



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2015; 3 (2): 290-296

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Received: 18-01-2015

Accepted: 19-02-2015

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Pollen spectrum of stingless bees honey (Hymenoptera: Apidae), Paraná State, Brazil

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Abstract

The objective of this study was to identify the plants that contribute to the honey composition of stingless bees using pollen analysis. We collected 30 stingless bees honey samples. The pollen analysis was performed following the standard method of acetolysis. We identified 55 pollen types belonging to 27 families. The predominant pollen types were *Casearia*, *Eucalyptus*, *Galactia*, *Miconia*, *Morus*, *Myrcia* I, *Syagrus* and Rubiaceae type. The pollen types most frequent among the samples were *Syagrus*, *Eucalyptus*, *Mimosa caesalpiniaefolia*, *Psidium*, *Miconia*, *Morus*, *Leucaena* and *Inga*. The average number pollen types per sample the studies species ranged from 4.25 to 9.00, indicating the participation of several plant species in the honey composition of stingless bees. The occurrence of a predominant pollen type was recorded in 40% of the samples. The pollen spectrum of the honey samples analyzed were diverse, with significant contributions of Fabaceae (subfamily Mimosoideae), Asteraceae and Myrtaceae as the most diverse in species visited by stingless bees in the study area.

Keywords: honeybees, nectar, melissopalynology

1. Introduction

Stingless bees occur mainly in tropical and subtropical regions, and are adapted to different types of vegetation including forests, savannas, fields and mountains. Honey and harvested pollen change in composition depending upon availability plant species flowering. Melissopalynology of pollen loads and honey of stingless bees allows recognizing bee floral preferences in different localities and the local vegetation types [1]. Each plant species has flowers with a distinct pollen type that can be studied to determine the floral source of honey. The pollen analysis is of great importance for quality control of honeys, which can include numerous pollen grains and honeydew, elements that, together, can provide information about their floral origin [2, 3].

The Neotropics bee fauna is rich and diverse, but little studied, well with the trophic resources used by these bees. Deforestation and introduction of exotic competing bee species are considered the main threats to most stingless bees [4]. Human intervention, resulting from agricultural activities, construction of highways, application of insecticides, among others, dramatically reduces the availability of floral resources and conducive areas for the nesting of these insects [5]. Therefore, environmental protection and conservation measures are important for the maintenance of the flora used by bees.

The study of pollen spectrum of honey renders information about its floral origin, providing subsidies to beekeepers for the preservation and expansion of the flora in a region and, consequently, for the management of bee colonies. Thus, the objective of this study was to identify the plants that contribute to the honey composition of stingless bees using the pollen analysis.

2. Material and Methods

Samples, consisting of 50 mL of honey, were obtained from beekeepers during the production peak production of honey at the municipality of Guaraqueçaba, Paraná State, Brazil (25°17'15"S; 48°19'1"W) where bee colonies had already been structured. We collected 30 samples in February 2012 of species of nine Meliponini species: *Cephalotrigona capitata* (Smith, 1854), *Melipona bicolor* (Lepelletier, 1836), *Melipona marginata* (Lepelletier, 1836), *Melipona mondury* (Smith, 1863), *Melipona quadrifasciata* (Lepelletier, 1936), *Melipona*

scutellaris (Latreille, 1811), *Melipona seminigra* (Friese, 1903), *Scaptotrigona xanthotricha* (Moure, 1950) and *Tetragonisca angustula* (Latreille, 1811).

To collect the samples, disposable syringes were used. The samples were placed in properly marked plastic containers, packed in thermal bags, and sent to the Laboratory of Useful Insects at the Department of Entomology and Acarology of the College of Agriculture “Luiz de Queiroz”, São Paulo University (USP), for laboratorial analyses.

We used the acetolysis method [6] for identification of pollen types. The resulting sediment was mounted on slides with glycerin jelly. The identification of pollen types in the samples was determined by comparison with the reference database of the Laboratory of Useful Insects at the Department of Entomology and Acarology of College of Agriculture “Luiz de Queiroz” of the São Paulo University and with descriptions in specialized literature such as Barth [7], Barth *et al.* [8, 9] and Moreti *et al.* [10, 11].

The qualitative analysis was performed by means of successive count of up to 1.000 pollen grains/sample determining percentages and classes of occurrence, according to Louveaux *et al.* [12]: predominant pollen (PP) (>45% of the

total of grains), secondary pollen (SP) (16-45%), important minor pollen (IP) (3-15%) and minor pollen (MP) (<3%). After the analyses, we determined the relative frequency of each pollen type in the samples: $f = [(n_i/N) \times 100]$ where, f = relative frequency of pollen type i in sample; n_i = number of pollen grains of pollen type i in sample; N = total number of pollen grains in sample.

3. Results and Discussion

We identified 55 pollen types belonging to 27 families (Table 1 and 2). The richest families in pollen types were Fabaceae (subfamily Mimosoideae) (10.91%) followed by Asteraceae and Myrtaceae, 7.27% each. The predominant pollen types were *Casearia*, *Eucalyptus*, *Galactia*, *Miconia*, *Morus*, *Myrcia* I, *Syagrus* and Rubiaceae Type (Figure 1). *Syagrus* (93.33%), *Eucalyptus* (56.67%), *Mimosa caesalpiniaefolia* (56.67%), *Psidium* (56.67%), *Miconia* (53.33%), *Morus* (53.33%), *Leucaena* (50.00%) and *Inga* (40.00%), were the most frequent pollen types in the honey samples studied (Figure 2). The occurrence of a predominant pollen type was recorded in 40% of the samples (Table 1 and 2).

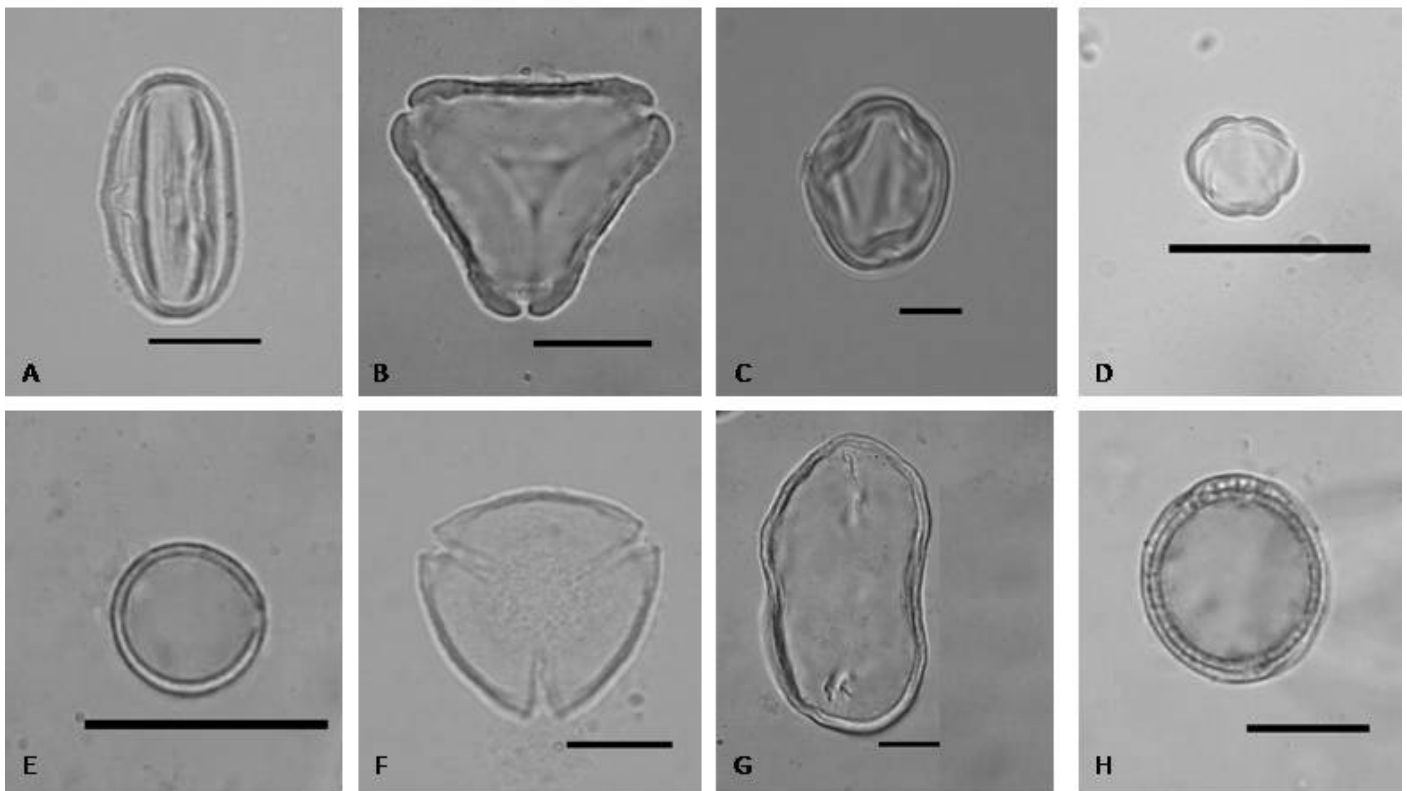


Fig 1: Photomicrograph (1.000x) of the predominant pollen type in the honey samples of stingless bees: A – *Casearia*; B – *Eucalyptus*, C – *Galactia*; D – *Miconia*; E – *Morus*; F – *Myrcia* I; G – *Syagrus* and H – Rubiaceae type. Range 10 μ m.

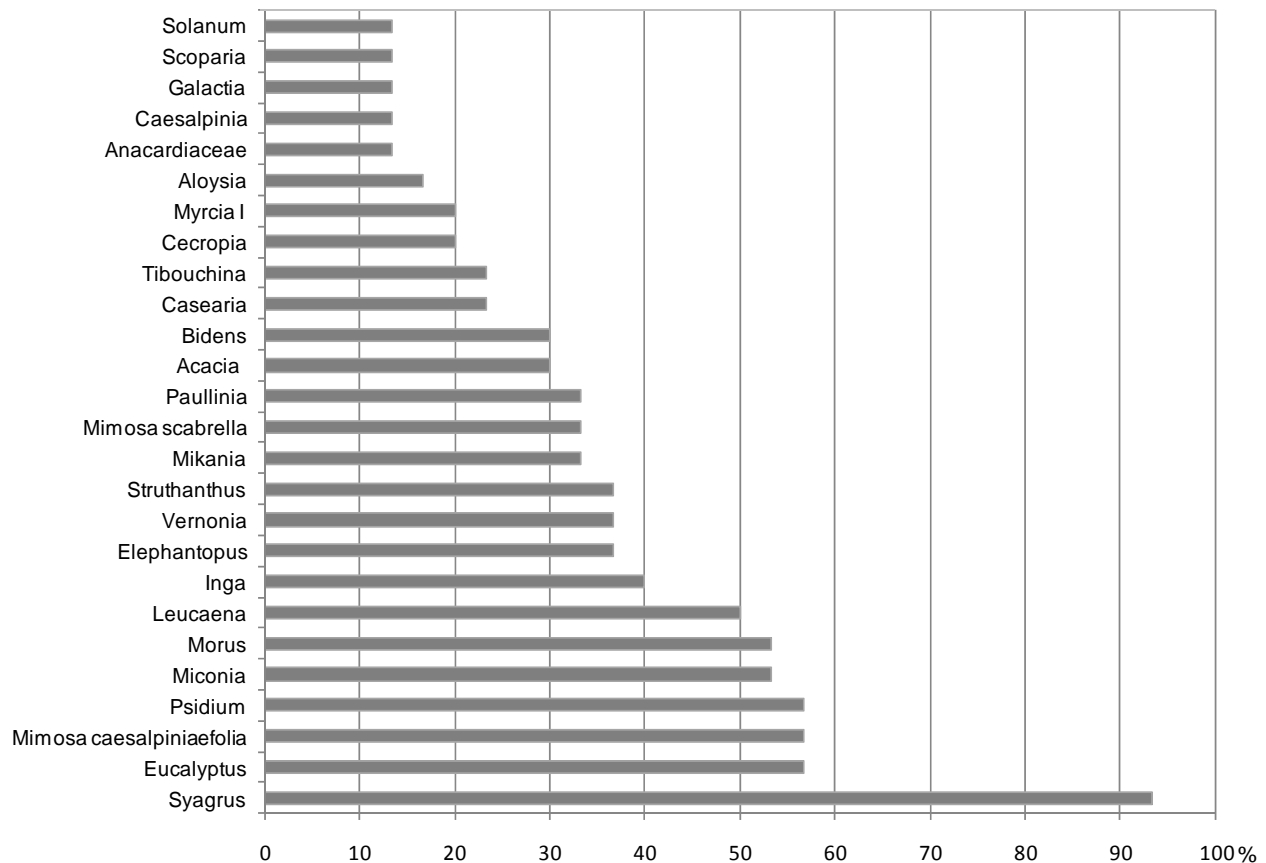


Fig 2: Frequency of occurrence of pollen types ($\geq 13\%$ frequency) in honey samples of stingless bees obtained from the municipality of Guaraqueçaba, Paraná State, Brazil.

The average number pollen types per sample the studies species was 9.00 to *Melipona bicolor* samples, 8.30 to *Melipona marginata*, 8.00 to *Cephalotrigona capitata*, 6.00 to *Melipona seminigra*, 5.66 to (*Scaptotrigona xanthotricha*), 5.50 to (*Melipona scutellaris*), 5.00 to (*Tetragonisca angustula*), 4.66 to (*Melipona mondury*) and 4.25 (*Melipona quadrifasciata*), indicating the participation of various plant species in the honey composition of these stingless bees.

The pollen spectrum of honey samples from Paraná State, according to Ramalho *et al.* [13], can be summarized in *Allophylus*, *Baccharis*, *Campomanesia*, *Cecropia*, *Citrus*, *Eucalyptus*, *Matayba*, *Mimosa scabrella*, *Paspalum* and *Vernonia*. We observed a presence in the pollen spectrum of honeys from Guaraqueçaba, Paraná State four pollen types cited by Ramalho *et al.* [13] (Table 1 and 2). In the municipality of Curitiba, Paraná State, Brazil, Borsato *et al.* [14] identified 14 pollen types, among which *Mimosa scabrella*, *Mimosa verrucosa*, *Eucalyptus*, *Myrcia* and *Solanum* are predominant or secondary pollen types in the honey pollen spectra produced by the species *Melipona marginata*, *Melipona quadrifasciata* and *Melipona bicolor*, similar to the results observed in this study (Table 1). Borsato *et al.* [14] observed in honey samples from Guaraqueçaba, produced by bees *Melipona mondury*, *Melipona quadrifasciata* and *Melipona bicolor*, the presence of Melastomataceae type as predominant pollen and *Syagrus* type as secondary pollen. These findings show similarity of the floral sources used by stingless bees in this municipality. Melastomataceae showed the types *Miconia* and *Tibouchina* in all classes of occurrence in the studied samples of stingless bees in this region.

Abreu *et al.* [15] in a study of *Melipona quadrifasciata* honey found the most frequent pollen types in samples of honey and pollen of were Myrtaceae (*Eucalyptus*, *Myrcia*), Melastomataceae and Solanaceae. According these authors,

there is no doubt that Myrtaceae (*Eucalyptus*) pollen play an important role in the diet of *M. quadrifasciata*. This information corroborates the results found in the analysis of honey *M. quadrifasciata* (Table 1), where *Eucalyptus* and *Myrcia* pollen types occur as the predominant pollen.

The pollen spectrum of the analyzed honeys indicates the diversity of plants visited by stingless bees in the area where the honey was produced. The largest number of pollen types identified were classified as isolated pollen, representing the samples of *Melipona marginata* 92,31%, *Tetragonisca angustula* 92.00%, *Cephalotrigona capitata* 89.48%, *Melipona mondury* 86.21%, *Melipona bicolor* 84.62%, *Melipona seminigra* 79.42%, *Melipona scutellaris* 78.73%, *Scaptotrigona xanthotricha* 72.23% and *Melipona quadrifasciata* 67.86% (Table 1 and 2).

Martins *et al.* [16] found that, although Fabaceae, subfamily Mimosoideae showed pollen types of four species in honey samples of *Melipona fasciculata*, only two types contributed significantly to honey composition, *Mimosa caesalpiniaefolia* and *Mimosa pudica*. According to these authors, *Melipona fasciculata* honey showed in its composition nectar of few plant species, ruderal and native. Some species of the genus *Mimosa* are pollen sources (*Mimosa scabrella*), nectar (*Mimosa bimucronata* and *Mimosa invisa*), or (*Mimosa dalleoides*, *Mimosa pudica* and *Mimosa velloziana*) for Meliponini [17]. Carreira *et al.* [18] explains who *Mimosa* plant is ruderal, polliniferous, occurring in abundance in various locations, and most plants bloom year round, which renders the species an inexhaustible floral source.

Asteraceae, the second most representative family in the samples, is also cited as important nectar and pollen source, *Emilia coccinia*, *Mikania cordifolia*, *Vernonia scorpioides* and *Vernonia* sp. are listed as plant species used as floral sources by africanized honeybees and stingless bees [19]. Ramalho *et al.*

[17] identified 41 species of Asteraceae used as sources by *Apis mellifera*, Trigonini and *Melipona*, thus constituting one of the richest and most visited this family by social bees in different regions. Asteraceae is considered one of the rich in species and, thus, one of the most visited by social bees in different regions of Brazil [17]. This information supports the results found in this study. The Asteraceae family consists of perennial herbs, sub shrubs and shrubs, but also occur annual herbs, vines and trees. Due to its extraordinary power of environmental adaptation can be found in many more habitats, preferably in open grasslands and in varying weather conditions in tropical, subtropical to temperate.

The sources used by bees depend on their availability at the collection site. However, in the same area, different bee species exhibit variable niche extensions suggesting that their preferences for certain species can determine the extent of this niche [20].

The cost/benefit balance in foraging supports the premise of floral preferences, because due

to economic issues, the bees may also show a floral constancy or “temporary specialization” [21]. In general, stingless bees forage with abundant flowering and longer lasting plants however they diversify and collect pollen from other less attractive sources [22].

The generalist habit of stingless bees is considered a necessary mechanism. Therefore, it is accepted as a pattern for bees in the Apini and Meliponini, with large perennial colonies, high production rates of offspring, which require large amounts of food throughout year [23].

The pollen analysis of the stingless bee honey indicated the generalist characteristic of these species because of the diversity of plant species identified in the pollen spectrum. Thus, pollen analysis in honey, in stingless bee pollen and in propolis, could be added to studies on the plants visited by bees to obtain more information about meliponicultural pasture of a region.

Table 1: Pollen types identified in honey samples of stingless bees collected in Guaraqueçaba, Paraná State, Brazil: Mmo (*Melipona mondury*), Mse (*Melipona seminigra*), Msc (*Melipona scutellaris*) and Mqa (*Melipona quadrifasciata*). PP – predominant pollens (>45%), SP – secondary pollen (16–45%), IP– important minor pollen (3–16%), MP – minor pollen (<3%).

Family	Pollen types	Honey samples													
		Mmo 01	Mmo 02	Mmo 03	Mse 01	Mse 02	Mse 03	Msc 01	Msc 02	Msc 03	Msc 04	Mqa 01	Mqa 02	Mqa 03	Mqa 04
Amaranthaceae	<i>Alternanthera</i>												IP		
Anacardiaceae	Anacardiaceae Type										MP				IP
	<i>Spondias</i>					MP									
Arecaceae	<i>Syagrus</i>	PP	PP	SP	MP	IP	IP	IP	MP	IP	IP		MP	IP	MP
Asteraceae	<i>Bidens</i>				MP	MP							IP		MP
	<i>Elephantopus</i>								MP	MP			MP	MP	MP
	<i>Mikania</i>	MP	MP		MP	MP		MP	MP	IP					
	<i>Vernonia</i>	MP	MP	MP		IP									
Bignoniaceae	Bignoniaceae Type								IP	MP					
	<i>Tabebuia</i>	MP													
Bombacaceae	<i>Pachira</i>			MP											
Cecropiaceae	<i>Cecropia</i>				IP			IP	IP				SP		MP
Fabaceae/Caesalpinioideae	<i>Delonix</i>								MP						
Fabaceae/Mimosoideae	<i>Acacia</i>								MP	MP	MP			MP	
	<i>Inga</i>				MP	MP							IP		
	<i>Leucaena</i>			MP	MP								IP	IP	IP
	<i>Mimosa caesalpiniaefolia</i>	IP	MP	MP	IP	MP	IP			MP	IP		MP	MP	MP
	<i>Mimosa scabrella</i>				SP		SP	IP	IP		IP			MP	IP
Flacourtiaceae	<i>Casearia</i>											IP			
Loranthaceae	<i>Struthanthus</i>								MP						
Melastomataceae	<i>Miconia</i>			SP	SP	PP	SP	SP	SP	SP	SP		SP	SP	IP
	<i>Tibouchina</i>				IP	IP	IP					SP			

Family	Pollen types	Honey samples													
		Mmo 01	Mmo 02	Mmo 03	Mse 01	Mse 02	Mse 03	Msc 01	Msc 02	Msc 03	Msc 04	Mqa 01	Mqa 02	Mqa 03	Mqa 04
Moraceae	<i>Morus</i>	MP	MP	MP	IP				SP	IP		SP			
Myrtaceae	<i>Eucalyptus</i>	IP	IP	IP	MP			IP	IP	IP	IP	IP		PP	IP
	<i>Myrcia I</i>							SP	SP	SP			PP	IP	SP
	<i>Psidium</i>	IP	IP	IP	IP	SP	SP				IP				
Poaceae	Poaceae Type I	IP			MP	MP						MP	IP		
	Poaceae Type II	MP	MP	MP											
Rubiaceae	Rubiaceae Type						MP								
	<i>Borreria</i>										MP				
Rutaceae	Rutaceae Type								MP						
Sapindaceae	<i>Paullinia</i>			MP	MP			MP		MP	MP				MP
Solanaceae	Solanaceae Type							SP							

Table 2: Pollen types identified in honey samples of stingless bees collected in Guaraqueçaba, Paraná State, Brazil: Cca (*Cephalotrigona capitata*), Mbi (*Melipona bicolor*), Mm (*Melipona marginata*), Sxa (*Scaptotrigona xanthotricha*) and Tan (*Tetragonisca angustula*). PP – predominant pollen (>45%), SP – secondary pollen (16–45%), IP– important minor pollen (3–16%), MP – minor pollen (<3%).

Family	Pollen types	Honey samples															
		Cca 01	Cca 02	Mbi 01	Mbi 02	Mbi 03	Mbi 04	Mm 01	Mm 02	Mm 03	Sxa 01	Sxa 02	Sxa 03	Tan 01	Tan 02	Tan 03	Tan 04
Amaranthaceae	<i>Alternanthera</i>											MP					
	<i>Gomophrena</i>					MP											
Amarylidaceae	Amarylidaceae Type														MP		
Anacardiaceae	Anacardiaceae Type		MP						MP								
	<i>Schinus</i>					IP	MP										
	<i>Spondias</i>	IP															
Apiaceae	Apiaceae Type						SP			MP		SP					
Arecaceae	<i>Syagrus</i>	IP	IP	MP	IP	IP	IP	IP	IP	MP		IP	IP	MP	MP	IP	IP
Asteraceae	<i>Bidens</i>	MP	IP	MP				MP	SP								
	<i>Elephantopus</i>													MP	IP	MP	
	<i>Mikania</i>					IP	IP								IP		
	<i>Vernonia</i>	MP		MP	IP	IP	MP	MP	IP								
Bignoniaceae	<i>Stenolobium</i>		MP			MP	MP										
Bombacaceae	<i>Pachira</i>											MP					
Cecropiaceae	<i>Cecropia</i>								IP								
Combretaceae	<i>Terminalia</i>									IP							
Cucurbitaceae	Cucurbitaceae Type					MP											
Euphorbiaceae	Euphorbiaceae Type							IP									

Family	Pollen types	Honey samples															
		Cca 01	Cca 02	Mbi 01	Mbi 02	Mbi 03	Mbi 04	Mm 01	Mm 02	Mm 03	Sxa 01	Sxa 02	Sxa 03	Tan 01	Tan 02	Tan 03	Tan 04
Fabaceae/Caesalpinioideae	<i>Caesalpinia</i>			MP		MP			MP		PP						
	<i>Delonix</i>													IP	MP		
	<i>Senna</i>									MP					MP		
Fabaceae/Faboideae	<i>Desmodium</i>		IP														
	Faboideae													MP	IP		
	<i>Galactia</i>	PP	PP							PP		SP					
Fabaceae/Mimosoideae	<i>Acacia</i>												MP		MP	MP	MP
	<i>Inga</i>	MP	MP	MP		MP			MP	MP	MP	MP	MP				
	<i>Leucaena</i>	MP	MP	MP				MP	MP	MP	IP	IP	MP			MP	MP
	<i>Mimosa caesalpiniaefolia</i>	MP	IP	IP	IP		MP	MP									
	<i>Mimosa scabrella</i>	MP	IP	SP			IP										
	Mimosoideae															MP	
Flacourtiaceae	<i>Casearia</i>								IP				SP	PP	SP	IP	IP
Loranthaceae	<i>Struthanthus</i>			IP		MP	MP	MP			IP	SP	IP	MP			MP
Malvaceae	Malvaceae Type							MP									
Melastomataceae	<i>Miconia</i>			SP	SP	SP	IP							IP			
	<i>Tibouchina</i>			IP				SP	IP								
Moraceae	<i>Morus</i>	SP			SP	MP	MP	SP						IP	PP	SP	SP
Myrtaceae	<i>Eucalyptus</i>		IP				SP	IP	MP					MP			MP
	<i>Myrcia</i> II														MP		
	<i>Psidium</i>	IP		SP	SP	SP	SP	IP	SP		IP	IP	IP				
Piperaceae	<i>Piper</i>					IP	IP					IP					
Poaceae	Poaceae Type I	MP	MP			MP									MP		MP
	Poaceae Type I			MP													
Rubiaceae	Rubiaceae Type					MP										PP	SP
Rutaceae	Rutaceae Type				MP	MP			IP				SP				
	<i>Zantoxylum</i>				IP	IP	MP										
Sapindaceae	<i>Cupania</i>			MP													
	<i>Paullinia</i>					MP			MP								
Scrophulariaceae	<i>Scoparia</i>											IP	SP			IP	IP
Solanaceae	<i>Solanum</i>									MP			IP			IP	IP
Verbenaceae	<i>Aloysia</i>											MP					

4. Conclusion

The pollen spectrum of the honey samples analyzed were diverse, with significant contributions of the families Fabaceae (subfamily Mimosoideae), Asteraceae and Myrtaceae as the most diverse in species visited by stingless bees in the study area.

5. Acknowledgments

The authors wish to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

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