Behavior of *Apis mellifera* L. Africanized honeybees in sunflower (*Helianthus annuus* L.) and evaluation of *Apis mellifera* L. colony inside covered area of sunflower

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ABSTRACT. This research was carried out to evaluate the behavior of africanized honeybees, *Apis mellifera* L. (Hymenoptera, Apidae) and the development of a colony inside a covered area of sunflower, *Helianthus annuus* L. (Asteraceae) crop. It was used an area marked with 8 m x 3 m, and a second area in which a honeybee colony was installed inside a covered area with wire screen of 8m x 3m, two meters high in the highest part. The honeybees were constantly on sunflower during all day, however the Halictidae bees had been the most frequent in pollen harvesting, but these bees were seen until 11 a.m. In covered area, it occurred a reduction of 34.74% in swarm weight, 38.08% in honey, 100% in worker pupae, and 99.37% in egg-larvae. The honeybees gathered more nectar than pollen during the day. The nectar forager has more effect upon crop pollination than pollen foragers and pollen/nectar foragers, in sunflower.

Key words: honeybees, *Apis mellifera*, sunflower, *Helianthus annuus*, pollination.

RESUMO. Comportamento de *Apis mellifera* L. africanizada em flor de girassol (*Helianthus annuus* L.) e avaliação do desenvolvimento de uma colônia de *Apis mellifera* em área de girassol coberta. O presente experimento objetivou avaliar o comportamento polinizador da *Apis mellifera* L. (Hymenoptera, Apidae) africanizada e o desenvolvimento da colônia em área coberta de girassol, *Helianthus annuus* L. (Asteraceae). Foi utilizada uma área somente demarcada de 8 m x 3 m, e o desenvolvimento da colônia dentro de uma gaiola telada com 8 m x 3 m com dois metros de altura na sua porção mais alta. A abelha mais constante visitando o girassol durante todo o dia foi a *Apis mellifera*, embora os halictídeos tenham sido os mais freqüentes coletores de pólen, sendo estes vistos somente nas manhãs até às 11h. Na área coberta, houve uma redução de 34,74% no peso do enxame, 38,08% de mel, 100% no número de pupas de operárias e uma redução de 99,37% no número de ovos/larvas. As abelhas coletaram mais néctar do que pólen uniformemente ao longo do dia. No girassol, as abelhas coletoras de néctar têm influência na polinização da cultura maior do que as coletoras de pólen e néctar/pólen.

Palavras-chave: abelhas, *Apis mellifera*, girassol, *Helianthus annuus*, polinização.

Introduction

Cross-pollination had its importance recognized long time before its biological meaning was understood. In the beginning of 1909, the most progressive and farming innovators began to rent honeybees colonies for pollination. The knowledge about the pollination control opened a new era for the beekeeping. Now, it can be affirmed that no other pollinator insect can be more easily administered and manipulated than *A. mellifera*. For this reason, *A. mellifera* continues being recognized as an important insect for humans on honey production as well as for the pollination of crops (Gojmerac, 1980).

Offering food is an important factor for the colony development. Roberts (1979) demonstrated that, according to the age of the plant, there is a variation on sugar concentration of the nectar, decreasing along the time, as well as on the nectar gathering frequency by the insects.

The pollination service and the honey production may be enhanced if the beehives closeness in relation to the crop. The performance of honeybees in the pollination depends on many factors, such as pollen and nectar availability, climate conditions and physical aspects of the land (Free, 1993).

The ideal condition is to place the colonies in the middle of the crop, although that distribution depends on the farmer's handling (Free, 1993). Some authors, for example Cirnu (1960), mentioned by McGregor (1976) and Free (1993), recommend two colonies per hectare for sunflower pollination. While Furgala (1954) and Sundeleac (1965), mentioned by McGregor (1976) and Free (1993), considering that one colony per hectare is enough for sunflower pollination.

According to McGregor (1987), the production of sunflower seeds needs insects pollination. The area of cultivation of sunflower seems to increase every year and it is necessary a pollination program of insects. It is expected an increase of its production with the use of an efficient pollination program using honeybees.

Basualdo *et al*. (2000), studying the pollen harvesting by Africanized and European honeybees (*A. mellifera* L.) in a field of sunflower for hybrid seeds, in Argentina, observed that Africanized honeybees gathered the largest amount of pollen, concluding that they can be the most efficient bees for the pollination of sunflower crop for the commercial production of seeds.

This experiment was carried out to evaluate the behavior of bees, mainly Africanized honeybees in sunflower flowers, *Helianthus annuus* L. (Asteraceae) and to evaluate the development of *Apis mellifera* (Apidae) colony in a specific sunflower area.

Material and methods

The experiment was carried out at Fazenda Experimental de Iguatemi at Universidade Estadual de Maringá (UEM) - Paraná state. Maringá is a city located in the northwest of Paraná, with an average annual temperature of 21.9ºC, and altitude of about 554 m (source: Department of Meteorology of UEM). The *H. annuus* sunflower variety Embrapa 122-V 2000 used, was developed by the Empresa Brasileira de Pesquisa Agropecuária through its National Soybean Research Center (Embrapa - Soja).

This research was developed from October 1999 to February 2000 in an area of 1986 m^2, which was demarcated (24 m^2 each), with three replications. The pollination cages were installed, made out of nylon with screen of two millimeters, three meters wide, eight meters long and two meters high in the highest part, protecting an area of 24 m^2. The treatments consisted of T1 - area just demarcated, discovered and freely visited by insects; T2 - cage with a colony of *A. mellifera* honeybees - area only visited by honeybees; T3 - cage without honeybees - area not visited by insects like control, the seeds produced were by auto pollination.

The cages were built some days before the flowering and outlying at the end of the flowering, close to honeybee colony. The sunflower culture was evaluated daily from the whole flowering phase to the seeds ripening phase. These sunflower seeds were planted on October 20, according to recommendations of Embrapa Soja. Ten days before the flowering, the toss and the divisions of the areas were set. Cages were built seven days before the flowering to allow the complete vegetative development of the plants. The sunflower culture was evaluated daily during the whole flowering phase to the phase of the seeds ripening.

The evaluations of bee behavior on the sunflower flowers were done in a daily base during the period, 20 minutes every hour, taking note of the bee species in activity in the schedule, the type of harvest that the honeybees *A. mellifera* were gathering (nectar and/or pollen) and the time spent in this activity.

The insects visiting the crop were captured with an entomological net. The bees and other collected insects were separate and placed in recipients isolated for conservation, for posterior identification and estimate of the most frequent species. The samples have been sent to Departamento de Biologia Geral at Universidade Estadual de Londrina for identification.

The average number of achenes, the average number of florets and the average number of bees expected in the experimental area were calculated, according to the methods described by McGregor (1976) and Free (1993).

The development of the colony was evaluated through combs mapping, according to the adapted method of Al-Tikrity *et al.* (1971), counting the area occupied by worker egg/larvae, worker pupae, honey, pollen, drone egg/larvae and drone pupae. The nucleus had its combs mapped in the beginning of the experiment, when it was placed in the cage, 15 days after and at the end.

The nucleus was weighed with and without adult honeybees to obtain the estimate of the adult honeybees number in the colony, and a hundred workers were weighed for an average weight worker.

In the last mapping, twelve grams of pollen of the colony were collected and frozen for posterior bromathological analysis. The crude protein and ether extract analysis of the grains were realized in the samples of the three treatments, following the method described by Silva (1990).

The data were statistically analyzed using a completely randomized design. After the variance analysis, the averages were compared, using the Tukey's test (Pimentel Gomes, 1990), using the GLM procedure of Statistical Analysis System (Sas Institute, 1998).

Results

The following bees species were found visiting the flowers of sunflower crop:

a) Family Apidae

a.1) Subfamily Apinae (88.47%)

Collected species: *Apis mellifera* L.

a.2) Subfamily Meliponinae (8.78%)

Collected species: b.1) *Trigona hialinata* Lep.

b.2) Scaptotrigona bipunctata Lep.

c) Family Halictidae (2.65%)

Unidentified species

d) Family Andrenidae (0.11%)

Collected species: *Oxaea flaveacens* Klug

The Halictidae were the most frequent bees for food harvest (101.28 ± 94.25 seconds, only pollen), followed by *A. mellifera* (71.45 ± 83.36 seconds, pollen and nectar). Along the day, the honeybee remained in the flowers from 11 to 115 seconds, in both, uncovered demarcated area and the honeybees cage (Figure 1).

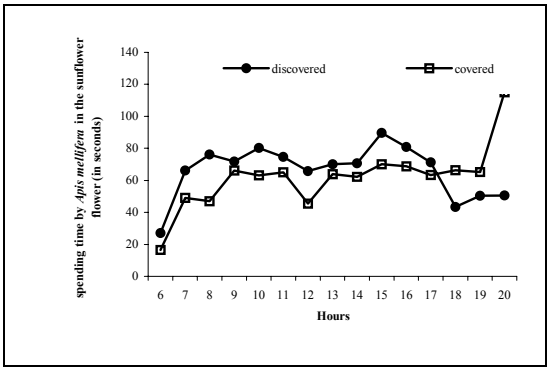

Figure 1. Time of food harvest (in seconds) of the *Apis mellifera* workers in sunflower (*Helianthus annuus* L.)

Considering the type of harvest on the sunflower heads, the main activity was nectar gathering (69.39%). The nectar/pollen and pollen gathering were below that level (16.46% and 14.18%, respectively).

It was observed that the pollen collectors went quickly over the florets, in zigzag and they scrubbed their body with their legs periodically, accumulating the pollen in the corbicula. The nectar collectors settled among the florets, forcing the head to reach the nectary. When leaving the sunflower, the worker honeybees flew fast, with the head turned and sometimes landed on the sunflower head again, continuing the harvest of pollen and/or nectar. This behavior occurred more frequently in pollen harvesting. Seemingly when they used the resources or the load for transport was already complete, the working honeybees went straight to their beehive.

The number of nectar worker collector honeybees was high during the whole day, and the highest activity was at 2 p.m. (Figure 2). The pollen and nectar/pollen collectors presented a similar behavior with higher gathering of pollen at 9 a.m. and nectar/pollen at 5 p.m.

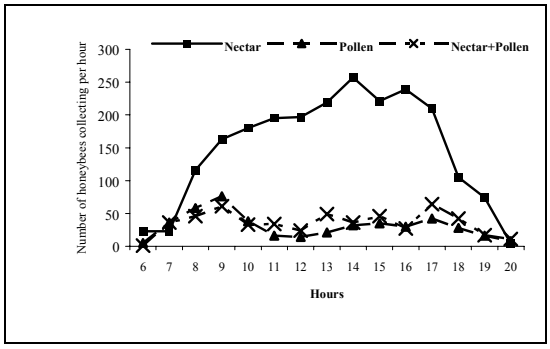

Figure 2. Number of *Apis mellifera* honeybees gathering nectar, pollen or nectar + pollen by hour in sunflower flowers *Helianthus annuus*

There was statistical difference among the types of harvest of food by the honeybees and there was interaction between treatment and type of harvest, although there was no significant difference between treatments (Table 1). The unfolding of the interaction treatment and type of harvest is showed in Table 2. There was significant difference among time of harvest inside the treatments. The time of nectar gathering was longer in uncovered area (72.88 ± 85.34 seconds) and the time of pollen gathering longer in honeybees covered area (54.55 ± 56.69 seconds).

The development of this *A. mellifera* colony in sunflower covered area decreases in weight. The

queen posture of male eggs did not occur in the period. The weight of colony in the beginning of the experiment was greater (0.95 kg with an estimate of 15833.33 workers) than at the end (0.62 kg with an estimate of 10333.33 workers). There was a progressive decrease in the development of the colony and consequently a decrease in weight. The variation in larvae and food occupied area is showed in Table 3. Initially, there was an increase in the pollen reservation, between the first and the second mapping.

Table 1. F Values with its respective probability (P), coefficient of variation (CV%) and averages of the time of food harvest (nectar, pollen and nectar + pollen) in uncovered demarcated area and in the cage system with honeybees *Apis mellifera*, adjusted for the hour as covariate

Variation source	Time of harvest (seconds)
Treatments (T)	0.77 P=0.3801
Type of harvest (H)	31.76 P=0.0001
Interaction T*H	5.91 P=0.0028
Hour (covariate)	0.03 P=0.8634
CV (%)	109.18
Means uncovered area[1]	71.45 ± 83.36
cage system with honeybees[2]	62.75 ± 62.86
Type of harvest	
Nectar	66.47 ± 75.06
Pollen	49.59 ± 54.35
Nectar and Pollen	86.02 ± 79.79

[1]area just demarcated of 8 m x 3 m, discovered and freely visited by insects; [2]cage with 8 m x 3 m contends in its interior a colony of honeybees *A. mellifera* - area only visited by honeybees

Table 2. Unfolding of significant interaction (presented in Table 1) with F values with its respective probability (P), coefficient of variation (CV%) and averages of the time of food harvest (nectar, pollen and nectar and pollen)

Variation source	uncovered area[1]	cage system with honeybees[2]
Type of harvest	16.89 P=0.0001	22.44 P=0.0001
CV (%)	115.56	98.81
Means Nectar	72.88 ± 85.34 b[3]	58.27 ± 59.99 b
Pollen	43.37 ± 50.71 c	54.55 ± 56.69 b
Nectar and Pollen	89.05 ± 89.80 a	83.82 ± 71.78 a

[1]area just demarcated of 8 m x 3 m, discovered and freely visited by insects; [2]cage with 8 m x 3 m contends in its interior a colony of honeybees *A. mellifera* - area only visited by honeybees; [3]Means followed by different letters, in the same column, differs statistically by the Tukey's test

Table 3. Percentage of occupied area with eggs/larvae, pupae, honey, pollen, brood (eggs/larvae + pupae) food (honey and pollen) and total area in the beehive inside covered area with sunflower

	Eggs/Larvae	Pupae	Honey	Pollen	Brood	Food	Total
17/12/1999	18.14	2.52	30.95	0.46	20.65	31.41	52.06
31/12/1999	2.46	0.29	21.45	1.32	2.75	22.77	25.51
10/01/2000	0.11	0.00	19.16	0.74	0.11	19.91	20.02
Mean values	6.90	0.93	23.86	0.84	7.84	24.69	32.53

The bromathological analysis of the gathered pollen showed 17.64% of crude protein, 0.13% of ether extract and 1.74% of ashes in dry matter.

Discussion

The values found in the evaluation of the pollinator behavior in uncovered area were 69.39% of nectar gathering, agreeing with Moreti (1989) that, in her research about sunflower, found that *A. mellifera* was the bee with larger unfailing in schedule terms and type of harvest. It was found a greater activity at 2 p.m., in disagreement with Moreti (1989) that found a higher activity between 8 and 10 a.m. However, it is in accordance with Bailéz *et al.* (1987) that sunflowers in Argentina found higher activities of *A. mellifera* in the afternoon, after 2 p.m. It means that in this research we had more nectar collector and these worker honeybees are very important to this variety of sunflower in Maringá region.

In three varieties of sunflower, in three different times - 9 a.m., 11.30 a.m. and 4.30 p.m. - Schinohara *et al.* (1987) concluded that *A. mellifera* was the most frequent bee mainly at 4:30 p.m. Butignol (1984) and Marchini *et al*. (1984) verified in their researches that *A. mellifera* was the predominant bee on the sunflower. Butignol (1984) found 91.6% of *A. mellifera* among the collected insects. Kerr *et al.* (1970), studying the biology of Africanized honeybees (*A. mellifera scutellata*), Italian (*A. mellifera ligustica*) and its hybrid ones, observed a higher activity of *A. m. scutellata* at about 3 p.m. We observed the presence of *A. mellifera* in flowering period of sunflower during the whole day, and the activity is higher in the afternoon, after 2 p.m.

The Halictidae only gathered pollen and carried these in the scope spending more time on this activity than other bees.

Stingless bees are important pollinators in several vegetable species, mainly for those which *A. mellifera* are not effective. There are few studies about the economic aspects of the pollination of those bees or even their usage as pollinator agents in cultivated areas (Velthius, 1989).

According to Heard (1999), the sunflower is one of the crops that is visited and pollinated partially or occasionally by stingless bees, but their effectiveness as pollinator is not determined by the sunflower culture. As it was observed in the present experiment, the frequency of stingless bees was high during the morning, but not during the whole day.

Ahmed *et al.* (1989), in research in Sudan, found higher percentage of *A. mellifera* among the pollinators. *Bombus* sp was the following. These authors concluded that *A. mellifera* is an important sunflower pollinator and the plants prevented from visitation had very little production. This fact is also found in the present study, in which the average

achene numbers in the honeybees cage (115) was higher than the treatment without it in cage (27).

Due to the food reserve decrease in the *A. mellifera* colony, there was a tendency of decrease in the queen posture in a specific environment, although a total stop did not occur. Dozet *et al.* (1993) who maintained a colony in the same isolated system reported that the queen stopped posture after 60 days inside the cage. In this experiment, the colony placed inside the cage suffered a drastic reduction in posture due to less pollen offering, at the end of flowering.

The gathered pollen analysis in this colony presented 17.64% of crude protein and 0.13% of ethereal extract. Malerbo (1991) found 19.63% and 1.91% for the crude protein and ethereal extract respectively, in Pêra-Rio orange variety. The protein concentration found in the present work did not differ from Malerbo and Nogueira-Couto (1992), which obtained 18.6% of protein in the gathered pollen, although the ethereal extract percentage showed inferior compared to the one of Pêra-Rio orange. This shows that the food gathered by the honeybees had quality, but it was not enough for the queen posture maintenance.

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