Suggested methodology to evaluate the effectiveness of artificial introduction of stingless beehives in the productivity of assai palm tree orchards

Giulia Rury Venturieri[1], Giorgio Cristino Venturieri[2], Giorgini Augusto Venturieri[1*]

1. Federal University of Santa Catarina, Agroecosystem posgraduate course, Florianópolis, Santa Catarina, Brazil.
2. Brazilian Enterprise for Agricultural Research. Lab. of Botany, Belém, Pará, Brazil.
*Corresponding author: giorgini.venturieri@ufsc.br

ABSTRACT. The assai palm tree (*Euterpe oleracea* Mart.), is native to the Amazon estuary region, and is cultivated extensively for its fruit, which is extracted as pulp and sold throughout Brazil, and increasingly to international markets. Fruit production was evaluated in an upland orchard at two discrete time points: before, and after the introduction of 28 colonies of the stingless bee species *Melipona flavolineata* and *Melipona fasciculata* (14 colonies per species). Initial results indicated that the bees had no influence on fruit yield. However, the experiment was considered as inconclusive due to methodological gaps, and is used here as a case study to discuss critically the validity of the applied methodology. Improvements in experimental design and parameters to be evaluated are suggested.

Keywords: *Euterpe oleracea*; *Melipona fasciculata*; *Melipona flavolineata*; Amazonia.

Sugestões metodológicas para avaliação da efetividade da introdução artificial de abelhas sem ferrão na produção de frutos de açaizeiros

RESUMO. O açaí (*Euterpe oleracea* Mart.) é uma espécie nativa da Amazônia e de seu fruto pode ser extraída uma polpa com propriedades antioxidantes. A sua produtividade foi avaliada em terra firme durante dois momentos: antes e depois da introdução de abelhas sem ferrão, para servirem como polinizadoras, sendo: 14 colônias de *Melipona flavolineata* e 14 de *M. fasciculata*. Não foram observadas diferenças quanto à produtividade, dando a impressão de que as abelhas não tiveram qualquer influência. Entretanto, o experimento foi considerado como inconclusivo devido a lacunas metodológicas, mas aproveitado como estudo de caso para discutir criticamente a validade da metodologia aplicada. Melhorias no desenho experimental e parâmetros a serem avaliados são sugeridos.

Palavras-chave: *Euterpe oleracea*, *Melipona fasciculata*, *Melipona flavolineata*, Amazônia.

1. Introduction

The assai palm tree (*Euterpe oleracea* Mart.) is native to the lowland forests of Amazonia and has recently been labelled as a 'superfood', because of its berries that contain high levels of anthocyanins, a natural source of antioxidants (RUFINO et al., 2010). For this reason, the fruit is now in high demand from both domestic and international markets. It is a self-incompatible monoecious species, and mainly pollinated by bees and flies (VENTURIERI et al., 2014).

The artificial introduction of *Melipona flavolineata* Friese and *M. fasciculata* Smith in beehives, has been previously suggested to improve yield in assai palm trees through more effective pollination of its inflorescences (VENTURIERI et al., 2014). Nevertheless, the simple introduction of pollinators in an orchard is no guarantee that increases in productivity will be achieved, because pollination involves multiple factors, including environmental influences on plants and pollinators; particularities of the plant, such as rewards offered to insect visitors; floral morphological barriers; floral phenology; and genetic inter-compatibility/incompatibility among plants. In addition, although pollination is a critical initial step in fruit production, subsequent environmental conditions and stressors (e.g. water and/or nutrient scarcity), greatly influence the physiological carrying capacity of the plant and thus, harvested fruit yield.

Despite intense visitation by insects, the rate of aborted female flowers in assai palm is typically high, reaching up to 60% by the 180th day after flower opening (VENTURIERI, 2016), an indicator of the existence of some bottleneck in the process, that could be associated with pollination. With better understanding of environmental factors that limit fruit yield in assai orchards, simple adjustments in the orchard management can be made to improve productivity.

In the present study, we use a case study, where fruit set was evaluated, before and after introduction of stingless beehives, to discuss improvements in the experimental design and parameters that must be evaluated to ascertain impacts of managed pollinators in assai palm tree orchards.

2. Material and methods

The experiments were conducted in the municipality of Santo Antônio do Tauá, Pará state, Brazil (1°03'26,27"S 48°08'33,73"W), in an eight-acre upland orchard. According to the Köppen classification system, the climate type is Af (PEEL et al., 2007). Tree clumps (multi-stemmed genets) were evenly-spaced in rows of 2 x 3.5m, and were seven years old at the time of study.

At the time of planting, no additional irrigation, fertilization or pruning of the palm stems had been conducted (extensive management protocols). The experiments were conducted in the transition between wet and dry seasons, when the rainfall in the region had begun to decrease and assai fruiting is at its lowest level. Two areas within the orchard were marked out for study; at the orchard edge, less than 50 m from an surrounding natural forest habitats, and in the orchard center, more than 50 m away from the orchard edge. Edge plots were mainly considered as being naturally pollinated because they were closer to the niche of the natural pollinators. In each area, 40 inflorescences were sampled, one per clump. Two evaluations were made in these areas, the first between March 14 and 15, 2012, prior to the introduction of stingless bee colonies; and the second aprox.

70 days later, between June 5 to 6, 2012, following the introduction of 28 beehives (14 of *M. fasciculata* and 14 of *M. flavolineata*) in the certer area, where evaluations were made 15 days after the introduction of the beehives so that they could be adapted. The number of newly formed fruits, *i.e.* those with diameter up to 0.5 cm, on nine rachillae, was counted on marked bunches in both areas 15 days after female flowers had their stigma exposed. The objective was to compare the productivity, both before and after the introduction of the colonies to determine the influence of managed pollinations on fruit productivity. Mean values and 95% confidence intervals of fruit production at the center and the edges of the orchard, were calculated at both time points. Descriptive statistics and graphs were generated using Statistica 6.0 software (Statsoft, Tulsa, Oklahoma, EUA).

3. Results and Discussion

Considering that averages at the center and edges were equal prior to the introduction of beehives, eventual differences found in the second assessment, after their introduction, where fruit set was marginally elevated in edge sites, could be considered a consequence of managed pollinator introductions. However, there was no difference in the number of fruits per rachilla sampled in the center or at the orchard edge, before, and after the introduction of the beehives, thus an indicative that the presence of beehives had no influence on the fruit productivity (Figure 1).
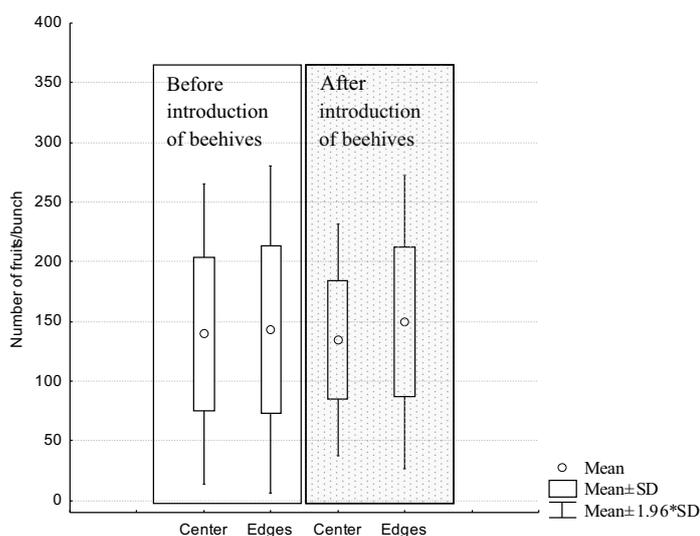

Figure 1. Number of fruits in nine rachillae from plants at the center and at the edges of an assai palm tree (*Euterpe oleracea*) orchard. / **Figura 1.** Número de frutos em nove ráquilas de plantas localizadas no centro e nas bordas de um pomar de açaizeiros (*Euterpe oleracea*).

Methodologically, this approach is limited, as the experiment was conducted in one area only, without replication, and was therefore unsuited to use of more rigorous statistical testing. The use of immature fruits as a parameter for evaluating the effectiveness of the introduced colonies in pollination is also not sufficient, because the fruit set could be under influence of various factors, including effects of environmental conditions on pollinator behavior, for example, the availability of alternative floral resources in the surrounding habitats (e.g. forested areas); the capacities of the plant to carry

fertilized flowers until fruit are mature; and the action of late genetic incompatibility reactions (VENTURIERI, 2016). Thus, study of both initial and final fruit set is recommended for assessment of pollinator contributions to fruit yield. Furthermore, upland areas are generally regarded as nutrient-poor and water deficient, hence, the impact of managed pollinator introductions could be limited due to either lack of adequate nutrients in the soil or water scarcity. Suggestions for further experiments would be as follows: to increase the number of replicate sites to enable use of statistical tests; an evaluation of the number of pollen grains deposited on stigmas of flowers to directly assess pollination function before and after beehive introductions; and accompaniment of fruit until harvest, in areas that received fertilizer and irrigation, and in areas that did not receive them, to assess the interaction between pollination and other possible limiting factors. It is also suggested that the growth of pollen tubes, on both styles of aborted flowers and blooms that remained on the rachillae are assessed, to quantify the effect of the beehives on transfer of compatible and incompatible pollen.

4. Acknowledgments

5. References

PEEL, M. C.; FINLAYSON, B. L.; McMAHON, T. Updated world map of the Köppen-Geiger climate classification. **Hydrology and Earth System Sciences**, v. 11, 1633-1644, 2007.

VENTURIERI, G. R. **Ecologia da polinização do açaizeiro (Euterpe oleracea) com e sem a introdução de colônias da abelha uruçu-amarela (Melipona flavolineata).** Dissertação (Mestrado) Universidade Federal de Santa Catarina, Florianópolis, 2016.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F.; MANCINI-FILHO, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. **Food Chemistry**, v. 121, 996-1002, 2010.

VENTURIERI, G. C.; SOUZA, M. S.; CARVALHO, J. E. U.; NOGUEIRA, O. L. Plano de manejo para os polinizadores do açaizeiro Euterpe oleracea (Arecaceae). In M. Yamamoto, M; P. E. Oliveira, M. C. Gaglianone (Ed.). **Uso sustentável e restauração da diversidade dos polinizadores autóctones na agricultura e nos ecossistemas relacionados: plano de manejo.** Brasília: Ministério do Meio Ambiente, p. 97-129, 2014.