Influence of Pollen on the Development of Africanized Bee Colonies (Hymenoptera: Apidae)

by

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ABSTRACT

This study aimed to assess the importance of quality and quantity of pollen on the development of colonies in different seasons. The field experiment was conducted at the Apiary of the Department of Entomology and Acarology of Luiz de Queiroz College of Agriculture, using five beehives of *A. mellifera*. In order to characterize the quality of pollen, researchers considered measures of total dry mass (g) and the physical-chemical and pollen composition of pollen load and bee bread samples. The development of hives was assessed according to the area covered by pollen, honey, and brood population in the hive (cm²), as well as fluctuating asymmetry of worker bee wings. Spearman's correlation was calculated among the assessed parameters. The value of ether extract of pollen loads was the only component that was related to the development of hives, its value increased as the area occupied by pollen in the hive decreased (-0.3200*), and as the difference of the number of hamuli of right and left hind wings increased (0.3317*). There was a positive relationship between the wealth (0.3150*) and evenness (0.3019*) of pollen composition and the size of brood population inside the hive. It was concluded that the development of the colony, mainly considering the area occupied by brood, is more successful with increased quantity, wealth, and evenness of collected pollen.

Key words: *Apis mellifera*, fluctuating asymmetry, physical-chemical composition, bee nutrition, bee pollen.

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INTRODUCTION

Pollen is removed from the anthers of flowers and carried by the forager bees to the hive inside their corbicula or pollen baskets, located on the hind legs of the workers. The corbicula is surrounded by a fringe of hair into which pollen is placed to form the load (Michener 1974). Pollen loads, retained by collectors installed at the entrance of the hive or stored in honeycomb cells (bee bread), indicate period of production and botanical and geographical origins of the product (Vanderhuck 1995, Marques-Souza 1996, Carvalho *et al.* 1999).

Pollen is used in the feeding of larvae, and also adult bees, soon after their emergence until the beginning of the preparation of the synthesis of royal jelly for feeding the brood and the queen (Crailsheim 1992). It is known that the amount and composition of pollen can change the behavior of adult bees, fecundity, life span of bees, the size of the brood stock and honey, colony population, the development of bees, and larval food quality (Waller *et al.* 1981, Schmidt *et al.* 1987, Sagili *et al.* 2005, Toth *et al.* 2005, Hoover *et al.* 2006, Mattila & Otis 2007, Human *et al.* 2007). It is noteworthy that Ioirich (1986) mentions that, in periods of protein resource shortage, and with no other source of protein, the colony may be extinct.

Considering the factors discussed above, this study aimed to assess the relevance of pollen quality for the development of Africanized bee (*Apis mellifera*) colonies in an area of Atlantic forest in the city of Piracicaba, SP.

MATERIALS AND METHODS

In order to characterize the quality of pollen, researchers considered as parameters: total dry mass (g), some nutritional components, and the plant origin of pollen load and bee bread samples. To assess the development of hives it was considered the size of the area covered by pollen, honey, and brood population in the hive, as well as fluctuating asymmetry of wings (module of difference between right and left wings) of active worker bees inside the hive. The field experiment was conducted at the Apiary of the Department of Entomology and Acarology of Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, where five beehives of *A. mellifera* (L.) were installed with a front pollen collector having mean retention rate of 45 ± 1.19 grains (± mean standard error).

Samples were collected in the fall (April 02, 2008 to May 28, 2008), winter (July 09, 2008 to Sept.03, 2008), spring (Oct.07, 2008 to Dec.02, 2008) and summer (Jan 09, 2009 to March 06, 2009), in a total period of 57 days for each season.

Pollen loads intercepted in the hives' entrances were collected every two days, cleaned by picking, and dried at 45°C for 48 hours as suggested by Funari *et al.* (1998a), the bee bread was collected every 15 days in a sorted manner within each hive. All samples were separated by fifteens and stored for further physical-chemical and pollen analyses.

Melissopalinological analysis of the samples was conducted with the use of acetolysis (Erdtman 1960) and the form of subsampling was recommended for the determination of the main pollen types occurring in a sample of heterospecific pollen (Modro *et al.* 2009). The identification of pollen types was based mainly on reference collection of microscope slides from the Palynology Library of the Insects Laboratory at the Department of Entomology and acarology of the Luiz de Queiroz College of Agriculture, as well as specialized catalogs in pollen morphology of several species of plants. Approximately 900 pollen grains per sample were identified and counted.

The values of the physical-chemical composition, in samples of pollen loads and bee bread, were obtained from tests carried out according to Silva & Queiroz (2002) in the Bromatology Laboratory of the Institute of Animal Science in Nova Odessa-SP. For pollen load samples, the percentage of dry matter, ether extract, mineral matter, crude protein, and total carbohydrates were measured. For the bee bread, percentages of dry matter and crude protein were determined. In samples with less than 2g, the value of dry matter was calculated from the other samples' mean moisture.

Within intervals of 15 days, during the collection period, the development of *A. mellifera* hives was assessed by measuring the area (cm²) of brood, honey and pollen using the technique of Al-Tikrity *et al.* (1971).

Ten active worker bees inside each hive were collected every two weeks and fixed in 70% alcohol. Temporary measuring slides were mounted, where fore and hind, right and left wings were detached and rectilinearly placed between two histological slides. The following left and right hind wing morphological characters were measured, according to Silveira *et al.* (2002): radial subcostal vein, width and length of marginal cell, maximum total length and width

of wing, length of cubital medial vein, length of cubital vein, length of anal vein, width of the 2nd medial cell. The total number of hamuli of the right and left fore wings was counted. Measurements were made using a Leica stereomicroscope, containing an ocular micrometer, and the results were converted to millimeter units.

Spearman correlation was carried out among the areas occupied with brood, honey, and pollen, fluctuating asymmetry of wings, values of physical-chemical composition and diversity indexes of Shannon-Wiever (H'), evenness expressed by Pielou (J') index, and Wealth, calculated by pollen composition. Statistical analyses were performed with the help of SAS statistical software (SAS Institute 2003) and the tests were applied to the 5% level of probability.

RESULTS AND DISCUSSION

The largest area occupied with brood and honey was during the fall, and summer had the largest area with pollen (Table 1). Sequentially analyzing from the beginning of field assessments, one can observe a marked reduction in occupied area inside the hive between fall and winter, remaining low during spring with an increase

Table 1. Mean area (cm²) occupied by brood, honey and pollen by beehive of Africanized bees (*Apis mellifera*) located in Piracicaba, SP, 2008-2009.

Season	Resource (cm²)		
	Brood	Honey	Pollen
Fall	341.49	389.33	104.00
Winter	189.87	126.08	46.14
Spring	256.87	90.33	42.94
Summer	265.14	82.75	128.86
Total (cm2)	1,052.79	688.48	321.94

in area of pollen in the summer. The occupation of a larger area with pollen in the summer associated with the availability of trophic resources are perhaps two of the factors responsible for increased area of brood and honey in the fall, based on a study conducted by Al-Tikrity *et al*. (1972).

The size of the bees showed similar absolute values among seasons, which confirms the results obtained by Herbert Jr. (1988) who found that nutritional stress did not influence the morphometric measurements of the bees, however, it is observed that the measures were slightly larger and more symmetrical in the fall (Table 2). Although the size of bees is a highly inheritable characteristic (Oldroyd *et al*. 1991), the size associated with greater measures of wind symmetry denote a more stable development of bees, whose subjects have bilateral symmetry. According to Woods *et al*. (1998), if the expression

of a bilateral character is produced by the same genome, then any asymmetry of the sides is a result of environmental disturbance.

By comparing Table 1's data with those from Table 2 it is possible to observe that the fall was the season that had largest occupied area in the hive and also greater symmetry and size of bees. However, the summer, which was the season with the lowest and smallest area showed low levels of symmetry when compared to bees collected in other seasons, a characteristic

Table 2. Mean values of the asymmetry measurements and float to the morphological characteristics of Africanized bees (*Apis mellifera*), Piracicaba, SP, 2008-2009.

Characteristics		Season*			
		Fall	Winter	Spring	Summer
Hamuli	N[1]	21.59±0.112	21.11±0.132	21.18±0.132	20.90±0.118
	A[2]	1.179±0.071	1.448±0.102	1.256±0.074	1.284±0.089
Total length	T[3]	7.91±0.013	7.88±0.014	7.90±0.015	7.86±0.013
	A	0.057±0.003	0.059±0.004	0.055±0.003	0.058±0.004
Total width	T	2.91±0.006	2.89±0.006	2.88±0.008	2.85±0.006
	A	0.038±0.002	0.045±0.003	0.044±0.003	0.045±0.004
Radial subcostal vein	T	3.80±0.007	3.78±0.009	3.78±0.009	3.78±0.009
	A	0.075±0.004	0.094±0.006	0.100±0.006	0.087±0.006
Cubital medial vein	T	2.08±0.005	2.06±0.005	2.05±0.006	2.05±0.006
	A	0.056±0.003	0.055±0.003	0.053±0.003	0.047±0.003
Cubital vein	T	1.77±0.004	1.78±0.004	1.79±0.004	1.78±0.004
	A	0.025±0.001	0.025±0.001	0.026±0.002	0.023±0.001
Anal vein	T	3.88±0.007	3.86±0.007	3.86±0.008	3.85±0.008
	A	0.040±0.002	0.047±0.003	0.043±0.003	0.045±0.003
Length of marginal cell	T	2.97±0.007	2.95±0.006	2.96±0.006	2.94±0.007
	A	0.036±0.002	0.037±0.002	0.034±0.002	0.037±0.002
Width of marginal cell	T	0.45±0.002	0.44±0.002	0.44±0.002	0.44±0.002
	A	0.028±0.001	0.030±0.002	0.028±0.002	0.029±0.002
Width of 2nd medial cell	T	0.81±0.002	0.80±0.003	0.80±0.003	0.78±0.003
	A	0.027±0.001	0.029±0.002	0.030±0.002	0.029±0.002

[1]N: mean number of hamuli per wing,[2]A: fluctuating asymmetry calculated from the module difference among the measures of right and left wings,[3]T: total size of the characteristic, measured in millimeters *Mean ± mean standard error.

that denotes deviations from perfect symmetry of subjects with bilateral symmetry (Soulé 1979).

These results are strengthened by the assertion that, fluctuating asymmetry and size of subjects may reflect the instability development caused by stress, which in this study may be due to the amount of food available (Scheiner *et al.* 1991, Clarke 1995, Nosil & Reimchen 2001, Kanegae & Lomônaco 2003).

The area occupied with brood decreased with time of hive exposure to the pollen collector (n = 45, p-value <1% r_s = -0.38854). While Pereira *et al.* (2006), attributed the reproductive diapause in hives with collectors to environmental stress, discarding the nutritional factor, the same trend also presented for the areas of pollen and honey, in this study, seems to support the assertion that the use of collectors in Africanized bee hives interferes with the development of hives (McLellan 1974, Duff & Furgala 1986a, 1986b, Shawer 1987, Funari *et al.* 1998b).

The value of ether extract of pollen loads was the only component that was related, significantly, with the development of hives, with greater value found with reduced area occupied with pollen in the hive, and with increased gap of the number of left and right hind wing hamuli (Table 3). Pollen with higher percentages of ether extract, when collected, may have been consumed more quickly than that with low percentages of such component, due to its phagostimulant function (Singh *et al.* 1999). Regarding the effect on hamuli fluctuation asymmetry, Parra (2007) mentions that the presence of linoleic acid, one of the essential fatty acids, is important in the formation of *Anagasta kuehniella* (Lepidotera) wings. However, this study cannot come to conclusions regarding the effect of ether extract composition on the development of bees (Table 3).

The larger the area of brood, the greater the amount of pollen collected by bees (Table 3) and the larger the area with pollen in the hive (n = 45, p-value <1% r_s = 0.4086). This result reinforces the hypothesis that the presence of brood in the hive (Pankiw *et al.* 1998, Dreller & Tarpy 2000), as well as the lack of stored pollen (Hellmich & Rothenbuhler 1986) stimulate forager bees to collect pollen. The pheromone of larvae or contact with the areas occupied by brood and stored pollen indicate the nutritional needs of the

Table 3. Spearman correlation (n = 45) between the quality and quantity of pollen collected and the development of Africanized bee (*Apis mellifera* L.) hives, in Piracicaba, SP, 2008-2009.

Development measures of hive		Bee bread	Pollen loads				MS(g)[1]
			Physical-chemical quality (%)				
		PB	PB	EE	MM	CT	
Area occupied in the hive (cm²)	PO	0.0429	0.2584	-0.3200*	0.2633	-0.2275	0.2234
	Honey	-0.0555	-0.0448	-0.1175	-0.1768	0.1123	-0.0212
	CR	-0.1121	-0.0579	0.0165	-0.1033	0.0023	0.5675***
Wind fluctuation asymmetry (μm)[2]	Ha	-0.2547	-0.1955	0.3317*	-0.2470	0.1147	0.0102
	Sc+R	0.1034	-0.0775	0.0977	0.1417	0.0129	0.0584
	Cm	-0.1139	-0.1735	-0.0726	-0.1423	0.2388	0.0329
	Co	0.0727	-0.0441	-0.1109	0.1991	0.0663	0.1170
	M+Cu	0.0210	-0.0087	0.0780	0.1137	-0.0175	-0.0584
	Cu	-0.0525	-0.0591	-0.1049	0.0855	0.0384	-0.0106
	An	0.1150	-0.1181	-0.0522	-0.0723	0.1089	0.1585
	La	0.1458	0.1023	-0.1641	0.2301	-0.0638	-0.0548
	Lm	-0.1038	-0.1707	-0.1124	0.0877	0.1787	0.1093
	2M	0.2135	0.1147	0.0114	0.0064	-0.1427	0.2198

Notes: Pollen (PO), brood (CR), crude protein (PB), ether extract (EE), mineral matter (MM), total carbohydrates (CT) and dry matter (MS) [1] Total amount of dry matter (g) of pollen loads collected in a 15-day period, [2] fluctuating asymmetry in right and left hind wings, measure in micrometers (μm): radial subcostal vein (Sc + R), length of marginal cell (Cm) total length of wing (Co); cubital medial vein (M + Cu); cubital vein (Cu); anal vein (An), full width of the wing (La), width of marginal cell (Lm), width of the 2nd medial cell (2M). Right and left fore wings: number of hamuli (HA), conventional signs used: * Significant at 5%, ***Significant at less than 0.01%.

hive and determine foraging behavior of bees, for the collection of pollen or nectar, even in the case of Africanized bees.

The area occupied with honey however, was not associated with the area of pollen (n = 45, p-value> 5%; r_s = 0.1882) nor with the area of brood (n = 45, p-value> 5% r_s = 0.0163), and that suggests that the search for sources of nectar occurs even in the absence of brood or with stored pollen, as proposed by Free (1967).

There was little relation between quality of pollen and development of the hive, which can be justified based on studies like the one conducted by Human *et al.* (2007), who noted the imbalance of the protein: carbohydrate ratio, which in the opinion of Schmidt (1985) would be more logical, in the case of a bee species that is extremely pollen oriented, in the use of resources and that feeds on pollen from a great diversity of plant species, so the cumulative effect of many compounds are responsible for the development and

Table 4. Spearman coefficient (n = 56) between pollen composition and area occupied in hives by Africanized bees (*A. mellifera*) in Piracicaba, SP, 2008-2009.

Pollen composition		Area occupied in the hive (cm²)		
		Pollen	Honey	Bee Springs
Bee bread	Wealth	0.2361	0.0408	0.3150*
	Diversity	0.0113	-0.0686	0.2174
	Evenness	0.0871	-0.0831	0.3019*
Pollen loads	Wealth	0.0631	-0.0347	0.2484
	Diversity	-0.0187	0.0130	0.0682
	Evenness	-0.0149	0.0286	0.0068

Conventional signs used: *Significant at 5%.

behavior of bees, and not some specific compounds, which usually occurs in monophagous species. In accordance with this assertion, Table 4 shows a positive relationship between wealth and evenness of pollen composition and the size of the area occupied by brood in the hive.

Although some studies show that the nutritional quality of pollen may determine the amount to be collected in foraging behavior, with an influential evoluting factor in food preference of bees, according to the needs of the hive (Pernal & Currie 2001, 2002), in this work, the amount of intercepted pollen in traps did not relate to its physical and chemical quality (n = 45, p-value> 5%). Free (1967) and Sagili & Pankiw (2007) also found no relationship between the amount of pollen collected and its values of carbohydrates and crude protein, respectively.

CONCLUSIONS

Brood and honey occupied a largest area in hives during the fall, and summer had the largest area with pollen.

The size of bees did not vary significantly among seasons.

The development of the colony, measured primarily by the size of the area occupied with brood, is more successful with increased amount of pollen collected, wealth, and evenness of pollen composition and less successful with increased time of exposure to pollen collectors.

The amount of pollen intercepted in traps shows no relationship to its physical and chemical quality.

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