; ALEIXO et al., 2017; HURTADO-BURILLO et al., 2017). *Scaptotrigona* species from the group *S. postica*Latreille, thereafter called *S. aff. postica* that occurs in the rain forest, state of Pará in the Brazil, and commonly known as “canudo” or “tucanaíra” is a commonly kept stingless bee species in the Amazon region, as it has great honey production potential. In addition, this species may be of significant value for use in the pollination of native crop species (VENTURIERI et al., 2012), due to visiting a great spectrum of plants, its tolerance of handling, populous colonies and the possibility to be multiplied on a large scale (RAMALHO, 2004; MENEZES, VOLLET-NETO, FONSECA, 2013).

Resources stored in the hives by bees may present a diversity of pollens in their composition, depending on the availability of flowering plant species near the hive. Melissopalinology identifies the pollens present in honey of bees and allows to recognize the floral relationship of bees, indicating their preferences in different locations and types of vegetation, which may help in identifying the floral origin (BARTH, 2013).

The use of native Amazonian bees can contribute to the sustainable development of regional fruit culture, increasing the productivity of fruits of the target crop and making use of local bee biodiversity, generating income, and preserving native species through their use.

In the present study, it was evaluated the yield of fruits in irrigated crop system of *Euterpe oleracea* under two situations, one under the influence of open pollination, promoted by the natural insect population of the area and another by the increase of *Scaptotrigona* aff. *postica* density, by the addition of hive boxes.

**MATERIALS AND METHODS**

**Experimental Area**

The work was carried out from January to September 2017, in an acai plantation cultivated in Santa Maria city, Pará State, Brazil (1º01’47.76” S and 47º35’08.95” W). The planting was done under dryland conditions, in a Yellow oxisoil medium type, irrigated with the water supply of 110 L/day/plant, with an application by rainfall control, mixed fertilization (NPK, micronutrients, and cattle manure), with a 3x3m spacing between clumps. The orchard was in the reproductive phase, with 6 years of implantation, and with the height of the trees not exceeding 15m.

A second area of 30 hectares, in the municipality of Igarapé-acu, State of Pará (1º01’46” S 47º35’03” W), also of monoculture of irrigated acai crop, in July 2015, was used to evaluate the crop fidelity of forager bees of *S. aff. postica* to the acai flowers.

**Crop fidelity**

For this experiment, 4 strong colonies were placed in the center of the cultivation area. After 8 days on site, at the time of greatest floral opening (9-10 am), the boxes had the entrance hole closed with a mesh and replaced by empty boxes for the
capture of the forager workers that returned to the colony. Of the total foragers, of the 4 boxes, only those with pollen load were evaluated, to verify the botanical origin.

To count the bees captured in the trap boxes, CO2 gas was used to make the bees fall asleep, a white background with 4 divisions was placed at the bottom of these boxes, two repetitions for each colony was done, photos were taken in high resolution for later counting on the computer screen (Figure 1b). Two repetitions for each colony was done, counting a total of 452 workers.

Microscope slides were made from samples of pollen loads from the bee corbiculae. The pollen was identified using an optical microscope, comparing it with the pollen slides made from acai anthers’ flowers (Figure 1d). After there was no doubt about the visual identification of pollen loads, all bees that contained whitish pink pollen basket were counting as acai origin (Figure 1a).

**Figure 1.** Use of of *Scaptotrigona aff. postica* to increase pollination of *Euterpe oleracea*. a) bee foragers arriving with acai pollen basket; b) bee sample in a trap box; c) communal shelter (meliponary); d) acai pollen from microscope slides and e) acai yield from crop plantation where colonies have been placed.

**Palynological analysis from pots of Scaptotrigona aff. postica**

The introduction of *S. aff. postica* at the acai palm plantation was carried out in January, two months before the beginning of the flowering of the plant, to adapt the colonies to the new environment, recovering from the stress of environmental change. It was used Vertical (Embrapa) hive model developed for housing *Scaptotrigona aff. postica* colonies (JAFFÉ et al., 2015; LEÃO et al., 2016). The hives were organized in a collective shelving system (Figure 1c).

We randomly selected 10 beehives of *S. aff. postica*, from 30, to verify the occurrence of pollen of acai tree or other species present in the environment. The palynological analysis was performed in triplicate from pollen and honey collected from internal food pots from February to May 2017.

An evaluation parameter of pollen load of pink-white color, proposed by Venturieri et al. (2014), as a visual indicator for the evaluation of pollen recently collected in the corbicle of workers returning to the colony.

Melissopalaminological studies were performed by standard methodology according to (LOUVEAUX et al., 1978), modified by Iwana e Melhem (1979), followed by the acetolysis method for pollen sediments, according to (ERDTMAN, 1960) and subsequent assembly of blades.

The pollen grains were quantified using light microscope (400x) on glycerin dispersion slides until the number of 500 pollen grains per sample.

The identification carried out by comparing the shape and measurement of the pollen with pollen reference (ANGGADHANIA et al., 2020).

**Fruit yield evaluation**

An area of 500 m² of acai palm plantation was measured through a fiberglass measuring tape where 30 colonies of *S. aff. postica* were installed at the center of property. The area was divided into four quadrants with three concentric ranges with the following distances: 1) from 0 to 50 meters, 2) from 65 to 115 meters and 3) from 120 to 180 meters from the central location where the hives were arranged (meliponary) (Figure 1c, Figure 2).

After 180 days of pollination, the approximate period of formation of the ripe fruit (OLIVEIRA, 2002), the productivity
characters were evaluated. The sample counting was made from thirty six plants randomly distributed in four quadrants and three ranges.

**Figure 2.** Layout of the study area and strips in acai (Euterpe oleracea) cultivation area: (f) ripe fruit collection.

For each mature bunch collected were measured: The Total Weight of fruits per bunch (TWF), expressed in kg; Number of Fruits per Rachilla (NFR), Average Fruit Diameter (AFD), expressed in millimeters (mm); average weight of 100 fruits (W100), expressed in grams (g), (n = 100); the total weight of ripe berries (WRB), the average weight of the hydrated fruits (WHF) in grams (g), fruit yield / fruit dry mass (MSF) in g/g.

The pulping of the acai fruits was performed according to the methodology of Rogez (2000) (Figure 3). The acai pulp was obtained with the ratio (1: 2 v/w), volume (L) of mineral water, and mass (kg) of the fruit. The dry matter content was quantified by drying (ADOLFO LUTZ, 2008). About 2 to 5 g of the pulp obtained was used, in porcelain crucibles and taken to the oven for 24 hours.

**Figure 3.** Pulping steps of acai fruits

**Fruit quality evaluation**

The total phenolic content of acai fruits was determined using the Folin-Ciocalteu colorimetric method (SINGLETON; ROSSI, 1965), adapted by Silva, Rogez, Larondelle (2007). Phenolic content was expressed in milligrams of gallic acid equivalent per 100 g sample (mg EAG / 100g). Quantifications were performed in duplicates and calculations were based on the calibration curve obtained with gallic acid (Extrasynthese, Genay, France). Anthocyanin quantification is based on the method of Askar and Treptow (1993) and optimized by Rogez (2000) based on dilutions with 1.0 and 4.5 buffers at different wavelengths 520 and 690 nm by differential spectrophotometry. The total content of anthocyanins (AT) was calculated as cyanidin-3-glucoside (mg/l) equivalents.

**Statistical analysis**

Firstly, the homoscedasticity and normality of the variable fruit production were verified, respectively by the Brown-Forsyth test and the Lilliefors test, both at p <0.05. No significant differences were observed, the ANOVA analysis of variance model was adopted, and when in at least one of the tests a significant difference was observed, the ANOVA - GLM and the t-student test were used (MYERS, 2012). To determine which factors contributed to the distinction of means, the DMS test was applied (p >0.05). The correlation between the variables was estimated through the Spearman correlation index (AYRES et al., 2007).

**RESULTS AND DISCUSSION**

The crop fidelity of Scaptotrigona aff. postica to the flowers of Euterpe oleracea

Pollen of E. oleracea (Figure 4a, 4b) showed a predominance of 57.87% indicating that the samples are of monofloral origin because they present a quantity of pollens >45%, Smilacaceae pollen - Smilax sp (Figure 4e) showed 26.37%, behaving as secondary pollen. Euforbiaceae pollen (Figure 4d) and Pollen of Psidium guajava (Figure 4e) showed 14.98% and 1.06%, respectively.

**Figure 4:** Pollens identified in honey samples from Scaptotrigona aff. postica. (a and b) Pollen from Euterpe oleracea, in ventral and dorsal disposition, respectively; (c) Smilacaceae pollen - Smilax sp.; (d) Pollen of Euforbiaceae species; (e) Pollen of Psidium guajava (Myrtaceae).

In the experiment where bee foragers returning to the colony, during the pick of opening hours of male flowers, it was found that 100% of the pollen loads were pollen from acai flowers (Figure 1a, 1b).

**Increased productivity in weight and number of fruits**

Based on the productive parameters of acai palm, it was observed higher total weight of fruit (TWF) per bunch, number...
of fruits per rachilla (NFR), average fruit diameter and average weight of one hundred fruits for plants located in the nearest radius to the hives (fi: 0-50 m), showing that pollination by S. aff. postica was very effective in a short distance (Figure 2).

The average weight of the bunch was significantly different (p <0.005) between the palm trees (fi) near the hives, compared to the medium (f2) and distant (f3), indicating a significant increase in acai yield with the densification of S. aff. postica colonies near to plants. Acai fruit bunches with pollination less than 50 m increased TWF and NFR by 3.30 kg and 35.8 fruits per rachilla, compared to 1.12 kg and 1.35 kg, 24.43 and 20.19 fruits per rachilla at 115 m and 180 m respectively (Table 1, Figure 5, Figure 6).

Table 1. Productive impact based on the distance between the bee hives and the inflorescences: TWF - Representative Average of the total weight of fruits per bunch; NFR - Number of fruits per rachilla; AFD - Average fruit diameter; P100 - Average weight of one hundred fruits, SD = standard deviation

<table>
<thead>
<tr>
<th>Distance</th>
<th>TWF (kg)</th>
<th>SD</th>
<th>NFR (un.)</th>
<th>SD</th>
<th>AFD (mm)</th>
<th>SD</th>
<th>P100 (g)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>fi: 0-50m</td>
<td>3.30a</td>
<td>1.20</td>
<td>35.84a</td>
<td>11.63</td>
<td>14.39a</td>
<td>0.61</td>
<td>1.69a</td>
<td>0.14</td>
</tr>
<tr>
<td>f2: 65-115m</td>
<td>1.12</td>
<td>0.69</td>
<td>24.43</td>
<td>10.71</td>
<td>12.58</td>
<td>0.83</td>
<td>1.11</td>
<td>0.21</td>
</tr>
<tr>
<td>f3: 130-180m</td>
<td>1.35</td>
<td>0.85</td>
<td>20.19</td>
<td>9.45</td>
<td>12.29</td>
<td>0.66</td>
<td>1.10</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*p-value was statistically significant p < 0.001

Figure 5. Productivity characters after Acai pollination, according to the distance of the S. aff. postica colonies (fi: 0-50m, f2: 65-115m, f3: 130-180m). TWF - Representative Average of the total weight of fruits per bunch; **p <0.001.

Figure 6. Differences in acai fruit production at different distances from 30 colonies of S. aff. postica where fi = 0-50, f2 = 65-115 and f3 = 130-180 m.

The statistically significant difference, with p <0.0001, between the number of acai fruits/rachilla between the nearby (fi) and medium and distant (f2 and f3) palm trees of native beehives, indicates greater pollination of stigmas per rachilla (Figure 7).

Qualitative parameters of acai fruit size
Estimates of the qualitative parameters on acai fruit are related to the development of average diameter per fruit (DMF) (Figure 8) and the average weight of 100 fruits (P100) expressed in g (n = 100) in irrigated acai tree (Figure 9). The influence of pollination showed significant growth in the fruit size closest to the hives of S. aff. postica. Although, the fruit size not necessarily implicate in higher fruit yield, hence fruits can produce a large size with finer shell pulp.

Figure 7. Productivity characters after Acai pollination, according to the distance of the S. aff. postica colonies (fi: 0-50m, f2: 65-115m, f3: 130-180m). NFR - Number of fruits per rachilla; **p <0.001.

Figure 8. Productivity characters after Acai pollination, according to the distance of the S. aff. postica colonies (fi: 0-50m, f2: 65-115m, f3: 130-180m). AFD - Average fruit diameter; **p <0.001.
With the artificial densification of *S. aff. postica*, there was a significant increase in fruit set, fruit weight and size in clumps near the hives. In Brazil, several crops have a major or essential dependence on pollinators to improve and increase production in fruit monocultures such as nuts, fibers, and oilseeds (GIANNINI et al., 2015).

Regarding the diameter and individual weight of the fruits a significant increase in these parameters was observed, when compared to fruits of more distant clumps, suggesting an increase in factors associated with fruit development.

It was observed in the present study a significant increase in the productivity of acai palms fruits. The area of impact on productivity was greater in the range up to 50 m away from the hives, decreasing with larger distance from the meliponaries. The estimates of the yield parameters obtained showed excellent potential for increasing acai yield in an irrigated crop system. The representative average of the total fruit weight per bunch (TWF) (Table 1), number of fruits per rachilla (NFR), demonstrated a considerable productive gain according to the distances from the hives of *S. aff. postica*. The increase ranged 2.5 times more for the total fruit weight parameter.

According to the results presented here, where the shortest distance between hives and plants resulted in a significant increase in fruit number, weight and size, we can infer that even having *S. aff. postica* a flight capacity above 200 m (ARAUJO et al., 2004), the concentration of their visits to the plants closest to their respective hives indicates that, under the high plant density under crop system conditions used in the area of this study, the acai plants presented sufficient food resources to the needs of the colonies of *S. aff. postica*.

The smaller radius of action of stingless social bee species relative to *A. mellifera* bees makes the control of the flow of genetic material more accurate, preventing focus field pollen from being transmitted to off-property areas, for example (SCHNELL; MACHADO, 2014).

The acai palm is a monocious species and predominantly self-incompatible (VENTURIERI et al., 2004), depends on cross-pollination for fertilization of its fruits. Converting the forest to agricultural crops cause a great reduction in the fauna of natural pollinators, the introduction of native bees from the Amazon is an appropriate option for the increase of production, especially when using *S. aff. postica*, which is already adapted to the Amazonian environment and the resources offered, pollen and nectar, by the acai plant.

According to Venturieri et al. (2014), acai male flowers begin their anthesis by offering more abundant resource than female flowers, which can be interpreted as an incentive for pollinators to first visit male flowers then they go to female flowers, whose climax of offering their nectar at its highest sugar concentration follows the decline in nectar production in male flowers, a period coinciding with the greater receptivity of stigma, later in the morning.

The small size of the *S. aff. postica* (5-6 mm) (ALVAREZ, 2016), compared to other species such as Africanized *A. mellifera* (10-13 mm) (SILVA, et al., 2020), and other larger stingless bees, such as *Melipona* spp (8-15 mm) (SILVA et al., 2011), favors more effective and driven pollination in nearby areas to the hive. A previous study in Belem showed that the acai tree is visited by various forage insects, including native bees, wasps, flies, beetles, and the European bee *A. mellifera*.

Among this great diversity of flower visitors, the species that would have the greatest potential for pollination of acai trees, in crop system in the Amazon Region, would be the species of the genus *Scaptotrigona*, due to the following factors: 1) size, compatible with the flowers of acai tree and the volume of resources offered; 2) behavior, visiting both male
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and female flowers when they are fertile; 3) amount of pollen transported in a suitable place for perfect transference to the stigma (ventral part and legs); 4) ease of handling, they have no sting, offering no harm to the horticulturist and their breeders, the keeping methods are well known in the region (meliponiculture); 5) short radius of action, being confined to the plantation area and 6) fidelity to the flowers of the acai.

Another highlight would be the production of honey and native bee pollen, with high added value, which can provide extra income to acai gardeners, especially because the greater flowering occurs in the inter-crop period of this crop.

The pollen samples analyzed from the pollen and honey pots in Scaptotrigona hives showed about 60% of the pollen corresponding to the plant species Euterpe oleracea. It was identified the floral origin of both pollen and honey as predominantly from acai. Other native plant species from the Amazon region, such as Smilax sp., and Psidium guajava also presented great frequency along the samples of S. aff. postica food source.

Therefore, generally, honey is recognized as monofloral honey when the content of the majority of the pollen is more than 45% of the total pollen (RAMIREZ-ARRIAGA et al., 2011; OLGA et al., 2012; MARTÍN ARROYO et al., 2017).

For Schnell and Machado (2014), this potential importance in pollination, and safe and easy handling, due to the absence of sting, has caused many species of native Meliponini bees to be increasingly studied to be used both in greenhouse plantings, contributing to the increase in the production and quantity of fruits and seeds. The possibility of using other bee species besides the domestic bee also helps to mitigate the health problems that are currently severely decreasing Apis mellifera populations.

In qualitative terms of acai, the values of total anthocyanins and polyphenols evaluated in fruits were not statistically significant. The fruit dry mass content (FDM) did not present significant differences (DIAS, 2012).

The estimates of the yield parameters obtained showed excellent potential for increasing acai yield in an irrigated cultivation. The representative average of the total fruit weight per bunch (TWF) (Table 1), number of fruits per rachilla (NFR), demonstrated a considerable productive gain according to the distances from the hives of S. aff. postica.

CONCLUSION

The use of Scaptotrigona aff. postica provided a significant increase of up to 2.5 times more in the production of acai fruit, among plants located between 0 and 50 meters away from bee colonies. This increase occurred in both fruit size and number of fruits per bunch. These results contribute to both the acai palm horticulture and the importance of using native species of meliponine bees for pollination services.

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