Acta Scientiarum. Animal Sciences
ISSN: 1807-8672
Editora da Universidade Estadual de Maringá - EDUEM

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Acta Scientiarum. Animal Sciences, vol. 42, e47421, 2020
Editora da Universidade Estadual de Maringá - EDUEM

DOI: https://doi.org/10.4025/actascianimsci.v42i1.47421

Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v42i1.47421

Bees biodiversity, forage behavior and fruit production in gherkin crop (*Cucumis anguria* L.)

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ABSTRACT. The objectives were to evaluate the biodiversity of bees, forage behavior and their effect on fruit production in the gherkin crop (*Cucumis anguria* L.) in the campus of the University Center Moura Lacerda in two years. The frequency and type of collection of the insects in the flowers was observed by counting from 8:00 a.m. to 5:00 p.m., in the first 10 minutes of each time, for three distinct days in each year. The percentage of fruiting was quantified in 25 female flowers covered with nylon compared to the 25 female flowers uncovered in the two years. The flowers were visited by the Africanized honey bees *Apis mellifera* and the native bees *Plebeia* sp., *Exomalopsis* sp. and *Melissodes* sp., and the Africanized honey bees presented higher frequency and constancy with a higher number of visits in the male flowers compared to the female ones and these visits occurred between 8:00 a.m. and 3:00 p.m. Without the visitation of the bees there was no fruit production, and both the Africanized honey bee and the native ones when collecting nectar and pollen, visited both female and male flowers, carrying pollen in their body, being considered important pollinators of this culture.

Keywords: Africanized honey bees; *Apis mellifera*; native Brazilian bees; pollination.

Received on April 9, 2019.
Accepted on May 7, 2019.

Introduction

Costanza et al. (2017) reported that pollination is considered an ecosystem service that provides benefits to humans, which include the maintenance and genetic variability of native plant populations that sustain biodiversity and ecosystem functions, diverse supply of fruits, seeds, bee products, among others, and the promotion of cultural values related to traditional knowledge. In food production, the global economic valuation of the ecosystem service of pollination was estimated at € 153 billion (Gallai, Salles, Settele, & Vaissière, 2009). In Brazil, it is estimated that pollination related to agricultural production has an annual value of US $ 12 billion (Giannini, Cordeiro, Freitas, Saraiva, & Imperatriz-Fonseca, 2015; Malerbo-Souza & Silva, 2011).

In the tropical communities, Ollerton, Winfree, and Tarrant (2011) concluded that 94% of the plants are pollinated by animals, most of them pollinators such as bees, flies, butterflies, moths, wasps, beetles and thrips. Among these insects, bees are the most abundant group of pollinators in agriculture visiting over 90% of the 107 main agricultural crops (Klein et al., 2006). Among the agricultural crops, a group of plants studied for pollination is the Cucurbitaceae family which mostly have separate female and male flowers, being dependent on pollinators to ensure the production of their fruits (Serra & Campos, 2010).

The Cucurbitaceae family has several species of economic importance with about 90 genera and 750 species adapted to the tropical and subtropical regions. Several cucurbits are used in food: *Cucurbita pepo* (Vidal, Jong, Wien, & Morse, 2010), *C. moschata* (pumpkin), *C. maxima* (moranga or jerimum), *Sechium edulis* (chayote), *Citrullus lanatus* (watermelon), *Cucumis melo* (melon), *C. sativus* (cucumber), and *C. anguria* (gherkin) which have a high commercial value besides the bush (*Luffa cylindrica*) used as a bath sponge (Krug, Alves-dos-Santos, & Cane, 2010).

Gherkin is a plant originated in African, rich in zinc, an important mineral for the proper functioning of all tissues of the body, as well as for the metabolism of sugar and proteins; it presents antioxidant activity in the fight against free radicals; has no toxic effect on the animal body and has considerable amounts of total phenolic compounds capable of degrading free radicals. It grows sub-spontaneously in areas cultivated with other species such as beans and corn, and cultural practices are not carried out, which results in low fruit yield and quality. Being a very resistant plant during its development, pesticides are not used, being considered an organic vegetable that can be consumed "in natura" and in the form of preserves (Souza Neta et al., 2018).

This plant has a low growth habit with an angular stem, rigid and hairy; every 5 to 7 cm, has curled tendrils that leave the petiole of the leaves, which have deep lobed cuts and have alternate arrangements, not having stipules; at each internode, there are two to four male flowers, pale yellow in color, and a female flower, which has an infertile ovary developed, which may or may not fructify (Souza Neta et al., 2018). After 50 days of sowing, flowering and fruiting begin and harvesting takes place around 60 to 70 days after sowing extending for three months or more (Modolo & Costa, 2003).

The objectives of this study were to evaluate the bees biodiversity, their forage behavior in flowers and the effect of these visits on gherkin production in Ribeirão Preto, SP, Brazil.

Material and methods

This experiment was carried out in the campus of the University Center Moura Lacerda, in the city of Ribeirão Preto, SP, Brazil, in two consecutive years (2015 and 2016). The altitude of the site is 544 meters with the following geographical coordinates: 20º33'26'' south latitude (S) and 48º34'04' 'west longitude (W), with temperate subtropical climate Köppen and Geiger (1928) climate classification: Aw), annual average temperature of 21°C and average annual rainfall of 1,500 mm.

The gherkin crop was installed in 2015 September and 2016 September and the beginning of the flowering occurred from October extending until December in both years. The total area of the plot was 30.0 square meters, being 10.0 meters long and 3.0 meters wide. Two to three seeds per plant were planted with spacing of 0.5 meters between plants and 1.0 meter between the lines, and only organic fertilizers (earthworm humus and cow manure) were used to conduct the crop.

For each year studied, the attractiveness of the male flowers to the female flowers was evaluated, during the observation of the frequency of the visits, the percentage of insects present in the male and female flowers throughout the day was observed. The insects visiting the gherkin flowers were photographed and identified in comparison to the entomological collection of the authors. The foraging behavior of these insects was evaluated through visual observations during the day, in the experimental period (flowering), in both years.

The frequency of visits and the type of collection (nectar and/or pollen) of the visiting insects were evaluated during the day in the gherkin flowers. These data were obtained by counting within the first 10 min of each time, from 7:00 a.m. to 6:00 p.m., with three replications, during three distinct days, between October and December, in both years (2015 and 2016). This was done by walking around the flowerbed, noting the insects present in the flowers and what they were collecting (nectar or pollen) (Malerbo-Souza & Silva, 2011; Malerbo-Souza, Silva, Andrade, Farias, & Guedes Medeiros, 2018).

The constancy of the species was determined by the formula: C = (P x 100) / N where: P = number of samples containing the species (total daily samples) and N = total number of collections made. According to the percentages obtained, the species were separated into the following categories: constant species - present in more than 50% of the collection species present in less than 25% of the collections (Marchiori & Linhares, 1999). To evaluate the percentage of fruiting, 25 female flower buds were protected from nylon to prevent the visitation of insects (covered treatment) and 25 female flower buds were marked and remained uncovered (uncovered treatment), and the fruits obtained in both treatments were harvested, counted and weighed with precision digital scale, during the experiment, in the Institution's laboratory (Gamito & Malerbo-Souza, 2006).

All data were analyzed using the statistical program Biostat, to analyze the differences between the averages, in the years studied, the Tukey test was used at 5% and to analyze the frequency of visitation of the insects to the flowers, during the day, we used regression analysis by orthogonal polynomials, thus obtaining equations suitable to the observed patterns, under the conditions of the experiment.

Results and discussion

The flowering started 30 days after planting and its duration was 55 ± 3 days, the male and female flowers remained open for one day (Figure 1), initiating the opening close to 7:30 a.m. and closure close to 4:00 p.m.. The relationship between male and female flowers was, on average, 5.55 male flowers for each female one. After 75±6 days, the culture showed yellowing of its leaves and showed no more flowers being eradicated.

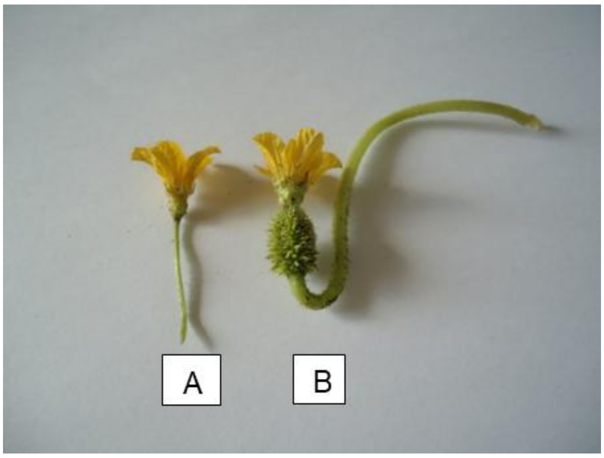

Figure 1. Side view of the male (A) and female (B) flower of the gherkin (*Cucumis anguria*) in Ribeirão Preto, SP, Brazil, 2016 (Personal collection).

Firstly the emission of male flowers was observed, remaining an increased number compared to female flowers throughout the flowering period. This characteristic was also observed in the watermelon (Malerbo-Souza & Silva, 2011; Souza & Malerbo-Souza, 2005), pumpkin (Lattaro & Malerbo-Souza, 2006) and cucumber (Nicodemo, Malheiros, De Jong, & Couto, 2012). The greater number of male flowers compared to female flowers ensures a greater flow of pollen in the crop, ensuring pollination, provided there is the pollinating agent. The greater number of male flowers besides serving as an initial attraction for visitors for supplying pollen and nectar are important in determining the flow of pollen in the population. The presence of separate female and male flowers (monoecious plant) shows the need for the transfer of pollen grains by biotic vectors, especially by bees. The attractiveness of a flowering plant can be influenced by endogenous plant characteristics such as the amount of nectar and pollen available to bees, and the concentration of flowers in the plant (Ramalho, Silva, & Carvalho, 2007).

Only insects were observed visiting the male and female flowers of gherkin. The observed (Figure 2) insects were Africanized honey bees *Apis mellifera* (72.2%, on average) and native bees (27.8%, on average) being *Plebeia* sp. (16.7%), *Exomalopsis* sp. (8.3%) and *Melissodes* sp. (2.78%). Sporadic visits of lepidopterous were observed on gherkin flowers.

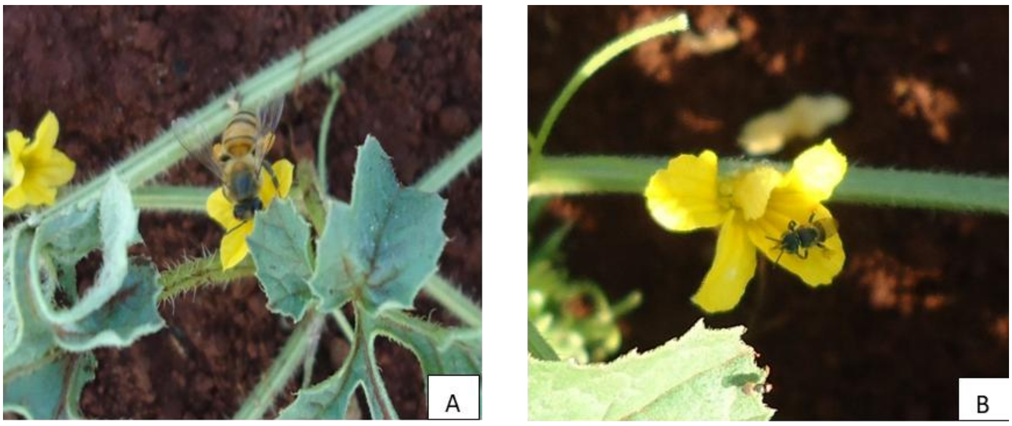

Figure 2. Africanized honey bee *Apis mellifera* (A), stingless bee *Plebeia* sp. (B), solitaries bees *Exomalopsis* sp. (C) and Melissodes sp. (D) (as fotos dessas abelhas foram removidas, inseri-las novamente) collecting pollen on the male flowers of the gherkin (*Cucumis anguria*) in Ribeirão Preto, SP, Brazil, 2016 (Personal collection).

On average, in the two years observed, Africanized honey bees visited the gherkin flowers from 8:00 a.m. to 3:00 p.m. decreasing their frequency throughout the day obeying the equation $\hat{Y} = -0.5545x + 5.6909$, where the variable $\hat{Y}$ is the number of bees estimated and the variable X is the time of the day in which the visits occurs. The Africanized honeybees had a higher occurrence of visits in male flowers (84.6%) compared to the female ones (15.38%) in gherkin (Figure 3). Africanized honey bees visited from 8:00 a.m. to 3:00 p.m. to collect nectar and pollen in male flowers decreasing their frequency throughout the day: $\hat{Y} = -04545x + 4.7273$. Africanized honey bees visited the flowers from 8:00 a.m. to 2:00 p.m. in the female flowers for nectar collection decreasing their frequency during the day ($\hat{Y} = -0.1x + 0.9636$, $R^2 = 0.2420$). Lattaro and Malerbo-Souza (2006) also observed high foraging activity of Africanized honey bees, at 8:00 am, predominantly for pollen collection, observing a low number of bees visiting female flowers in *C. maxima* in Ribeirão Preto, SP.

The male flowers were visited for both pollen and nectar collection and the female flowers for nectar in the two years. In the Cucurbitaceae, female flowers produce nectar and the male flowers produced pollen and nectar, but in a smaller quantity, a strategy to attract pollinators. Carneiro Neto et al. (2018) stated that the male flowers of the gherkin produced nectar in greater quantity after 1:00 a.m., and suggested that it could be due to the lower availability of pollen in these flowers needing a greater volume of nectar to attract floral visitors. These authors also evaluated the average number of pollen grains per anther and observed that for the Liso variety it was 1,843.66 ± 67.67, with an average of 5,530.98 grains per flower, and for the Crioulo variety it was 2,099, 33 ± 78.62 per anther, and in average 6,297.99 grains per flower, presenting a significant difference.

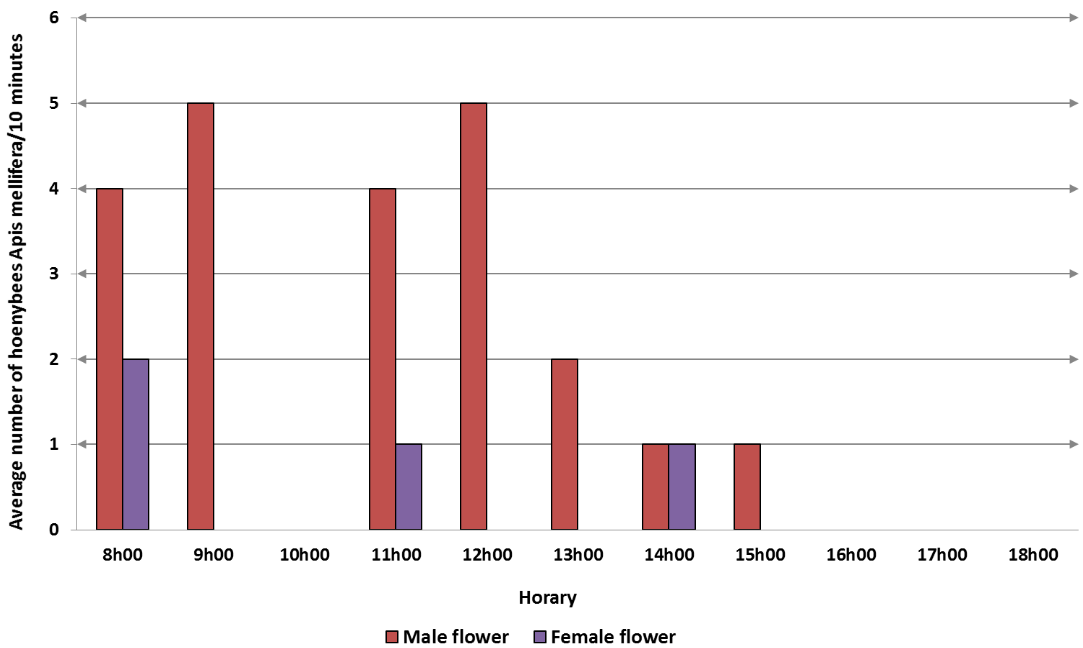

Figure 3. Average number of Africanized honeybees *Apis mellifera* in the male and female flowers of the gherkin crop (*Cucumis anguria*), in Ribeirão Preto, SP, Brazil, in 2015 and 2016.

The Africanized honey bees landed on the flower petals and walked to the corolla tube to access the nectar. In male and female flowers, these bees access to the nectary occurred by introducing the gloss. When collecting nectar in the male flowers, his body touched the anthers and the pollen adhered to his hair and when visiting female flowers this pollen was deposited on the stigma. These bees went from flower to flower, sometimes on the same plant, others on different plants. Due to their size in relation to the size of the flowers of the gherkin, when they reached the nectary, they touched the reproductive parts of the flower and visited several flowers, both male and female, being considered pollinators of the gherkin. The stingless bees *Plebeia* sp. and the solitary bee *Exomalopsis* sp. were observed visiting the male and female flowers of the gherkin and, although they were in smaller numbers and the stingless bee *Plebeia* sp. being smaller than the *A. mellifera*, were considered pollinators of the gherkin because they carried pollen in their body and they touched the stigmas of the female flowers.

Costanza et al. (2017) stated that in order to be classified as a pollinator of a plant species it is necessary that the potential pollinator be attracted by the flowers of the crop, which shows constancy to that species, having adequate size and behavior to remove the pollen of the stamens and to deposit them in the stigmas, carrying in their body much viable and compatible pollen, and visiting the flowers when the stigmas are receptive. In the gherkin, Africanized honeybees and native bees met most of these requirements being considered efficient pollinators, moving rapidly zigzagging the flowers when collecting nectar and pollen possibly making pollen dispersion more efficient.

The Africanized honey bee was considered a constant species in male flowers (62.5%) and an accidental species in the female flowers (9.1%) of the gherkin. Native bees were considered accidental species in the gherkin flowers (6.1%, in both species). Although they were accidental species, the constancy index was important in the fruiting of the gherkin and was observed that in the collection of nectar and pollen, the native bees visited several flowers in the experimental area before flying out of the crop. This behavior may have promoted the transfer of genetic material from one plant to another characterizing it as a pollinator (Oliveira & Rech, 2018).

In the two observed years (2015 and 2016), in the female flowers that remained covered with nylon, without the visitation of the insects, there was no fruiting, proving that no self-pollination occurred in the gherkin crop, as it occurs in other Cucurbitaceae (Lattaro & Malerbo-Souza, 2006; Malerbo-Souza & Silva, 2011; Nicodemo et al., 2012; Souza & Malerbo-Souza, 2005). In the female flowers that were visited by insects there was, on average, 69.0 ± 2.0% of fruiting and the average weight of the fruits was 22.7 ± 2.80 g, and there was no significant difference between the years. It is evidenced that the production of gherkin depends on the cross pollination performed by bees and verified the importance of the preservation of these pollinators, be they Africanized or native bees. There is a need for farmers to be aware of the value of bees for gherkin production to preserve and/or reforest the areas close to their plantations, with species of plants that bloom in different months of the year, this is necessary for the maintenance of the colonies of bees, both Africanized honey bees and native bees. Many farmers exterminate solitary bees like *Xylocopa* spp. effective pollinators of yellow passion fruit (*Passiflora edulis f. flavicarpa*) thinking they are beetles. Deforestation, burning and intensive use of agrochemicals, often incorrectly used, cause destruction of the natural habitat of pollinators, poisoning both bees and their products, such as honey and pollen (Pires et al., 2016).

Data from the Thematic Report on Pollination, Pollinators and Food Production in Brazil, launched by the Brazilian Platform for Biodiversity and Ecosystem Services (Wolowski et al., 2019) showed that bees are the largest group of pollinators and represent about 48% of all species identified as floral visitors of crops linked to food production. These species are associated with 132 (92%) crops being recognized as pollinators of 91 of them and constituting exclusive pollinators of 74. Pedro (2014) stated that the Africanized honey bee was considered a potential pollinator of 54 of the 86 crops used in food production, while stingless bees were registered as floral visitors of 107 crops and as pollinators of 52. In addition, farmers should be informed to avoid applications of pesticides in the flower stage

Conclusion

The gherkin crop is visited by different bee species both Africanized honey bees *Apis mellifera* and native bees like stingless bees (*Plebeia* sp.) and solitary bees (*Exomalopsis* sp and *Melissodes* sp.). These bees prefer to visit the flowers from 8:00 a.m. to 3:00 p.m. and when collecting nectar and pollen, they visit both female and male flowers, carrying pollen in their bodies, characterizing them as effective pollinators of that culture. The flowers that were not visited by the bees did not produce fruits proving the necessity of these pollinating agents for the production of fruits.

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