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Botanical Origin of Protein Sources Used by Honeybees (*Apis mellifera* L.) in Atlantic Forest

by

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ABSTRACT

Productive and reproductive traits of beehives are influenced by climate and food availability in the region where the bees are reared or maintained, thus honey and pollen storage, egg-laying conditions of the queen as well as comb occupation are subject to seasonal variations. The present study was conducted in the apiary of the Department of Entomology and Acarology, College of Agriculture Luiz de Queiróz, ESALQ/USP, in the municipality of Piracicaba, in an area containing fruit trees, ornamental plants and a fragment of a native forest. The objective was to identify protein sources used by honeybees (*Apis mellifera*) over a whole year (2010-2011) in remnants of the Atlantic forest, information that can be used in the conservation and restoration of degraded areas. For sample preparation, the acetolysis method was adopted (Eredtman 1952) and the quantitative analysis was performed by counting successive samples of 900 grains per sample which were grouped by botanical species and/or pollen types. The results show that the bees used various plant types in the area, including ruderal species, to maintain their colonies. *Apis mellifera* seeks food sources in all plants in the surroundings of the apiary, including herbaceous, shrubs, trees, native or introduced. *Eucalyptus* sp. played an important role as a food source in all seasons due to its wide availability around the apiary and its high flower production. The most frequent pollen types (greater than 10% of the sample) were *Anadenanthera* sp., *Acacia* sp, *Miconia* sp. and *Eucalyptus* sp. in winter; *Philodendron* sp., *Mikania cordifolia*, *Parthenium* and *Eucalyptus* sp. in spring; *Alternanthera ficoidea*, *Chamissoa altissima* and *Eucalyptus* sp. in summer; *Philodendron* sp., *Raphanus* sp. and *Eucalyptus* sp. in autumn.

Key words: pallinology, pollen, beekeeping.

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INTRODUCTION

Pollen is the male gamete of flowers of angiosperms, necessary for egg fertilization and, therefore, for seed formation (Raven 2001). It also serves as a reward for floral pollinators (Schlindwein *et al.* 2005), mainly bees, which use pollen as a protein source of their diets (Zerbo *et al.* 2001). Honeybees, *Apis mellifera*, use pollen as a food source in all developmental stages of the colony (Basim *et al.* 2006), therefore, protein content and availability the pollen are essential factors for the colony (Zerbo *et al.* 2001). Pollen is the sole protein source (Roubik 1989) necessary for the proper functioning of the hypopharyngeal gland in worker bees (Crailsheim 1990).

Pollen grains vary in shape, size, color, appearance, morphology and can, thus, be used to identify plant gender and species (Schmidt & Buchmann 1993; Almeida-Muradian *et al.* 2005). Pollen grain identification is made through its outer wall, chemically stable and morphologically varied, which allows the identification of various pollen types through the pollen analysis (Salgado-Labouriau 1973, Barth 2004). This identification can be used as indicator of the geographical and botanical origin of honey (Barth 2004), since the pollen types found in these products are considered "fingerprints" of bee foraging habits (Wittmann & Schlindwein 1995).

Knowledge of bee foraging habits can be an important tool for beekeeping development (Pearson & Braiden 1990), determining the site of apiary implantation and honey production (Ashman *et al.* 2004; Biesmeijer *et al.* 2006). This type of study can also be used for reforestation and conservation of areas and for the establishment of ecological corridors (Modro 2009).

This work aimed to identify the plant species used as protein sources by honeybees (*Apis mellifera*) in remnants of the Atlantic forest, information that can be used in conservation and restoration programs of degraded areas.

MATERIALS AND METHODS

Experimental area

Samples were collected at the beginning and end of each of the four seasons during a whole year between 2010 and 2011, at the apiary located in the Department of Entomology and Acarology the College of Agriculture "Luiz de Queiroz", near a fragment of the Atlantic forest, in the municipality of Piracicaba (22°52'33"S, 47°38'30"W, altitude 546m), São Paulo state, Brazil.

Collection of samples

Five hives were selected under similar population conditions and in the first and last weeks of each season (spring, summer, autumn and winter), we installed pollen collectors that allowed the entrance of the bees, but intercepted the pollen collected, which fell into a tray and remained until it was removed.

Pollen Analysis

We used the acetolysis method (Erdtman 1952) for identification of pollen types. The method forces the exit of the cellular content of the pollen grain, leaving only the cell wall. Afterwards, slides were mounted on glycerinated gelatin.

The grain characterization was made by comparison with a reference slide collection of the Department of Entomology and Acarology of the College of Agriculture "Luiz de Queiroz" (ESALQ/USP) and the specialized literature (Barth 1970 a, b, c, Moreti *et al.* 2002, Roubik & Moreno 1991).

After the grain identification, we carried out a quantitative analysis by counting 300 grains per slide, 900 per sample (analysis made in triplicate). The quantitative analysis aims to determine the proportion of contribution of each pollen type, identifying and grouping it according to its occurrence as dominant pollen (DP), when it accounts for more than 45% of total pollen grains counted; accessory pollen (AP, 16-45%) and isolated pollen (IP, up to 15%). The IP was subdivided into significant isolated pollen (SIP, 3-15%) and occasional isolated pollen (OIP, less than 3% of the grains observed) (Louveaux *et al.* 1978).

The main pollen types were photomicrographed through a Zeiss microscope coupled to a digital camera.

The experimental design was completely randomized with five replicates (represented by hives). Data were analyzed using the MIXED procedure, SAS (Statistical Analysis System), to determine the structure of variance and covariance matrix. The significance level for the variance analysis was 5%. The data were transformed to Log (X+1) to achieve homoscedasticity in the variance analysis.

RESULTS AND DISCUSSION

In the study period, 68 pollen types belonging to 19 botanical taxa were found (Table 1). Modro (2011) conducted a study in the same place and found similar results with regard to plant families, however, the author observed a total of 81 taxa. This difference can be explained due to the seasonality of some plant species and the presence of cropped areas in the region of collection.

The main pollen types classified in terms of statistical significance were: in spring, *Mikania cordifolia* and *Eucalyptus* sp; in summer, *Alternanthera ficoidea* (dominant); in fall, *Eucalyptus* sp as accessory pollen, and in winter there was no statistical difference among the pollen types (Table 1).

According to Renner (1968), most plant species have flowers that do not produce pollen or nectar throughout the day, but only at certain hours. Thus, bee activity, food type and increased collection time, according to Butler (1945); Moffett & Paker (1953), Bennett & Renner (1961), depend on the following characteristics: genetic trait of the hive, the amount of nectar available, sugar content in flowers, time of day, environmental factors and plant species. Therefore, variations in collection sites greatly affect the collection of floral resources by bees.

The families Fabaceae, Asteraceae and Arecaceae showed the greatest number of species in winter, Arecaceae and Asteraceae in spring and Asteraceae in autumn. The main pollen species were: *Acacia* sp. and *Anadenanthera* sp. (Fabaceae), *Alternanthera ficoidea* and *Chamissoa altissima* (Amaranthaceae), *Philodendron* sp. (Araceae), *Eucalyptus* sp. (Myrtaceae), *Mikania cordifolia* and *Parthenium* sp. (Asteraceae) and *Raphanus* sp. (Brassicaceae).

The difference in numbers of pollen types along the seasons shows the generalist habit of these bees, however, there seems to have a preference for some plant species that appear in all seasons such as *Eucalyptus*, *Raphanus*, *Astrocaryum* and *Philodendron*, although none of them are dominant. The preference of *Apis mellifera* for *Eucalyptus* pollen has been observed by other authors, whenever *Eucalyptus* was present in the vicinity of the apiary (Almeida-Anacleto 2007 and Carvalho *et al.* 1999, in Piracicaba, São Paulo State; Modro 2006 and Barreto 1999, in Viçosa, Minas Gerais State; Luz *et al.* 2007 in Engenheiro Paulo de Frontin, Rio de Janeiro State, and Moreti *et al.* 2011 in Pindamonhangaba, São Paulo State – all municipalities in Brazil).

Table 1 - Average percentage of pollen types collected by *Apis mellifera* in Piracicaba, São Paulo state, Brazil, in the four seasons in 2010-2011. Means followed by different capital letters in the columns differ by Tukey-Kramer (P <0.05). [1]Means in parentheses are averages transformed to Log (X+1) [#] statistical data on produced pollen. DP -dominant pollen; AP – assistant pollen, SIP – significant isolated pollen, OIP - occasional isolated pollen (X -no collection).

Family	Species	Season			
		Spring	Summer	Autumn	Winter
Amaranthaceae	*Amaranthus* sp.	X	X	X	1.46±8.54 OIP (0.36±0.24)A
	Alternanthera ficoidea	3.70±14.76 SIP (0.27±0.42)AB	59.89±6.61 **DP** (1.63±0.18)A	4.39±6.08 SIP (0.69±0.17)B	X
	Chamissoa altissima	X	16.88±10.61 **AP** (1.24±0.19)AB	X	X
Anacardiaceae	*Tapirira* sp.	X	X	2.13±14.78 OIP (0.49±0.42)AB	X
Araceae	type1	2.10±8.53 OIP (0.42±0.24)AB	X	1.71±8.53 OIP (0.43±0.24)AB	1.77±8.67 OIP (0.41±0.24)A
	Philodendron sp.	20.04±6.61 **AP** (1.01±0.19)AB	5.55±6.69 SIP (0.68±0.19)AB	14.97±8.65 SIP (0.89±0.24)AB	9.85±6.72 **SIP** (0.72±0.19)A
Arecaceae	type 1	1.00±10.45 OIP (0.28±0.30)AB	X	5.31±8.94 SIP (0.67±0.24)AB	2.34±8.66 OIP (0.47±0.24)A
	Astrocaryum sp.	4.46±8.53 SIP (0.59±0.24)AB	2.99±10.44 OIP (0.54±0.30)AB	6.78±6.12 SIP (0.66±0.17)AB	1.91±6.73 OIP (0.33±0.19)A
Asteraceae	*Bidens pilosa*	X	X	0.87±6.74 PIO (0.26±0.19)B	0.25±14.77 PIO (0.08±0.42)A
	Mikania cordifolia	34.10±7.56 **AP** (1.48±0.21)A	X	5.22±8.53 SIP (0.59±0.24)AB	1.05±10.71 OIP (0.31±0.29)A
	Parthenium sp.	30.96±10.45 **AP** (1.05±0.30)AB	X	4.70±6.13 SIP (0.64±0.17)AB	0.15±10.45 OIP (0.04±0.30)A
	Type 1	X	X	3.04±10.71 SIP (0.60±0.29)AB	X
	Sonchus oleraceus	X	X	0.55±8.67 OIP (0.18±0.24)B	X
Brassicaceae	*Raphanus* sp.	1.62±10.44 OIP (0.36±0.30)AB	1.69±10.44 OIP (0.36±0.30)B	21.62±5.03 **AP** (1.07±0.14)AB	0.87±7.46 OIP (0.37±0.21)A
Cucurbitaceae	*Momordica* sp.	X	X	1.54±8.67 OIP (0.39±0.24)AB	0.42±10.71 OIP (0.14±0.29)A
Fabaceae	*Acacia* sp.	X	X	0.70±10.69 OIP (0.06±0.29)B	17.59±8.54 **AP** (1.11±0.24)A
	Anadenanthera sp.	X	X	X	16.59±8.54 **AP** (0.12±0.24)A
	Caesalpinia peltophoroides	x	X	X	1.22±8.54 OIP (0.34±0.24)A
	Mimosa caesalpinifolia	8.40±7.39 SIP (0.77±0.21)AB	X	X	0.04±10.45 OIP (0.04±0.30)A

Table 1 (continued).

Family	Species	Season			
		Spring	Summer	Autumn	Winter
	Fabaceae type1	x	X	1.43±10.45 OIP (0.38±0.30)AB	X
Lythraceae	*Lagerstroemia indica*	2.32±8.54 OIP (0.51±0.24)AB	X	X	X
Malvaceae	*Paquira* sp.	X	X	X	0.15±10.45 OIP (0.06±0.30)A
	Dombeya sp.	X	0.27±10.45 OIP (0.11±0.30)B	0.72±8.53 OIP (0.20±0.24)B	X
Melastomataceae	*Miconia* sp.	X	X	X	12.39±10.45 **SIP** (1.13±0.30)A
	Morus nigra	9.24±8.53 SIP (0.98±0.24)AB	6.08±7.39 SIP (0.63±0.21)AB	1.29±10.44 OIP (0.30±0.30)AB	
Myrtaceae	*Eucalyptus* sp.	31.34±5.03 **AP** (1.12±0.14)A	14.83±4.79 PII (0.87±0.13)AB	37.52±4.79 **AP** (1.53±0.13)A	18.79±5.66 **AP** (0.99±0.16)A
Piperaceae	*Piper* sp.	2.32±7.47 OIP (0.42±0.21)AB	1.89±7.47 OIP (0.40±0.21)B	X	6.02±8.65 **SIP** (0.75±0.24)A
Poaceae	Type 2	0.51±6.13 OIP (0.16±0.11)B	X	X	X
	Oryza sp.	X	X	2.11±10.71 OIP (0.46±0.29)AB	X
Rutaceae	*Citrus* sp.	X	X	1.03±10.45 OIP (0.28±0.30)AB	X
	Prunus persica	X	8.14±8.54 SIP (0.87±0.24)AB	X	X
Sapindaceae	x	X	X	0.29±10.71 OIP (0.11±0.29)B	X
Solanaceae	Type Solanaceae	0.96±10.45 OIP (0.28±0.30)AB	4.54±10.45 SIP (0.74±0.30)AB	X	X
Scrophulariaceae	*Scopania* sp.	X	5.48±10.45 SIP (0.57±0.30)AB	X	X

Quantitatively, the *Eucalyptus* sp. grains showed some frequency in almost all seasons. This may be associated with the availability of these resources in the collection site and the high pollen production of this plant species that makes this type of resource more easily collected by bees. This fact is consistent with Bawa (1983) and Castro (1994) who state that the attractiveness of flowering can be influenced by the amount of pollen produced, the concentration and abundance of flowers, number of competing insects, the distance between the flowering sites and nest, and innate preference for the species. However, these factors are still subject to variation such as flower size, relative humidity, soil moisture, temperature, altitude, time and daylight duration.

This generalist habit can occur due to the protein need of the colony, since the protein content may vary with the botanical origin. According to Rouston *et al.* (2000), protein variation may not be directly related to the attractiveness of pollinators, since the pollen of zoophilic species is not richer in proteins than in anemophilous species. According to Schmidt & Buchmann (1993) bees collect pollen from many plant species and thus have good nutritional balance and high dilution of toxic potential of alkaloids and other toxins.

The summer was the only season that showed dominance of a plant species, despite the 12 different types found. Bees sought preferably the species *Alternanthera ficoidea*, which is an herbaceous plant originating from the Americas (Mears 1977), showing the importance of plants often considered weeds for honey production (Modro 2011). It is interesting to observe that this same plant appears as SIP in spring and summer, which indicates that it could become an important bee forager in other seasons. The preference for this pollen type, which, at times, appears in small amounts changing to dominant in others, may be related to the energy bees spend to seek other food sources, since this species occurs in abundance in the surroundings of the apiary (Modro, *et al.* 2007; Modro, *et. al.* 2011).

Pollen types that had a frequency lower than 10% may be considered secondary, because Ramalho *et al.*, (1985) believe that sources between 1 and 10% of pollen have little attractiveness as food source. These pollen types, however, may be useful as a suplement to food needs of the colony and become important for maintaining balance at other times of year.

CONCLUSION

Apis mellifera bees seek food sources in all plants in the surroundings of the apiary, regardless of the plant type, whether herbaceous, a shrub, a tree, native or introduced.

The *Eucalyptus* sp played an important role as bee forager in all seasons due to its wide availability in the vicinity of the apiary and its large production of flowers.

The most frequent pollen types (greater than 10% of the sample) were: *Anadenanthera* sp, *Acacia* sp, *Miconia* sp and *Eucalyptus* sp during winter;

Philodendron sp, *Mikania cordifolia*, *Parthenium* sp and *Eucalyptus* sp in spring; *Alternanthera ficoidea*, *Chamissoa altissima* and *Eucalyptus* sp in summer; *Philodendron* sp, *Raphanus* sp and *Eucalyptus* sp in autumn.

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