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Pollen spectrum of the honey of uruçu bee (*Melipona scutellaris* Latreille, 1811) (Hymenoptera: Apidae) in the North Coast of Bahia State

Larissa Silva Souza[1], Cátia Ionara Santos Lucas[3], Pâmela de Jesus Conceição[3], Joana Fidelis da Paixão[2*] and Rogério Marcos Alves[2]

*[1]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Campus Senhor do Bonfim, Senhor do Bonfim, Bahia, Brazil. [2]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Campus Catu, Rua Barão de Camaçari, 118, 48110-000, Catu, Bahia, Brazil. [3]Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil. *Author for correspondence. E-mail: joana.paixao@ifbaiano.edu.br*

ABSTRACT. Regional-level studies of floral resources used by social bees for honey production can contribute to the improvement of management strategies for bee pastures and colonies, by identifying the most visited flowers and thus characterizing the various geographical origins of honey. The objective of this study was to investigate, through pollen analysis, the types of pollen and nectar sources used by the uruçu bee (*Melipona scutellaris* L.) in the North Coast of Bahia. Honey samples were taken monthly from five colonies in an apiary from August 2010 to July 2011. Pollen analysis of honey was performed by using the acetolysis method, followed by qualitative and quantitative analysis of pollen grains. Fifty pollen types belonging to 40 genera and 17 families were identified. The results indicate predominance of pollen types belonging to the families Fabaceae and Myrtaceae, which suggests that the bees preferred foraging from trees and shrubs. These plants should be included in regional reforestation projects in order to improve management of this bee species and honey production.

Keywords: stingless bees, Meliponinae, pollen collection, Atlantic Forest, trophic niche.

Espectro polínico do mel da abelha de uruçu (*Melipona scutellaris* Latreille, 1811) (Hymenoptera: Apidae) do Litoral Norte do estado da Bahia

RESUMO: Estudos em nível regional dos recursos florísticos utilizados por abelhas sociais para a produção de mel podem contribuir para a melhoria das estratégias de manejo do pasto meliponícola e das colônias, através da identificação das flores mais visitadas, e dessa forma caracterizar a origem geográfica do mel. O objetivo desta pesquisa foi investigar, por meio da análise polínica, os tipos de vegetação fornecedora de néctar e pólen para a abelha uruçu (*Melipona scutellaris* L.) no Litoral Norte da Bahia. Foram realizadas coletas quinzenais em 5 colônias de meliponário, de agosto de 2010 a julho de 2011. A análise polínica do mel foi conduzida através do método da acetólise, seguida por análise quali-quantitativa dos grãos de pólen. Foram identificados 50 tipos polínicos pertencentes a 40 gêneros e 17 famílias. Os resultados apontam para a dominância dos tipos polínicos pertencentes às famílias Fabaceae e Myrtaceae, sugerindo que essas abelhas preferem forragear em árvores e arbustos. Esses tipos vegetais predominantes devem ser incluídos nos projetos regionais de reflorestamento, visando favorecer o manejo dessa espécie de abelha e melhorar a produção de mel.

Palavras-chave: abelhas sem ferrão, Meliponinae, coleta de pólen, Mata Atlântica, nicho trófico.

Introduction

Bees belonging to the family Apidae, subtribe Meliponina are known as stingless bees and exhibit eusocial behavior. The *Melipona* genus includes the largest number of species of this group, being found in the Neotropical region (South and Central America and the Caribbean Islands) (SILVEIRA et al., 2002).

Most of these bees feed on products obtained from flowers. The main source of protein for adult bees and their larvae is pollen collected from flowers by foragers. After collection, the bees store the pollen in the corbicula. When they return to the colony, the bees deposit pollen in pots or combs, compressing the product with the head to get a compact mass. This material undergoes transformations under the action of temperature, humidity and salivary enzymes (NOGUEIRA-NETO, 1997), and mixed with nectar to form the bee bread (by honey bees) or 'samburá' (by stingless bees) (MENEZES et al., 2013).

Considering the importance of nectar and pollen producing plants in the development of rational beekeeping, Moreti et al. (2000) reported that efforts should be made to identify the species of interest to beekeeping and honey production in each region.

The plants visited by bees for nectar collection can be identified by analyzing the different types of pollen found in honey (AIRA et al., 1998). This information is important for beekeepers, as it enables them to employ strategies that will maximize the bees' exploitation of the trophic resources in a particular geographic region, especially in areas of natural vegetation. Therefore, pollen analyses carried out on a monthly basis can provide a valuable beekeeping calendar for honey producers (LUZ et al., 2007).

Regional-level studies are required in order to identify the plant species visited by bees, particularly the stingless bee *Melipona scutellaris* Latreille, 1811. The breeding of this bee species is mainly conducted in anthropized areas; therefore, it is important to study the native and exotic plant species that provide the resources for these bees.

The aim of this study was to identify pollen types present in the spectra of honey samples taken from *M. scutellaris* colonies in the municipality of Camaçari, Bahia, during a production cycle.

Material and methods

The study was performed in an apiary in Camaçari (North Coast of Bahia), in which *M. scutellaris* were housed. The area is anthropized and characterized by remnants of ombrophilous forest and the presence of exotic plants (SEI, 2013). The apiary was located at 12° 48' 35.99" S and 38° 15' 24.37" W. Five colonies were used for the trials; these were kept separate from the other colonies in the apiary and were not fed artificially during the experimental period (August 2010 to July 2011).

Honey was collected monthly from selected honey pots. To ensure that the samples represented the honey produced each month, the pots were observed biweekly and compared with a picture of the colony that was taken during the previous harvest. Images were captured using a Canon SX50HS digital camera. This procedure ensured that we sampled only pots with honey that was newly deposited by the bees, and prevented resampling from pots used in previous sampling periods. Monthly a 50-mL honey sample was collected from each colony using a disposable syringe. Samples were stored in individual tubes.

Qualitative analysis of pollen spectra involved comparison of the pollen types present in the honey samples and those present in the flowering plants in the study area. The pollen types were also compared with to pollen identified in reference slides from the Pollen Collection at the Insecta Center, Universidade Federal do Recôncavo da Bahia (UFRB), and descriptions in specialized literature, such as Barth (1970a, b, c, 1971, 1989, 1990, 2004) and Roubik and Moreno (1991).

The floral buttons were collected during biweekly visits to the study area. During these visits, the presence of bees on flowers and the resources collected were recorded. Samples of blooming plant species were collected for identification in the UFRB herbarium, and pollen extracted from floral buttons of these samples was mounted on microscope slides.

For quantitative pollen analysis, all honey samples collected in a given month were pooled. Slides were prepared from the samples of honey collected based on a method by Erdtman (1960). The slides were visualized using an optical microscope (Olympus Microscope Model CX41), and were photographed (Olympus E330-Adu digital camera, 1.2x); 1000 pollen grains were counted for each sample (Barth, 1989). The relative frequency of each pollen type was established using the formula: $f = (ni\ N^{-1}) \times 100$, where f is relative frequency of pollen type i in sample j; ni is the number of pollen grains of pollen type i in sample j; N is the total number of pollen grains in sample j. Pollen types were classified according to frequency: dominant pollen (> 45% of total grains) (DP), accessory pollen (16 – 45%) (AP), important isolated pollen (3 – 15%) (IIP), and occasional isolated pollen (< 3%) (OIP) (LOUVEAUX et al., 1978).

Results and discussion

The pollen types found in the collected honey samples are depicted in Table 1. Most of the plant species had been previously identified and were part of the regional flora reference collection, which allowed for the identification of the pollen in collected honey at the genus and species level. We identified 50 pollen types, belonging to 40 genera and 17 families. We found three additional pollen types that we were unable to identify.

The number of pollen types from each family found in collected honey samples is shown in Figure 1. Plant families Fabaceae (15 types, including 10 types from subfamily Mimosoidae) and Myrtaceae (nine types) were represented by the greatest number of pollen types in the spectrum of uruçu bee honey. Other families that contributed to the formation of the pollen spectrum in honey were Anacardiaceae (four types), Sapindaceae (three types), Solanaceae (three types), Arecaceae (two types), and Verbenaceae (two types). Myrtaceae and Fabaceae (Mimosoideae) were found in samples collected in almost every month of the study period (11 months) (Table 1).

The predominance of pollen from Fabaceae and Myrtaceae species in honey samples was expected (Figure 1), because previous studies have shown that pollen from these families is present in honey samples of stingless bees, especially in anthropized areas, where many species of Myrtaceae have been planted for food and reforestation (CARVALHO et al., 2001; RAMALHO et al., 2007).

The Fabaceae (Mimosoideae) family includes species that provide plenty of resources (pollen and nectar) to bees. According to Ramalho et al. (1990), several species of the genus *Mimosa* are sources of pollen and/or nectar.

The Sapindaceae family includes species of vines and small trees that grow in forest areas and produce fruits. Members of this family are used as ornamental plants and in timber production for carpentry, furniture, and firewood (ARAÚJO; COSTA, 2007). Species belonging to this family are considered nectar plants (VIDAL et al., 2008) and are often represented by few pollen grains in honey pollen spectra, as observed in the present study. Although Sapindaceae pollen grains were found in honey samples collected in the North Coast of Bahia region, the species of this family may not have contributed effectively to the production of the honey

Table 1. Frequency of pollen types (%) in honey samples collected from *Melipona scutellaris* in anthropized areas of Camaçari-Bahia in 2011 and 2012.

		Months											
Family	Pollen type	Aug/11	Sep/11	Oct/11	Nov/11	Dec/11	Jan/12	Feb/12	Mar/12	Apr/12	May/12	Jun/12	Jul/12
Anacardiaceae	*Spondias macrocarpa* Engl.	36.45	32.47										72.34
	Tapirira guianensis Aubl.	1.48	11.34										
	Schinus terebinthifolius Raddi				15.84	20.8							
Arecaceae	*Cocos nucifera* L.					0.49	1.59			4.95	26.84		
	Syagrus sp. Mart.				1.34							2	
Apocynaceae	*Himatanthus sp.* Willd. ex Schult.		1.03										
Burseraceae	*Protium sp.* Burm.f.									2.16			
Bromeliaceae	*Hohenbergia sp.* Schult. & Schult.f.						2.65						
Bignoniaceae	*Tabebuia ochracea* (Cham.) Standl.									0.36			
Boraginaceae	*Heliotropium sp.*L.											5.3	
Fabaceae-Caesalpinioideae	*Bauhinia sp.*L.				0.41					1.68			10.49
	Caesalpinia echinata Lam.										0.48		
	Senna sp. Mill.	28.17											
	Caesalpinia pulcherrima (L.) Sw.	0.84	14.95				26.72						
Fabaceae-Mimosoideae	*Leucaena leucocephala* (Lam.) de Wit		1.54	3.49									0.09
	Inga sp. Mill.												0.91
	Inga edulis Mart.			2.32		15.29				8.8	2.88		
	Mimosa sp. L.											1.23	
	*Pithecellobium dulce (*Roxb.) Benth.	0.21				0.73	2.11						
	Piptadenia moniliformis Benth.		26.28	74.42	8.24							2.13	
	Stryphnodendron sp. Mart.	0.21											
	Mimosa caesalpiniifolia Benth.	0.84			43.31	33.04		38.86		7.8			
	Albizia sp. Durazz.	0.42											
	Parapiptadenia pterosperma (Benth.) Brenan											1.68	0.23
Fabaceae-Papilionidae	*Pueraria sp.* DC.	3.38											
Guttiferae	*Vismia sp.* Vand.	2.38											
Melastomataceae	*Miconia sp.* Ruiz & Pav.											0.05	
Myrtaceae	*Syzygium samarangense* (Blume) Merr. & L.M.Perry							28.28		6.72			0.45
	Eugenia uniflora L.		4.12		7.2					7.8			0.87
	Myrcia crassifolia Kiaersk.					8.32							
	*Psidium sp.*L.										30.19	29.3	
	Eucalyptus torelliana F. Muell.				19.34	12.6	29.9		42.25	30.37	25.39	8.47	
	Myrciaria cauliflora (Mart.) O.Berg						20.89			6.09			
	Campomanesia dichotoma (O.Berg) Mattos	1.27											
	Algrizea minor Sobral et al.								7.75				
	Myrcia sp. DC.							32.86					
Pouteriaceae	*Pouteria caimito* (Ruiz & Pav.) Radlk.						13.2			2.76			
Rubiaceae	*Coffea arabica* L.											10.41	
Rutaceae	*Citrus sp.*L.											1.26	
Sapindaceae	*Allophylus sp.*L.										10.38		
	Cupania vernalis Cambess.								14.08	13.92			
	Cardiospermum corindum L.				0.41								
	Solanum paniculatum L.				0.21								
	Solanum sp. L.											2.52	
Verbenaceae	*Aloysia gratissima* (Gillies & Hook.) Tronc.	0.63	8.26	4.65	0.41				35.92	2.88	3.83	0.64	
	*Lantana sp.*L.											5.69	
Not identified	NI 1	0.41		15.12	0.62	4.77	1.05			0.36			
	NI 2				2.67	2.82	1.59			1.44			
	NI 3					1.1				1.92			

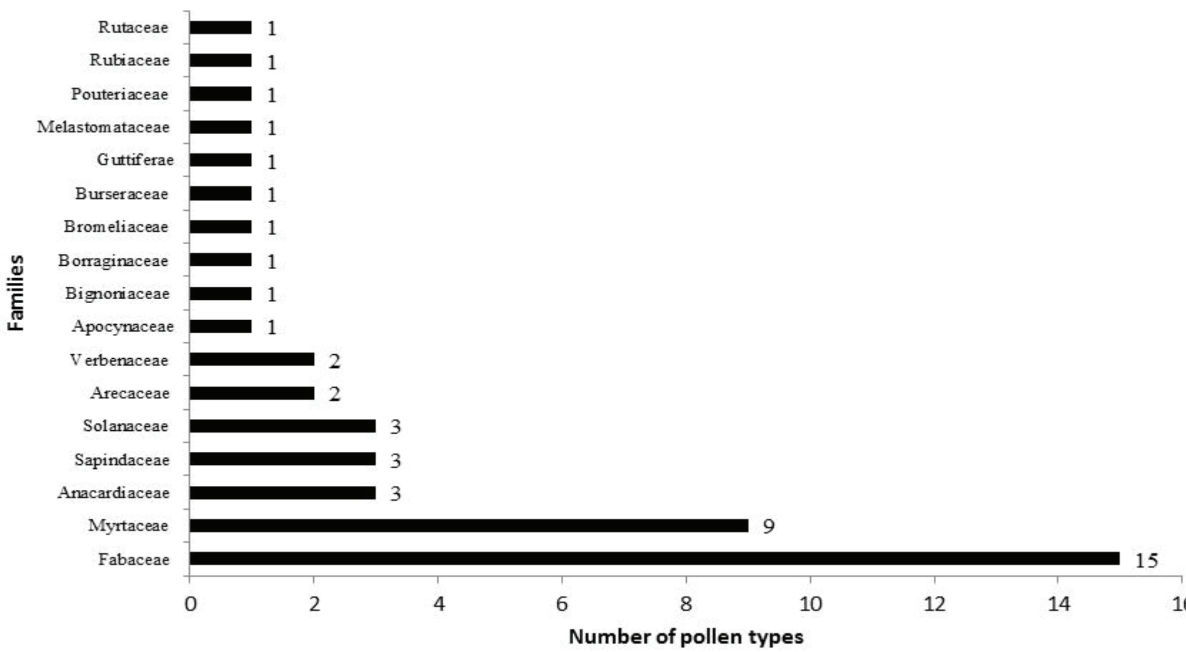

Figure 1. Distribution of pollen types in each family identified in *Melipona scutellaris* honey samples from the municipality of Camaçari, North Coast of Bahia.

Members of the Solanaceae family are mainly pollen plants that are common in anthropized areas and in transition zones. Species in this family are visited by bee species belonging to the genus *Melipona*, which collect pollen by vibrating the flower's anthers (BUCHMANN, 1983; ROUBIK, 1989). Members of the Anacardiaceae family include fruit trees that serve as resources for birds and other animals.

Similar results to these presented in the present study were found by Carvalho et al. (2001), who investigated the pollen types present in *M. scutellaris* honey in the municipality of Catu, Bahia State. The families most represented in the pollen spectra in honey from Catu were Myrtaceae (56%), Mimosaceae (25%), and Caesalpiniaceae (14%). Ramalho et al. (2007) studied the dynamics of the pollen sources collected by *M. scutellaris* and found that the bees collected pollen from the following families (in order of importance): Myrtaceae, Mimosaceae, Anacardiaceae, Sapindaceae, and Fabaceae. Martins et al. (2011) also found that pollen from the families Fabaceae (Caesalpinioideae) and Myrtaceae were the most represented types of pollen in the honey of *M. compressipes* bees in Maranhão.

The most frequently observed pollen types in the present study belonged to *Aloysia gratissima* (8 months), *Eucalyptus torelliana* (7 months), and *Mimosa caesalpiniifolia* (5 months). *E. torelliana* and *M. caesalpiniifolia* are exotic species that are used as ornamental plants and timber, and have extended blooming periods with high nectar and pollen production. The greatest diversity of pollen types was found in September or November (15 types) and January (14 types). This period coincides with the end of the rainy season and the beginning of the dry season in the region, when many plant species bloom and bee foraging increases.

The most frequently observed pollen types in each sample/month belonged to: *Spondias macrocarpa* (December 2011; January and February 2012), *M. caesalpiniifolia* (May, June, and August), *E. torelliana* (August, September, and October 2011; and April, May, and June 2011), and *Solanum stipulaceum* (November 2011 to January 2012). These species bloomed for extended periods and grew in dense clusters in the study area.

Mimosa caesalpiniifolia has great potential for urban forestry, hedging, and wood production (ALVES et al., 2002). This species blooms for eight months of the year and bees collect nectar from this species, especially during the rainy season when nectar production by other species is low. Barth (1970a) stressed that *M. caesalpiniifolia* pollen is often over-represented compared to the nectar produced because this species is mainly polliniferous. Luz et al. (2007) classified *M. caesalpiniifolia* pollen in *Apis mellifera* honey as IIP and OIP, which is observed mainly in May and June.

Spondias macrocarpa is a native tree that is considered an excellent source of pollen with long, massive blooms. *M. caesalpiniifolia* and *E. torelliana* are exotic species introduced in Brazil for the timber industry and as a supply of trophic resources to bees. *Solanum stipulaceum* is an invasive species that is found in deforested areas, provides pollen, and is pollinated by native bees (NUNES-SILVA et al., 2010).

Melipona scutellaris has been reported to visit several of the plant species for which pollen was identified in the present study (ALVES et al., 2006). Most pollen grains found in the present study were included in the isolated pollen class (IIP, < 15%), which is characteristic of Brazilian honeys (BARTH, 1970a). Honeys from different regions of the state of Bahia show characteristic *Acacia*, *Hyptis*, *Mimosa*, and *Myrcia* pollen types (BARTH, 1970c).

We found 15 IIP pollen types classified as IIP and 12 OIP pollen types (Table 2). Barth (2005) reported that nectar plants are more important than pollen plants for honey production, although some of the pollen found in honey is from anemophilous and polliniferous plants.

Evaluation of plants that provide trophic resources to bees (Table 2) helped in the identification of 44 plants, including 16 types of polliniferous plants, 18 types of nectar plants, and 10 types of pollen-nectar plants. This shows that the honey of *M. scutellaris* is of multifloral origin, with contributions from IIP pollen types, and that the bees collect both nectar and pollen from many different plant species.

DP (> 45% of the grains, Table 1) was found only in March (*P. moniliformis*) and January (*Spondias macrocarpa*), which are significant flowering periods for these species, whereas AP was found in all months, except January and March (10 months), and the largest number of AP pollen types was found in June. OIP was found in 10 months and most OIP types were found in January. IIP was found in 11 months, but not in June, and the largest number of AP pollen types was found in November.

Analysis of pollen types analyzed in terms of resources (pollen and nectar) collected by bees indicates that the only DP species that contributed to honey production was *P. moniliformis*, as it is primarily a nectar species. Four AP species produce nectar (*P. moniliformis*, *E. torelliana*, *M. caesalpiniifolia*, and *Aloysia gratissima*) and therefore, they likely contributed to the production of honey.

Barth (1989) reported that occasional pollen has little importance for the amount of provided nectar. Many plant species that have few grains of pollen in honey, contribute to honey production and determine the geographical origin of the resources collected by bees. For example, *Tapirira guianensis* (Anacardiaceae) is a common species in the study area (North Coast of Bahia) and is considered by beekeepers to be the best nectar-providing species to bees from December to February. The pollen of this species was underrepresented in the studied samples and was classified as IIP (February) and OIP (January). Barth et al. (2012), in their analysis of *M. scutellaris* honey, found that 32% of the pollen in honey was from *T. guianensis*. Verification of isolated and occasional pollen helps improve knowledge of plants that provide resources for bees.

However, Barth (2004) considered classification of the pollen types found in honey samples into frequency classes to be insufficient for complete understanding of the relative importance of plant species to bees, because nectar species may be under-represented in the spectrum. It is also necessary to consider which plant species produce nectar and pollen, the estimated productivity of these products, and the species most visited by bees, which can vary from one region to another. In most cases, studies are limited to pollen type, and it is not possible to determine plant species from these types (BARTH, 1989).

In this research Anacardiaceae, Mimosoideae, Myrtaceae, Sapindaceae, Verbenaceae, Rubiaceae, Caesalpinioideae and Burseraceae families were representative of PII and Anacardiaceae, Mimosaceae, Myrtaceae, Verbenaceae, Bromeliaceae, Burseraceae, Rutaceae, Caesalpinioideae and Sapindaceae were representative of PIO (Table 2). According to Carvalho et al. (2001), and Ramalho et al. (2007) these families are known as nectar supply. Although the nectar plants have a fundamental role in the production of honey, this study demonstrates that pollen arising from polliniferous species also contribute to the production of honey.

The assessment of pollen types present in collected honey (Table 1) and the growth patterns of the associated plants (Table 2) demonstrated that *M. scutellaris* had a preference for trees (30 species) and bushes (12 species) over vines (two species) and grass (two species). In this region, ruderal and invasive species represented by vines and grass are prevalent. However, even though such plants are abundant, the uruçu bee demonstrated a preference for foraging tree and shrub species. Martins et al. (2011) reported that the *M. fasciculata* honey collected in anthropized areas had nectar from few ruderal and native plant species, whereas Luz et al. (2007) found pollen from native, ruderal, and exotic plants in the honey produced by *Apis mellifera* in areas with strong human influence in Morro Azul do Tinguá (RJ).

Table 2. Growth patterns and resources collected from plant species found in honey samples from *Melipona scutellaris* in the North Coast of Bahia. SV – stratum vegetation, P = pollen, N = nectar, NP = nectar and pollen, T = trees, B = bushes, H = herbs, V = vines.

Families	Species	Plant resource	SV
Anacardiaceae	*Spondias macrocarpa*	P	T
	Tapirira guianensis	NP	T
	Schinus terebinthifolius	N	T
Arecaceae	*Cocos nucifera*	P	T
	Syagrus sp.	P	T
Apocynaceae	*Himatanthus* sp.	N	T
Burseraceae	*Protium* sp.	N	T
Bromeliaceae	*Hohenbergia sp.*	N	H
Bignoniaceae	*Tabebuia ochracea*	N	T
Boraginaceae	*Heliotropium* sp.	N	H
Guttiferae	*Vismia* sp.	N	B
Fabaceae-Caesalpinioideae	*Bauhinia* sp.	NP	T
	Caesalpinia echinata	N	T
	Senna sp.	NP	B
	Caesalpinia pulcherrima	P	T
Fabaceae-Mimosoideae	*Leucaena leucocephala*	P	T
	Inga sp.	N	T
	Inga edulis	N	T
	Mimosa sp.	P	H
	Pithecellobium dulce	P	T
	Piptadenia moniliformis	N	T
	Stryphnodendron sp.	P	T
	Mimosa caesalpiniifolia	NP	T
	Albizia sp.	P	T
	Parapiptadenia pterosperma	NP	T
Fabaceae-Papilionidae	*Pueraria* sp.	NP	V
Melastomataceae	*Miconia* sp.	P	B
Myrtaceae	*Syzygium samarangense*	P	T
	Eugenia uniflora	NP	T
	Myrcia crassifolia	NP	T
	Psidium sp.	P	T
	Eucalyptus torelliana	NP	T
	Myrciaria cauliflora	P	T
	Campomanesia dichotoma	P	T
	Algrizea minor	P	T
	Myrcia sp.	NP	B
Pouteriaceae	*Pouteria caimito*	P	T
Rubiaceae	*Coffea arabica*	N	B
Rutaceae	*Citrus* sp.	N	B
Sapindaceae	*Alophylus* sp.	N	T
	Cupania vernalis	N	B
	Cardiospermum corundum	N	V
Solanaceae	*Solanum stipulaceum*	P	B
	Solanum paniculatum	P	B
	Solanum sp.	P	B
Verbenaceae	*Aloysia gratissima*	N	B
	Lantana sp.	N	B

Conclusion

The growth of apiculture in Brazil has led to increased interest in the quality of honey. Analysis of pollen types in honey samples from the Northeastern region and comparison of the pollen with the regional flora is required in order to characterize the botanical origin of the honey and improve honey quality. In this study, the vegetation of interest was identified, and a preliminary beekeeping calendar was prepared for producers in the region.

Melipona scutellaris colonies were exposed to variations in the supply of flowers and their honeys contained pollen grains from a variety of plant species. Therefore, we can conclude that honeys from this species, which forages in the Camaçari region, are multifloral (wild), with characteristics that vary with the availability of blooms.

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