Ground corn collecting behavior by *Apis mellifera* L. workers as a possible risk to biocommunity health¹

Comportamento de coleta de pólen por operárias de *Apis mellifera* L. como um possível risco para saúde da biocomunidade¹

SEBASTIÃO LAROCA²

This is our third contribution on honeybee (Apis mellifera L.) behavior. Our first was in 1978 in collaboration with Mark Winston (Laroca & Winston. 1978) on workers collecting pollen from the body of *Bombus* pennsylvanicus males on the flowers of tall thistle [Cirsium altissimum (L.) Hill] (Lawrence, KS, USA). This discovery was interesting and proved important because thereafter THORP AND BRIGGES (1980) observed foraging females of other bee groups stealing pollen from other species of bees in a behavioral category for such interactions which they called *cleptolecty*. Apparently, cleptolecty occurs with more intensity when floral resources are scarce. LAROCA & ALMEIDA (2009), while dissecting nests of Ptilothrix plumata in a savanna (the Cerrado) of Jaguariaíva (a municipality in the state of Paraná), observed females performing the same category of behavior, i. e. stealing pollen from the interior of a neighbor nest of its conspecific bee. In this case, pollen was naturally scarce in the area, since the bee was olygolectic and the number of flowers of the single visited species was limited and the anthesis period very short (not more than three hours per day). Our second contribution on Apis mellifera L. was done in Lawrence, Kansas (USA),

¹In honor of Professor Joaquim Carlos Sena Maia, qualified master of biometry of several generations of undergraduate and graduate (MsC and PhD) students at UFPR, with whom we had the pleasure of joint classes teaching in ecological methods at the course of Biological Sciences (Entomology) (UFPR). ²Professor Senior at UFPR and invited volunteer scientist (from 2012 to 2015) of the Entomological biodiversity Laboratory at Instituto Oswaldo Cruz (RJ). E-mail: slaroca@slaroca.com.br.

based on the observation of workers visiting flowers of yellow sweet clover (*Melilotus officinalis*) and white sweet clover (*Melilotus alba*) to detect changes of behavior correlated with solar radiation. In conditions of low relative air humidity of Lawrence the foraging workers tended to escape heat stroke by moving toward shaded areas of flower spicks during the warmer periods of day and then leaving these areas when solar radiation was lower. It may be fair to suggest that this process might have anticipate the acquisition of honeybee language discovered by the Nobel Prize laureate, Karl von Frisch (see von Frisch, 1953). We have to consider that the clumped distribution of workers due to insolation at feeding sites favors interactions such as cleptolecty. Collection of corn products (flour meal and coarser ground corn) has been observed by several beekeepers, bird watchers, and small farmers in various regions of Brazil. Such abnormal interactions disturb community of producers due to losses caused in their production.

Despite the fact that these problems appear widely in the media of the country, there has not yet been a scientific study of the occurrence. We still do not know what the use (or uses) of the corn products inside the hives are, but our suggestion is that they are used as food for immatures, just as pollen is. However, an alternative hypothesis is that the corn products are used in the construction of the nest. These hypotheses must be tested and the results analysed regarding the consequences for the bees of the use of this alternative transgenic material.

The influence of the background color of the two recipients (dishes), one black and white, was also evaluated. In addition we observed the time spent by the workers on fine ground corn and coarser corn, as well as the hovering time spent above the dishs containing the corn.

According Jander (Jander, 1976) the movement involved in pollen manipulation in bees possibly is derived from body cleaning behavior observed in more primitive arthropods.

As this theme is new, we present the results of these simple experiments, with the hope that they will serve as a basis for new and deeper studies.

METHODS

The study site was located in the urban area of Curitiba (the capital city of the state of Paraná in southern Brazil), a city with limited floral resources. Observations were done from February 26 to April 12 (2020) when the air temperature ranged from 20 to 30° C and the relative humidity of the air from 40 to 70 %. To attract the bees we used fine (almost powder) and coarser ground corn. The attractants (small dishes with 200 ml of corn products) were placed two meters above the soil surface. The goal of the

experiments was to compare behavioral acts of honeybee workers involved in the activities of collection and manipulations of corn products in the study arena. The data gathered and its treatment included: measures of time of walks on the corn product as well as the time spent flying over the dishes; statistical comparisons between corn products of different color (light cream and yellow); and those parameters reflecting the granularity of the corn — fine (powder, with grains much less than 0.15 x 0.3 mm) and coarser ground corn (grains averaging 0.40 x 0.66 mm) (Table 1).

Direct visual observations were made and videos and photographs were taken at the location of the corn products. We also photographed dead workers to check the load of ground corn accumulated on their corbiculae.

RESULTS

GROUND CORN GATHERING BEHAVIOR

(Figs 1 to 4)

Direct observations established that worker bees began collecting immediately upon reaching the food source. The gathering process is stereotyped: the honeybee worker walks in a very active, fast, and irregular way, especially on the coarser ground corn (sometimes even falling on the corn if grains are coarser), then it flies. The flight periods (hovering) over the food source are frequent and long, but vary according to corn products granularity. We observed the details of those movements. While walking on the ground corn, with intense front legs movements, a worker nearly cover its front parts (ventral head, including proboscis, antenna, and mandible, as well as the thorax and especially the mesepisternum) with corn powder. The movements of the front leg are somewhat convergent, apparently canalizing the ground corn primarily to the middle of the mesepisternum.

After this phase, the bee hovers over the dish. By this flying behavior, the honeybee passes the pollen temporarily accumulated on its head (including proboscis, mandibles, and antenna) to its front legs (tibia and basitarsus) and then to its thorax (lateral and ventral parts), to its middle legs (tibia and tarsus), to the internal part of posterior basitarsi, and finally to each ipsolateral corbicula. Ground corn temporarily on the front legs also passes to the middle legs, ending by the same process on the corbicula. The ground corn accumulated on the corbiculae is similar in appearance to yellow pollen load. To pack the ground corn load more firmly on the corbiculae, the worker bees use the internal parts of their middle ipsolateral tibia and basitarsus. During the flight phase, proboscis extensions as well as apertures of mandibles are frequently observed. The granularity of the load is regularly fine (similar to granularity of refined sugar, or of fine grains of pollen), because it is selected

	yellow coarser ground corn wideness (mm)	yellow coarser ground corn lenth (mm)
	0.75	1.20
	0.65	0.85
	0.50	0.50
	0.40	0.65
	0.50	0.75
	0.40	0.75
	0.50	0.90
	0.30	0.40
	0.30	0.45
	0.45	0.75
	0.15	0.30
	0.35	0.45
	0.40	0.60
	0.55	0.80
	0.35	0.60
	0.35	0.50
	0.80	1.00
	0.15	0.55
	0.40	0.55
Σ	8.2	12.55
n	19	19
mean	0.4342	-
variance	0.0480	0.6605
standard deviation	0.2192	0.1405
	0.2402	0.3748

Table 1. Measures of ground corn (coarser) dimensions (width and length).

during the passes between setae that are involved in the process of loading; coarser grains are rare. The completion of a ground corn load by each worker takes roughly four to five minutes. The frequency at which bees approach this food source on warmer days (approximately 23 to 31° C) is higher from 9:30 am to 12:30 pm and from 3:00 pm to 5:00 pm.

During the collecting activity, significant time is spent in the flights to pass the ground corn (specially the fine corn) from ventral parts of the head and thorax to the corbiculae. Time spent hovering over the food source reached almost 82 % of the total collecting time in the case of the fine ground corn, while on coarser corn it was only about 36%. The interference between workers over the food source during the flight phase by "accidental" encounters is relatively high. This is presented in the following in the synthesis of a sample of measurements of time a bee spent on the food source and hovering above it.

RESULTS OF EXPERIMENTS

1. TIME EXPENDITURE

EXPERIMENT 1 (Table 2) — In this experiment fine (with a high proportion of powder consisting of grains less than 0.15 mm wide and 0.3 mm long) yellow ground corn was used. Hypothesis: H_0 : the average time spent on the corn is equal to the mean time hovering over the corn source; H_A : the average time spent on the fine ground corn is different from the mean time hovering over the corn source. Results favor the alternative hypothesis; in other words, the mean time flying tends to be longer.

EXPERIMENT 2 (Table 3) —Coarser granularity yellow ground corn was used. Hypothesis: H_0 : the mean time spent on the corn is equal to the mean time hovering over the ground corn source; H_A : the mean time spent on the coarser ground corn is different than the mean time hovering over the corn source. Results favor the alternative hypotheses; in other words, the mean time on the corn tends to be longer than the hovering time.

The overall conclusion from these experiments is that honeybee workers change behavior depending on the granularity (fine or coarser) of ground corn, spending more time on the coarser ground corn, which reflects in the energy budget involved in these activities which has to be analysed in future studies.

EXPERIMENT 3 (Table 4) — In this experiment we used fine granularity ground corn, yellow and light cream, in two different scenarios: a dish of light cream corn in the presence of another dish with the same quantity of yellow ground corn, and also Hypothesis testing: H_0 : the mean frequency of workers in the light cream ground corn in the presence of

	time in seconds 1.27 2.33 2.46 4.02 4.20 4.23 4.8 4.84 4.86 5.04 5.15 5.59 6.51 7.05	time in seconds 0.81 0.58 0.40 0.57 0.57 0.70 2.58 1.81 0.68 0.96 0.75 0.70 0.55 1.30
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	5.59 6.51	0.70 0.55
	6.51	0.55
	7.05	1 30
	7.09	2.20
	7.31	1.10
	7.37	0.44
	7.63	0.71
	9.02	1.07
	9.93	0.45
	10.42	1.58
	12.00	0.39
	12.21	1.23
	12.78	1.37
	13.48	0.98
	18.04	1.03
Σ	189.63	25.51
n	26	26
mean	7.2896	0.9812
SS	395.4118	7.7798
variance	19.8850	0.3112
standard deviation	4.4592	0.5578
t 40.9070; P (> 0.001 (2) 25		

Table 2. Honeybee workers' activity on fine corn meal (Curitiba, Brazil, April 4, 2020, during the period of 12:05 pm to 1:16 pm — 24.5° C, RU 43 %, wind vel. 0).

	flight (hoverin time in second		ls
	2.62	7.96	
	7.37	3.6	
	5.45	4.13	
	2.83	19.64	
	6.97	5.41	
	7.79	18.34	
	6.82	13.99	
	9.41	15.94	
	9.67	18.08	
	3.69	4.47	
	4.20	10.21	
	2.52	7.7	
	3.50	4.79	
	0.72	4.1	
	2.66	3.12	
	2.50	4.38	
	2.09	12.17	
Σ	80.81	158.03	
n	17	17	
mean	4.7535	9.2959	
SS	120.663	552.6907	
variance	7.541437	34.5432	
standard deviation	2.746168	5.8773	
4.0829; P (> 0.001 (2	2), 48, therefor	re highly significant	

Table 3. Honeybee workers' activities on coarser corn ground (Curitiba, Brazil, April 4, 2020, during the period of 9:03 am to 9:30 am 23.5 to 24.9° C).

Table 4. Honeybee activities (number of visits) in each dish (yellow and light cream) (fine granularity) (Curitiba, Brazil, March 16, 2020, during the period of 10:10 am to 10:30 am - 26.8 to 27.5° C, RU 65 %).

	yellow ground corn (fine): number of visit	light cream ground corn (fine): numbe of visit
	12	0
	16	0
	13	2
	3	0
	10	1
	12	0
	8	0
	15	0
	3	0
	11 7	0 0
	5	0
	3 7	0
	23	0
	14	0
	9	0
	22	0
	60	0
	18	0
	44	1
Σ	312	4
n	20	20
mean	15.6	0.2
SS	3646	3.2
variance	191.8947	0.1684
tandard deviation	13.8546	0.1884 0.4104

U 0.05 (2), 20, 20 = 319; Since U > U 0.05(2), 20, 20; H_o rejected.

Acta Biol. Par., Curitiba, 50 (1-4): 39-54. 2021.

Table 5. Honeybee activities (number of visits) to light cream dish (fine granularity) (Curitiba, Brazil, Abril 10, 2020, at approx. 10:48 to 10:58 am -20° C). Situation one: number of visits on the dish with light cream ground (fine); situation two: number of visits on the dish with yellow ground corn.

	number of workers on light cream dish in presence of yellow dish in its side number	number o workers on light cream ground (fine replacing the dish of yellow one (5 min after)
	1	5
	2	3
	1 1	5 3 3 3 2
	0	2
	1	4
	0	5 5
	0 0	6
	0	4
	0	5
	0 0	3 1
	0	4
	0	3 2
	1	2
	1 1	4
	1	
	1	3 2 2
	1 1	2 1
	0	3
	1	3
	1	4
Σ	15	81
n	25	25
mean	0.6	3.24
SS	8	44.56
variance	2.8284	1.8567
standard deviation	1.6818	1.3626
Mann-Whitney test		
Z = 5.59 Since U' > U 0.05	5(2),25,25; H _o rejected.	

Table 6. Honeybee workers activity on fine corn meal using two background color (dishs white and black) (Curitiba, Brazil, April 15, 2020, during the period of 12 h 5 min pm to 12: h 40 min pm — appr. 21° C).

	number of workers in the white dish	number of workers in the black dish
	1	2
	0	2 3
	2	1
	1	2
	1	2
	3	1
	1	1
	1	1
	1	1
	0	3
	5	0
	2	3
	2	3
	2	2
	3	1
	2	1
	4	1
	2	2
	4	2
	4	1
	1	2
	3	0
	3	0,
	2	3
	4	0
soma	54	38
n	25	25
mean	2,16	1,52
SS	41,36	24,24
variance	1,7233	1,01
st deviation	1,3128	1,005

Mann-Whitney test

Z = 1,9 Since U'< U 0.05(2),25,25; H_{o} accepted.

Acta Biol. Par., Curitiba, 50 (1-4): 39-54. 2021.

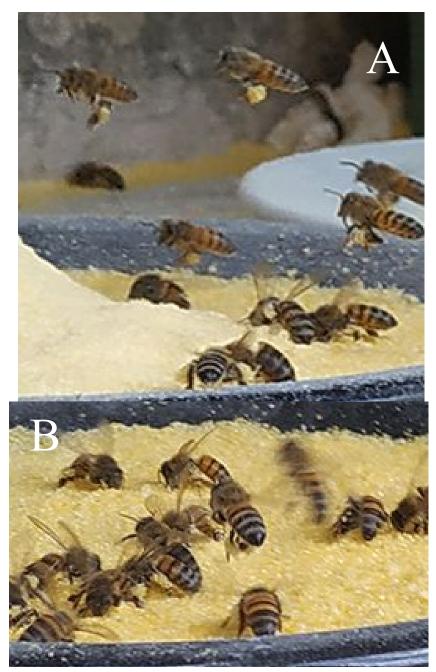


Fig. 1. Behavior of honeybee workers (*Apis mellifera* L.). A, on and over the fine ground corn and B, on coarser ground corn. In A it is seen a good number of workers flying (hovering) on corn.

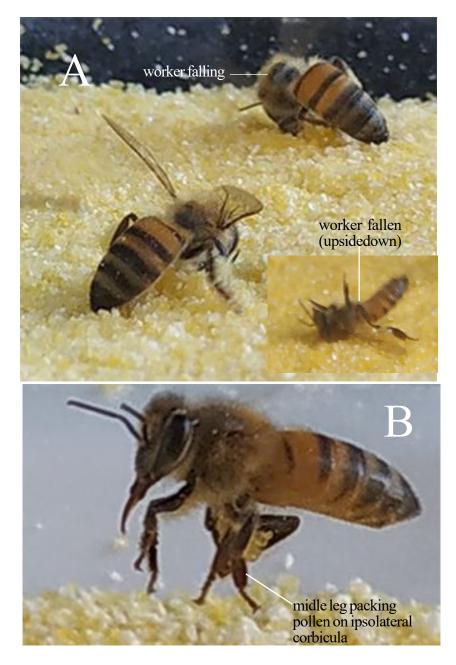


Fig. 2. Behavior of honeybee workers (*Apis mellifera* L.) on coarser ground corn. A, activities of honeybee workers showing the tendency to fall observed on coarse ground corn: B, honeybee worker with its proboscis extended and packing pollen with its midle leg on ipsolateral corbicula.



Fig. 3. Behavior of honeybee workers (*Apis mellifera* L.) on coarser ground corn. A, honeybee worker with an almost complete ground corn load. B, still beginning to collect.



Fig. 4. Behavior of honeybee workers (Apis mellifera L.) — honeybee worker with a complete corn load.

of corn is in the place previously occupied by the yellow corn. H_A : the means are different. H_A accepted. Therefore, honeybee workers have a clear preference for yellow ground corn.

EXPERIMENT 4 (Table 5)—In this experiment we used fine granularity ground corn to test the variation of the number of workers on dish with light cream corn in the presence of a dish with yellow corn with the number of workers on light cream ground corn, but replacing (five minutes later) the dish of yellow ground corn. H_0 : mean number of honeybee workers is equal on both dishs of light cream ground corn. H_A : mean numbers of honeybee workers are different on both dishs of light cream ground corn. H_0 : mean numbers of both dishs of light cream ground corn. H_A : mean numbers of honeybee workers are different on both dishs of light cream ground corn. H_0 : mean numbers of both situations.

EXPERIMENT 5 (Table 6) — In this experiment we used yellow fine granularity ground corn in two different colors of background (dishs white and black). H_0 : mean number of workers on the ground corn is equal in both dishs. H_A : mean number of workers on the ground corn is different in the dishs. H_0 is accepted. Therefore white and black backgrounds attract equally honeybee workers.

COMMENTS

Apis mellifera is an important organism for human beings because it produces honey, propolis, wax, royal jelly, and because it pollinates several cultivated species of plants and therefore is a source of income to beekeepers, small farmers, and other industries. Current observations, as well as video and reports published on the internet raise several scientific questions that demand answers. The first one is the rarefaction of flowering plants, as consequence of the destruction of nature by large agro-business, which are causing the bees to exploit alternative food sources, a behavior that is affecting the quality and production of honey, propolis, and perhaps even of royal jelly.

An example of this is what happened in a small farm in the municipality of Descalvado (in the state of São Paulo) where the bees invaded the food supply (ground corn) of chickens, causing the chickens to decrease the frequency of their feeding, consequently impacting meat production and hence the income of the farmers (see <https://globoplay.globo.com/v/3535121/>, acessed in February).

Athird repercussion occurred in the state of Minas Gerais, in which Marcello Bahtts (compositor, musician, nature conservationist and keeper of meliponids) made an impassioned defense of nature, bees, and the species of plants on which these insects depend. He attributed this serious problem to the impact of agriculture practiced on the large farms [(see video at <https://www.youtube.com/ watch?v=qRsNz5i9ySc>, acessed in February 26, 2020).]

It seems probable that ground corn carried to hives is deposited in the same receptacles as pollen. If so, it is of interest to know what the composition of this food is in its final form when it is administred to larvae. Another important question worthy of further study is the possible contamination of honeybee hives with the virosis of birds including chickens with possible impacts in the biotic community health.

Aknowledgments — Our gratitude to Dr. Paul Decelles (Johnson County Community College, Overland Park, Kansas, USA); Drs. Marcia Chame, Jane Costa as well as Sandor Christiano Buys (Laboratório de Biodiversidade Entomológica do Instituto Oswaldo Cruz, RJ) for reading the manuscript and giving us important suggestions.

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Recebido em 10 de dezembro de 2020.