Pollen analysis of honey and pollen collected by *Apis mellifera linnaeus*, 1758 (Hymenoptera, Apidae), in a mixed environment of *Eucalyptus* plantation and native cerrado in Southeastern Brazil

C. M. G. Simeão[a,b], F. A. Silveira[a], I. B. M. Sampaio[c] and E. M. A. F. Bastos[b]*

[a]Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais – UFMG, Av. Presidente Antônio Carlos, 6627, Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil

[b]Laboratório de Palinologia, Serviço de Recursos Vegetais e Opoterápicos, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias, Rua Conde Pereira Carneiro, 80, Gameleira, CEP 30510-010, Belo Horizonte, MG, Brazil

[c]Departamento de Ciência Animal, Escola de Veterinária, Universidade Federal de Minas Gerais – UFMG, Av. Presidente Antônio Carlos, 6627, Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil

*e-mail: embastos@funed.mg.gov.br

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Abstract

Eucalyptus plantations are frequently used for the establishment of bee yards. This study was carried on at Fazenda Brejão, northwestern region of the State of Minas Gerais, Brazil. This farm is covered both with native *Cerrado* vegetation (Brazilian savanna) and eucalyptus plantations. This paper reports on the botanic origin of pollen pellets and honey collected from honeybee (*Apis mellifera)* hives along a thirteen-month period (January 2004 to January 2005). The most frequent pollen types found in the pollen pellets during the rainy season were *Trema micrantha* (Ulmaceae), *Copaifera langsdorffii* (Fabaceae), an unidentified Poaceae, unidentified Asteraceae-2, *Cecropia* sp. 1 (Cecropiaceae) and *Eucalyptus* spp. (Myrtaceae); during the dry season the most frequent pollen types were *Acosmium dasycarpum* (Fabaceae), *Cecropia* sp. 1 (Cecropiaceae) and *Eucalyptus* spp. (Myrtaceae). Pollen grains of *Baccharis* sp. (Asteraceae), *Cecropia* sp. 1 (Cecropiaceae), *Copaifera langsdorffii* (Fabaceae), *Mimosa nuda* (Fabaceae), *Eucalyptus* spp. (Myrtaceae) and *Trema micrantha* (Ulmaceae) were present in the honey samples throughout the study period.

Keywords: Apis mellifera, Cerrado, pollen, honey, palynology.

Espectro polínico do alimento coletado por *Apis mellifera* linnaeus, 1758 (hymenoptera, apidae), em ambiente contendo plantações de *Eucalyptus* e cerrado nativo no sudeste brasileiro

Resumo

Plantações de *Eucalyptus* são, frequentemente, utilizadas como locais de instalação para colmeias. Este estudo foi realizado na Fazenda Brejão, localizada no noroeste de Minas Gerais, Brasil. Esta fazenda é coberta por Cerrado nativo (savana brasileira) e por plantações de eucaliptos. Este trabalho indica a origem botânica de bolotas e mel coletados em colmeias de *Apis mellifera* por um período de 13 meses (Janeiro/2004 a janeiro/2005). Os tipos polínicos mais frequentes nas amostras de pólen durante a estação chuvosa foram *Trema micrantha* (Ulmaceae), *Copaifera langsdorffii* (Fabaceae), Poaceae, Asteraceae não identificada 2, *Cecropia* sp. 1 (Cecropiaceae) e *Eucalyptus* spp. (Myrtaceae); na estação seca, os tipos polínicos mais frequentes foram *Acosmium dasycarpum* (Fabaceae), *Cecropia* sp. 1 (Cecropiaceae) e *Eucalyptus* spp. (Myrtaceae). As espécies *Baccharis* sp. (Asteraceae), *Cecropia* sp. 1 (Cecropiaceae), *Copaifera langsdorffii* (Fabaceae), *Mimosa nuda* (Fabaceae), *Eucalyptus* spp. (Myrtaceae) e *Trema micrantha* (Ulmaceae) estiveram presentes durante todo o período amostrado.

Palavras-chaves: Apis mellifera, Cerrado, pólen, mel, palinologia.

1. Introduction

The *Cerrado* is an important Brazilian ecosystem with diverse phyto-physiognomies such as forest, savanna and field (Brandão, 2000). It is considered one of the 25 biodiversity hotspots in the World (Mittermeier et al., 2000) for its exceptional concentration of endemic species and experiencing exceptional loss of habitat because of human activities (Silva and Bates, 2002). Currently, only 20% of its primary vegetation is left and only 1.2% is protected in conservation units (Mittermeier et al., 2000). Some features of the *Cerrado,* such as vegetation diversity,

great richness in nectariferous/pollineferous plants and low density of trees, make this a favorable region for beekeeping (Bastos et al., 2003). According to Carvalho and Bego (1995, 1996) and Viana et al. (1997), *A. mellifera* is one of the most common flower visitors of the *Cerrado.*

Nowadays, large portions of the *Cerrado* has been substituted by eucalyptus plantations (generally extensive), with more than 67% of the planted area concentrated in southeastern Brazil (Bernardo et al., 1998). In the state of Minas Gerais, most of the 1.5 million hectares of planted forest is eucalyptus plantations (Silveira, 2004), of wich the species used for the production of cellulose, wood and charcoal, are considered to be important pollen and nectar sources for the honeybees (Bastos, 1995; Bastos et al., 2003; Melo, 2004). For this reason, beekeepers frequently search for eucalyptus plantations to establish their bee yards.

The identification of plants visited by bees is an important indicator of nectar and pollen sources, permitting beekeepers to optimize the use of trophic resources, especially in natural vegetation (Howes, 1953). It is possible to identify the main melliferous and polleniferous plants exploited by bees in a delimited region through the identification of the pollen types found in the bee products (Barth, 1989).

Pollen grains is the natural source of proteins, lipids, vitamins and mineral salts for bees, being the only source of nitrogenated food available for bee larvae, and its absence may result in the hive extinction (Minckley et al., 2000). As for the energy source bees make use of nectar, transformed into honey (Doner, 1977).

The knowledge of the food sources most employed by bees is important to define strategies to enhance beekeeping productivity and profitability, as well as to certify the good quality and provenance of honeys (Bastos, 1996; Moreti et al., 2000).

Thus, the aim of the present study was to identify the food sources exploited by honeybees colonies established in firebreaks between native *Cerrado* vegetation and eucalyptus plantations in the northwestern region of Minas Gerais through the palynological analysis of honey samples and pollen pellets collected in honeybee hives.

2. Material and Methods

This study was carried out in the Fazenda Brejão, a farm owned by V & M Florestal Ltda. located in the municipality of Brasilândia de Minas (17° 01' S; 45° 52' W), northwestern region of the State of Minas Gerais, Brazil. The native *Cerrado* vegetation covers 65% of the 35.899-ha area of the farm and the remaining 32% is covered by eucalyptus plantations (Scolforo et al., 2001). The regional climate is the dry-sub humid tropical of Holdridge, with an annual average temperature between 22 °C and 24 °C, and annual average precipitation between 700 mm and 1000 mm (Golfari, 1975). During the study period, the average temperature and precipitation in the region were of 30.7 °C and 1895 mm, respectively.

Samples of flowering-plant species were collected monthly, between January 2004 and January 2005, along firebreak margins and inside *Cerrado* reserves. The plants were identified by the staff of the herbarium of the Taxonomic Collections of the Universidade Federal de Minas Gerais (BHCB) and deposited in the Laboratório de Palinologia of the Fundação Ezequiel Dias (FUNED), in Belo Horizonte, Minas Gerais, Brazil. Pollen collected from the anthers of these plants were acetolized according to Erdtman (1960) and used to prepare slides, which were deposited in the FUNED-POL reference slide collection, where pollen of plants previously collected in the Fazenda Brejão were already represented.

Two bee yards were established along firebreaks between eucalyptus plantations and native *Cerrado* vegetation (Beeyard-1: 17° 0' 38" S; 45° 51' 42" W; altitude: 532 m. Beeyard-2: 17° 0' 46" S; 45° 49' 31" W; altitude: 551 m), each containing three productive colonies housed in Langstroth hives.

2.1. Obtaining and preparing pollen samples for analysis

Pollen was collected once a week with the aid of pollen traps placed in the hive entrances for 24 hours, during the 13-month sampling period. These pollen traps extract about 50% of the pollen loads brought to the colony by the worker bees (Nagamitsu and Inoue, 1999). Pollen pellets collected were transferred to numbered plastic vials, which were hermetically closed and kept in a refrigerator at 4 °C until use.

Pollen samples from a single colony were weighed, dehydrated, homogenized, crushed, and three aliquots of 0.1 g were separately acetolized and used to prepare microscopic slides according to Erdtman (1960). These slides were deposited in the FUNED-POL slide collection.

2.2. Obtaining and preparing honey samples

Monthly, all frames containing only honey-filled cells (operculated or not) loaded in the past 30-day period were centrifuged (Guerra and Méndez, 2004). The honey obtained was sieved, homogenized and stored in hermetically-closed plastic vials. The pollen contained in each sample was acetolized and mounted in microscopic slides, according to Louveaux et al. (1978).

2.3. Analysis of pollen and honey samples

The identification of the pollen grains, in the previously prepared slides, was obtained through morphological analysis under the microscope comparing with pollen descriptions from Erdtman (1952) and Laboriau (1973) and through comparison with the reference slides in the FUNED-POL reference pollen collection. The frequency of each pollen type in 1200 pollen grains counted in each slide was calculated.

Pollen grains identified in the honey samples were grouped in four frequency classes according to the usual proceedings in melissopalynology, as suggested by Louveaux et al. (1970) and Barth (1970a, b, c): predominant pollen (representing more than 45% of all grains in the sample); accessory pollen (representing between 15% and 44% of the grains); isolated pollen (between 3% and 14%)

and occasional isolated pollen (less than 3%). Sub and over-representation of specific pollen types were considered in the data interpretation (Barth, 1989).

The contribution of the different pollen types was presented here relative to the two seasons in which the year is clearly divided and which define the management practices in the apiaries: dry (May to October) and rainy (November to April).

3. Results

3.1. Pollen spectrum in pollen pellets

Sixty-nine pollen types were identified in the 127 samples obtained. They belong to 28 plant families, with Fabaceae accounting for the larger number of species (14) found in the samples (Table 1).

More pollen types were recorded in the dry season (50 types) than in the rainy season (42). During the rainy season, pollen of *Eucalyptus* represented 8.27% of all pollen grains observed in the pollen pellets and during the dry season this figure rose to 14.54%.

3.2. Pollen spectrum of the honey samples

Twenty-one honey samples were collected during the study. No honey was available in the colonies between March and July 2004 and October 2004 and January 2005, when the populations were in decline.

Forty-eight pollen types were recorded in the honey samples, with Fabaceae and Asteraceae families representing the largest numbers of different plant types (11 and nine, respectively). November was the month with the largest variety of pollen types (30), and February with the smallest (18) (Table 2).

Pollen of *Eucalyptus* spp. was present during all the sampling period, although never as a predominant type pollen (it was accessory in August and isolated pollen in January, February, September, November and December) (Table 2).

Table 1. Frequency of the pollen types present in pollen pellets samples collected in Fazenda Brejão, in the municipality of Brasilândia de Minas, State of Minas Gerais, Brazil, between January 2004 and January 2005.

Pollen types	Frequency of each pollen type (%)	
	Rainy Season (November-April) N = 61	Dry Season (May-October) N = 66
Anacardiaceae		
Astronium sp.	0.00	5.26
Schinus sp.	0.01	0.00
Tapirira guianensis	1.56	0.02
Araliaceae		
Schefflera sp.	0.62	0.27
Asteraceae		
Baccharis sp.	3.45	0.58
Echinocoryne holosericea	0.05	0.07
Eremanthus sp.	0.03	0.00
Eupatorium sp.	2.15	2.15
Ichthyothere cunabi	0.01	0.00
Mikania sp.	3.57	0.00
Vernonia sp.	0.04	0.01
Wedelia sp.	0.05	0.00
Asteraceae unidentified 1	0.07	0.15
Asteraceae unidentified 2	14.46	0.00
Asteraceae unidentified 3	0.00	0.03
Asteraceae unidentified 4	0.13	0.49
Bignoniaceae		
Arrabidaea florida	0.32	0.00
Memora nodosa	0.02	0.00
Bombacaceae		
Eriotheca sp. .	0.00	0.34
Cecropiaceae		
Cecropia sp. 1	11.60	25.73
Cecropia sp. 2	0.41	0.01
Combretaceae		
Terminalia sp.	0.00	0.02

N = number of samples.

Table 1. Continued...

Pollen types	Frequency of each pollen type (%)	
	Rainy Season (November-April) N = 61	Dry Season (May-October) N = 66
Convolvulaceae		
Ipomoea sp.	0.00	0.02
Jacquemontia sp.	0.00	0.02
Cyperaceae		
Cyperaceae unidentified 1	0.08	0.01
Dilleniaceae		
Davilla elliptica	0.00	3.04
Fabaceae		
Acosmium dasycarpum	0.00	34.79
Bauhinia pulchella	0.00	0.10
Bauhinia sp.	0.00	0.03
Canavalia sp.	0.01	0.00
Clitoria fairchildiana	0.00	0.01
Copaifera langsdorffii	16.94	0.00
Leucaena sp.	0.00	2.14
Mimosa nuda	0.47	0.76
Mimosa sp.	0.19	0.00
Stryphnodendron sp.	0.00	0.03
Stylosanthes scabra	0.01	0.01
Trifolium sp.	0.00	0.07
Fabaceae unidentified 1	0.00	0.02
Fabaceae unidentified 2	0.01	0.17
Lamiaceae		
Hyptis sp.	0.00	0.01
Lecythidaceae		
Cariniana sp.	0.07	0.00
Loganiaceae		
Antonia ovata	0.00	0.87
Loganiaceae unidentified 1	0.00	0.33
Loranthaceae		
Struthanthus flexicaulis	0.01	0.00
Lythraceae		
Lafoensia pacari	0.00	0.01
Malpighiaceae		
Byrsonima coccolobifolia	0.02	0.02
Byrsonima sp.	0.53	0.00
Malvaceae		
Malvastrum sp.	0.00	0.04
Sida linifolia.	0.03	0.00
Sida sp.	0.02	0.02
Malvacea unidentified 1	0.01	0.00
Melastomataceae		
Rhyncanthera sp.	0.00	0.12
Melastomataceae unidentified 1	0.00	0.10
Melastomataceae unidentified 2	0.00	0.02
Myrtaceae		
Eucalyptus sp.	8.27	14.54
Eugenia dysenterica	0.00	1.09
Myrcia sp.	0.01	0.03

N = number of samples.

Table 1. Continued...

Pollen types	Frequency of each pollen type (%)	
	Rainy Season (November-April) N = 61	Dry Season (May-October) N = 66
Myrtaceae unidentified 1	0.00	0.01
Onagraceae		
Ludwigia sp.	0.00	0.68
Palmae		
Mauritia flexuosa	0.05	0.00
Palmae unidentified 1	0.01	0.02
Poaceae		
Poacea unidentified 1	16.12	1.38
Rubiaceae		
Diodia sp.	0.25	0.02
Richardia sp.	0.01	0.00
Sapindaceae		
Serjania sp.	0.00	0.24
Solanaceae		
Solanum sp.	0.53	0.01
Ulmaceae		
Trema micranta	17.45	2.15
Verbenaceae		
Stachytarpheta sp.	0.01	0.00
Unidentified Families		
Unidentified	0.34	1.94

N = number of samples.

Table 2. Frequency of pollen types present in honey samples collected between January 2004 and December 2004 in Fazenda Brejão, municipality of Brasilândia de Minas, State of Minas Gerais, Brazil.

Pollen types	Frequency of pollen types (%)					
	Jan/04	Feb/04	Aug/04	Sep/04	Nov/04	Dec/04
	N = 4	N = 4	N = 2	N = 2	N = 7	N = 2
Anacardiaceae						
Anacardiaceae unidentified 1	0.24				4.19	
Astronium sp.	0.35	0.45	0.72	5.33	7.10	2.73
Tapirira guianensis			0.36		14.07	12.89
Araliaceae						
Schefflera sp.		0.23	1.27			
Asteraceae						
Achyrocline sp.	0.02					
Ageratum sp.		0.02				
Ayapana sp.		0.02				
Baccharis sp.	17.78	14.70	8.95	3.22	0.09	3.64
Eremanthus sp.		0.02				
Eupatorium sp.			0.81			1.21
Mikania sp.		1.02				
Tagetes sp.	0.02					
Vernonia sp.	0.04		0.27			
Bignoniaceae						
Arrabidaea florida	0.11	2.27	3.80			
Arrabidaea sp.	0.15					
Bombacaceae						
Eriotheca sp.			0.18	0.10		

N = number of samples.

Table 2. Continued...

Pollen types	Frequency of pollen types (%)					
	Jan/04	Feb/04	Aug/04	Sep/04	Nov/04	Dec/04
	N = 4	N = 4	N = 2	N = 2	N = 7	N = 2
Cecropiaceae						
Cecropia sp. 1	22.89	17.59	32.91	61.62	37.35	53.01
Combretaceae						
Terminalia sp.			0.27	2.62	0.44	
Cyperaceae						
Cyperaceae unidentified 1			0.36		0.05	
Fabaceae						
Acosmium dasycarpum				14.99	11.29	2.68
Bauhinia sp.					0.03	
Copaifera langsdorffii	5.74	27.91	3.07	0.20	0.10	0.30
Leucaena sp.			0.36	0.20	1.91	
Mimosa nuda	6.57	8.51	14.10	0.10	0.11	0.30
Mimosa sp.	0.09	0.25	0.45			
Stryphnodendron sp.			0.18		0.18	
Stylosanthes scabra						0.10
Trifolium sp.			0.99	0.25	0.11	
Fabaceae unidentified 1					0.82	
Fabaceae unidentified 2	0.19	0.02		0.10		
Lamiaceae						
Hyptis sp.	0.02		0.18	0.05		0.30
Lecythidaceae						
Cariniana sp.					0.08	0.30
Loganiaceae						
Antonia ovata			0.63	0.30	0.05	
Loranthaceae						
Struthanthus flexicaulis			0.18	0.10	0.27	4.75
Malpighiaceae						
Byrsonima coccolobifolia					1.29	0.35
Byrsonima sp.					0.78	
Melastomataceae						
Melastomataceae unidentified 1					0.03	
Myrtaceae						
Eucalyptus spp.	7.81	8.65	20.61	4.98	9.87	6.37
Eugenia dysenterica			0.18	1.96	0.80	0.30
Myrcia sp. DC.	0.02	0.07	0.18		5.00	1.01
Myrtaceae unidentified 1					0.10	
Onagraceae						
Ludwigia sp.					0.03	
Palmae						
Palmae unidentified 1					0.04	0.05
Poaceae						
Poaceae unidentified 1	6.35	11.35	3.62	0.35	0.03	1.16
Rubiaceae						
Diodia sp.	0.94	0.39	0.18	0.10		
Sapindaceae						
Serjania sp.	0.02		0.18	0.35	0.13	
Ulmaceae						
Trema micranta	29.94	4.45	4.25	2.52	3.65	8.49

N = number of samples.

Table 2. Continued...

Pollen types	Frequency of pollen types (%)					
	Jan/04	Feb/04	Aug/04	Sep/04	Nov/04	Dec/04
	N = 4	N = 4	N = 2	N = 2	N = 7	N = 2
Verbenaceae						
Stachytarpheta sp.	0.02					
Unidentified Families						
Unidentified	0.69	2.08	0.76	0.56	0.01	0.06
Total number of grains	5404	4407	1106	1988	7920	1979

N = number of samples.

4. Discussion

The pattern of resource exploitation observed in Fazenda Brejão is that of a generalist species, as observed previously by other authors for *Apis mellifera* (*e. g.* Ramalho et al., 1991; Viana et al., 1997; Strickler and Cane, 2003). However, in spite of the large amount of plant species visited, the honeybees concentrated their foraging activity in a few species, from which they gathered the largest amounts of pollen and nectar at given periods. This behavior of *Apis mellifera* has been previously described (*e.g.* Pedro and Camargo, 1991; Viana et al., 1997; Melo, 2004) and according to Lima (1995), it could be explained by the fact that the honeybee colonies concentrate their foraging activity in the most abundant flowering plants in any given time.

The fact that honeybees harvest a larger variety of pollen types during the dry season was already reported (*e. g.* Oliveira, 1998; Pedro and Camargo, 1991; Viana et al., 1997), and may be due to the low precipitation and air humidity, when honeybee foraging is favored (Szabo, 1980).

The occurrence of pollen grains of Myrtaceae and, especially, *Eucalyptus* spp. in the pollen samples of the honeybee and other bees is well known (Ramalho et al., 1991; Soares, 2003; Melo, 2004). This is because *Eucalyptus* produces massive amounts of flowers with large numbers of anthers, which produce large quantity of pollen, a characteristic of the plants most commonly visited by *Apis mellifera* (Moncur and Boland 1989; Ramalho et al., 1991).

Many species of the Asteraceae family common in antropic environments are frequent pollen sources for the honeybee and regarded as good bee plants (Bastos et al., 1995; Bastos et al., 1998).

Cecropia, a plant genus commonly considered to be anemophylous (Joly, 1979; Barth, 1989; Bastos et al., 1991; Bastos et al., 1993), was present as predominant pollen in September and December and as accessory pollen in January, February, August and November. Soares (2003) also observed large amounts of *Cecropia* pollen in honey samples of *Melipona* aff. *rufiventris* (Apidae, Meliponina) collected at Fazenda Brejão, especially during periods when food was most abundant for the bees. According to Barth (1989), pollen of anemophilous plants can be found in honey, sometimes in large amounts, as contaminants, but it is never used as a nectar source by bees. However, honeybees were observed visiting both male and female *Cecropia* inflorescences (personal observation) which is interesting because at least one *Cecropia* species is known to produce nectar (*e. g.* Andrade, 1984). An alternative explanation is that bees may be collecting honeydew produced by aphids commonly found on these plant's inflorescences. No analyses have been performed to verify whether the honey containing *Cecropia* pollen has the typical characteristics of honeydew.

An interesting finding was the relatively large amounts of the *Trema micrantha* pollen-types in the honey collected at Fazenda Brejão, contrasting with the small amounts generally encountered in other samples of Brazilian honey (Barth, 1989).

The frequent presence of Poaceae pollen found in the honey samples studied is probably a consequence of pollen from body bees, since plants of this family are not nectariferous (Barth, 1989) but bees are known to collect pollen from their flowers.

Our results indicate that although *Eucalyptus* is an important pollen and nectar source to honeybees, in bee yards established in areas of *Eucalyptus* plantations, nearby areas of native vegetation are also heavily exploited by the bees as floral resources.

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