Palynological analysis of geopropolis samples obtained from six species of Meliponinae in the Campus of the Universidade de Ribeirão Preto, USP, Brazil.

Ortrud Monika Barth[1]

Laboratório de Palinologia, Departamento de Botânica, Instituto de Biologia, Universidade Federal do Rio de Janeiro e Laboratório de Ultra-estrutura Viral, Departamento de Virologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

[1]Corresponding adress: Laboratório de Ultra-estrutura Viral, Departamento de Virologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Avenida Brasil 4365, 21045-900 Rio de Janeiro. E-mail: barth@ioc.fiocruz.br

Short title: Palynological analysis of geopropolis samples

Abstract

Geopropolis samples were obtained from the entrance tubes of the nests of six species of Meliponinae located in the same apiary at the Campus of the University of Ribeirão Preto-USP, state of São Paulo, Brazil, and submitted to pollen analysis after chemical treatment. The aim of the study was to recognize the vegetation and the environment around the apiary for interest of the bees, as well as possible foraging preferences. Tree species were frequently visited by the six bee species, outstanding *Eucalyptus* for all except for *Frieseomellitta varia*. *Melipona quadrifasciata* attended pollen of *Mimosa* species from grassland vegetation. Pollen grains of anemophilous plant species from *Cecropia, Pinus, Piper, Poaceae and Typha* occurred frequently in the geopropolis sediments. It was possible to characterize the environmental conditions and the vegetation around the apiary using pollen analysis of geopropolis samples, as well as the trophic preferences for some of the bees.

Key words: geopropolis, Meliponinae, pollen analysis, Ribeirão Preto, Brazil

Introduction

Singless bees (Apidae, Meliponinae) have a pantropical distribution, and are essential pollinators mainly in rainforests. Geopropolis is a special type of propolis or bee glue prepared by stingless bees. As formerly presented [4], geopropolis is a mixture of resins, wax and soil. It is quite different from propolis produced by *Apis mellifera* L. that does not contain earth but in addition plant trichomes.

Several authors analysed the trophic sources for the Meliponinae. In respect to regional studies in Brazil, Kerr et al. [11] investigated in the Amazon region, Cortopassi-Laurino and Ramalho [8], Guibu et al. [10], Kleinert-Giovannini & Imperatriz-Fonseca [12] and Ramalho et al. [14] in the Campus of the University of São Paulo (USP), Carvalho and Marchini [7] in the Piracicaba locality, state of São Paulo, Cure et al. [9] and Silveira et al. [17] in the Viçosa and Ponte Nova region of the state of Minas Gerais, respectively, and

Camargo and Mazucato [6] in the Campus of the Faculdade de Medicina de Ribeirão Preto, where the dominant vegetation around the Campus comprised crop plantations (sugar cane and coffee) and pastureland.

Palynological studies of geopropolis samples were unique [4]. Pollen grains were always present in propolis samples [1, 3, 5, 16, 18] but can be missed sometimes in samples of geopropolis [4]. Pollen grains may be introduced into propolis and geopropolis by worker bees during manufacture and may arise from bee loads from anemophilous plant species. The wind spreads pollen grains, that may stick on plant exudates before these are carried by the bees into the beehives and processed to propolis and geopropolis.

In a previous paper [4] we analyzed the pollen spectra of three geopropolis samples proceeding from the state of São Paulo, three from the state of Minas Gerais and one from the state of Espírito Santo, produced by three species of Meliponinae. Sand or small particles of soil were always present in these samples. Between the 64 pollen types identified, 22 plant taxa presented a pollen frequency higher than 3%.

In order to better understand pollen contents of geopropolis deposted at the nest entrance, we intend in the present paper to analyse some samples obtained from different species of Meliponinae, of which hives were located in a restrict area of the campus of the Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, state of São Paulo. The same nutritional sources were available to these bees considering the vegetation present in an action radius of 100m around the apiary. It will be of interest to know the behaviour of these Meliponinae in respect to the visited plant species during the time of geopropolis manufacture.

Material and Methods

Geopropolis samples (1 – 6) were obtained from the six entrance tubes of the nests of different species of Meliponinae. Two of these colonies were kept in wooden boxes in the apiary of the Department of Genetics, the other ones occurred in house walls or inside the earth next to the apiary (Table 1).

Table 1. Species of Meliponinae and beehives locality for geopropolis sampling inside the Campus of the Universidade de Ribeirão Preto, USP.

Sample registration number	Bee species	Common name	Beehieve locality
1	*Lestrimellita cfr. limao* Smith, 1863	Limão	Left side of the lake looking from the Department of Genetics
2	*Trigona recursa* Smith, 1863	Arapuá / trigona	Underground beehive next the Department of Genetics
3	*Tetragonisca angustula* Latreille, 1836	Jataí	Inside wall of the Department of Genetics
4	*Melipona quadrifasciata* Lepeletier, 1836	Mandassaia	Inside wall of the Department of Genetics
5	*Nannotrigona testaceicornis* Lepeletier, 1836	Iraí	Apiary of the Department of Genetics
6	*Frieseomelitta varia* Lepeletier 1836	Moça branca	Apiary of the Department of Genetics

Palynological processing of samples followed Barth [2] starting with 0.5g of scraped geopropolis extracted overnight with ethanol; in sequence, the sediment was treated with KOH and in addition with the acetolysis mixture. One slide was prepared just before acetolysis, in order to get information about the presence of trichomes or other organic residues that may be destroyed in further sequence. At the final step of the processing, three more slides were prepared using unstained or basic fuchsine-stained glycerin-jelly.

Pollen counts reached more than 300 pollen grains per sample and pollen classes established followed honey analysis standards [19]. Sample sediments were observed using light and polarized light microscopy.

Results

The six samples of geopropolis analysed showed different physical-chemical properties. Sample 1 was dark-brown, very hard and brittle, and presented the typical smell of propolis. It seems to be elder then all the other samples studied. Sample 2 was black, of sandy-brittle texture and smelling feces. Sample 3 was gray, sticky and had an acid smell. Sample 4 was dark red-brownish, sandy-brittle and of soft smell. Sample 5 was externally gray and brown inside, hard and sticky and had a strong typical smell of propolis. Sample 6 was black, hard and elastic and had a strong smell of smoke.

Observation of slides prepared before acetolysis, showed that brownish organic material was abundant only in samples 2 and 4, remaining even after further processing (Table 2). Persistent sandy crystals could be detected in samples 1 - 4. Typical red-brownish clay ("barro") was identified only in sample 4. Spores of fungi were always present. Plant trichomes, as commonly found in propolis samples, were never detected in geopropolis samples; this can mean that resins were obtained in an other way than chewing plant buds and sprouts in order to open plant resin channels as *Apis* do it.

Table 2. Evaluation of geopropolis of nest entrances for sediment constituents, except pollen grains, after acetolysis: (+++) = very frequent, (++) = frequent, (+) = few, (-) = not detected.

Bee species	Organic material	Sandy fragments	Spores and hyphae of fungi	Soot (burned organic material)
Lestrimellita cfr. limao	+	++ (crystals)	+	+
Trigona recursa	++	+ (crystals)	+	+
Tetragonisca angustula	+	+++ (sandy powder)	++	+++
Melipona quadrifasciata	+++	+ (crystals)	+	+
Nannotrigona testaceicornis	+	-	++	+
Frieseomelitta varia	+	-	+	+

Pollen grains were always well preserved in all samples examined after acetolysis (Fig. 1). The plant family or genus identification using pollen morphology could be attained in general, while plant species were more difficult to be identified, as several plant taxa may belong to a same pollen type. These taxa are presented in Table 3 in accordance to the plant habitus.

Table 3. Plant taxa identified and sample references.

Plant taxa	Samples					
	Lestrimellita cfr. *limao*	*Trigona recursa*	*Tetragonisca angustula*	*Melipona quadrifasciata*	*Nannotrigona testaceicornis*	*Frieseomelitta varia*
Trees/lianas						
Anacardiaceae	x	x	x		x	x
Anacardiaceae: *Tapirira*			x			
Apocynaceae			x			
Aquifoliaceae: *Ilex*				x		
Araliaceae		x				
Arecaceae	x	x	x		x	x
Asteraceae: *Gochnatia*				x		
Bignoniaceae	x	x	x		x	x
Bombacaceae:						
Ochroma pyramidalis						x
Pachira aquatica		x				x
Pseudobombax			x			
Caesalpiniaceae: *Cassia*			x	x	x	
Schizolobium			x			x
Cecropiaceae: *Cecropia*	x		x	x	x	
Combretaceae/Melastomataceae	x		x			
Cucurbitaceae	x				x	
Euphorbiaceae: *Alchornea*			x		x	
Fabaceae: *Doliocarpus*	x					
Humiriaceae: *Vantanea*						x
Loranthaceae				x		
Meliaceae		x			x	
Mimosaceae: *Anadenanthera*	x		x	x		x
Moraceae				x	x	
Myrtaceae	x			x	x	x
Nyctaginaceae	x		x			
Phytolaccaceae			x			
Polygonaceae: *Triplaris*						x
Rhamnaceae			x			x
Rutaceae: *Zanthoxylum*			x		x	
Sapindaceae					x	
Ulmaceae: *Celtis*		x				
Shrubs						
Boraginaceae: *Cordia*		x	x			x
Euphorbiaceae:						
Ricinus communis		x				
Malvaceae						x
Mimosaceae: *Mimosa*		x	x	x		x

caesalpiniaefolia						
Mimosaceae: *Mimosa scabrella*				x		
Piperaceae	x	x	x		x	
Ulmaceae: *Trema*	x		x			
Herbs						
Amaranthaceae: *Alternanthera*	x			x	x	
Asteraceae: *Artemisia*	x					
Bryophyta				x		
Campanulaceae	x					
Chenopodiaceae		x				x
Cyperaceae			x	x		x
Lythraceae: *Cuphea*		x				
Onagraceae: *Ludwigia*	x		x			x
Oxalidaceae		x				
Poaceae	x	x	x	x	x	x
Scrophulariaceae	x		x		x	
Typhaceae: *Typha*	x				x	x
Urticaceae		x			x	
Crops and exotics						
Myrtaceae: *Eucalyptus*	x	x	x	x	x	x
Pinaceae: *Pinus*		x		x	x	x
Poaceae: *Zea mays*						x
Polygonaceae: *Antigonon leptopus*		x				
Rutaceae: *Citrus*	x	x				x
Various						
Apocynaceae		x				
Asteraceae	x		x	x	x	x
Caesalpiniaceae	x		x		x	x
Euphorbiaceae	x					x
Fabaceae	x		x		x	x
Malpighiaceae	x					x
Mimosaceae: *Acacia*				x		
Solanaceae		x	x	x		x

Considering the most frequent pollen types (>45%) in the studied samples (Table 4), and in accordance to pollen classes established for honey sediment analyses, the unique dominant pollen type of *Eucalyptus* (Figs. 2,3) was detected only once (sample 2). Accessory pollen types were found in four samples: *Eucalyptus* in sample 1, *Cecropia* (Figs. 4-6) in sample 3, *Piper* (Figs. 10-14) in sample 5 and *Mimosa verrucosa* pollen type (Figs. 7-9) in sample 4. This last pollen type includes several species of *Mimosa*, commonly found in the grassland vegetation. Sample 6 was the most heterogeneous and presented the highest value of non-identified plant taxa (19%). A total of 21 pollen types occurring with frequency higher than 3% could be recognized.

Table 4. Most frequent (>3%) pollen types and plant taxa in geopropolis samples analysed. Pollen frequency: PD = dominant (>45%), PA = accessory (15-45%), PI = isolate (3-15%).

Pollen types/ plant taxa	Samples					
	Lestrimellita cfr. limao	*Trigona recursa*	*Tetragonisca angustula*	*Melipona quadrifasciata*	*Nannotrigona testaceicornis*	*Frieseomelitta varia*
Amaranthaceae:*Alternanthera*	PI				PI	
Anacardiaceae	PI	PI	PI		PI	
Arecaceae	PI		PI			
Asteraceae	PI		PI		PI	PI
Bignoniaceae	PI					PI
Caesalpiniaceae: *Caesalpinia*	PI					PI
Caesalpiniaceae: *Cassia*			PI			
Cecropiaceae: *Cecropia*			**PA**			
Euphorbiaceae	PI					
Malpighiaceae						PI
Mimosaceae: *Anadenanthera*						PI
Mimosaceae: *Mimosa scabrella*				**PA**		
Myrtaceae: *Eucalyptus*	**PA**	**PD**	PI	PI	**PA**	
Myrtaceae: *Myrcia*				PI		
Nyctaginaceae: *Bougainvillea*	PI					
Pinaceae: *Pinus*		PI			PI	PI
Piperaceae: *Piper*					**PA**	
Poaceae		PI	PI			
Rutaceae: *Zanthoxylum*					PI	
Scrophulariaceae			PI		PI	
Thyphaceae: *Typha*					PI	PI
Non-identified (serveral taxa)	10.6%	13.5%	12.6%	13.9%	6.7%	19.0%
Total of taxa identified	26	23	28	19	23	29

Discussion

Colour, consistency and smell of geopropolis samples examined changed in accordance to the bee species. Colour varied from a black, gray to a brown and reddish-brown aspect, the first two colors in respect to the use of common soil and the last two in respect to the presence of the characteristic violet regional soil ("terra-roxa") of the Ribeirão Preto region. Consistency of geopropolis samples changed from hard to elastic and sticky, and may depend upon its age and, on the other hand, of its composition when containing more or less quantity of wax. The smell was expressive and characteristic of each bee species. It changed from a typical propolis smell to an unpleasant sensation.

After acetolysis processing of the samples, a variable quantity of organic material may remain in the geopropolis sediments, as well as sand, soil particles and brown spores of fungi. These elements indicate different sources visited by each bee species for preparing its geopropolis.

Pollen grains are an indicative component of propolis phytogeographical origin. Pollen spectra of geopropolis from the Meliponinae are wider ranging, as it may be observed in Table 3. Nevertheless, only 21 pollen types occurred with a frequency higher than 3% and only 4 with more than 25% of the pollen sum. *Eucalyptus* pollen is the best regional representative, as it constitute the unique taxon of a higher than 45% frequency, and the dominant pollen category collected by *Trigona recursa*.

Considering the most frequent pollen types (>3%) in Table 4, forest taxa were represented by Anacardiaceae, *Anadenanthera* and Arecaceae. Open-land vegetation was visited by the Meliponinae as it could be demonstrated by the presence of pollen grains of commonly occurring species of Asteraceae, Poaceae, *Alternanthera*, Scrophulariaceae and *Thypha*, the two last indicating humid localities. The environmental characteristics of the region around the apiary could be established using the pollen analysis of the geopropolis samples, and were given by crops, introduced and exotic plant taxa, and by cultivated plants in the Ribeirão Preto region, as *Eucalyptus*, *Bougainvillea*, *Pinus* and *Psidium* (*Myrcia* pollen type).

In relation to the distribution of pollen types/plant taxa identified in the geopropolis samples analysed (Table 4), it can be observed that nearly 50% of these occurred only once. Considering the same pollen types occurring in different samples, it was possible to obtain an average factor of agreement that in the present study of six different bee species was 2.1 (the sum of pollen type correspondences between two or more samples in relation to the number of all samples studied). When compared with the 10 samples of geopropolis collected in different localities in Brazil and previously studied by Barth and Luz [4], this factor was only 1.6. Therefore, the average factor of agreement of pollen types/plant taxa is lower for samples of different regional origins and is larger for samples of a same region, even if obtained for different bee species.

Few pollen types, with a frequency of higher than 3%, were recognized in geopropolis samples of *Trigona recursa* and *Melipona quadrifasciata*, while the most numerous pollen types occurred in the sample of *Nannotrigona testaceicornis*. No pollen type of the dominant or accessory pollen category was present in the sample of *Frieseomelitta varia*. This observation can mean that this bee species was the most generalist in plant resources, while *Trigona recursa* was the most specialized, using *Eucalyptus* pollen grains in the sample. The presence of feces was very clear in this last sample.

The pollen spectra obtained indicated that the arboreal plant species were important resources for the bees. Nevertheless, the Campus of the University of Ribeirão Preto is surrounded by crop plantations, particularly sugar cane, coffee, and pastureland [6]; pollen types of herbaceous plants were less present in geopropolis samples as initially expected. Several wild herb species of Asteraceae, Amaranthaceae and *Mimosa* could be identified, and also plant taxa indicating humid soil conditions as Scrophulariaceae, *Thypha* and *Piper*. Between the trees, the most important were *Eucalyptus*, followed by *Cecropia*, Anacardiaceae, Arecaceae, Myrtaceae, *Anadenanthera* and shrubs of *Piper*. The anemophilous pollen grains of *Pinus* may be considered as a “wind contamination”.

A preference for *Eucalyptus* species of the Meliponinae as found in our study, was also observed by Kleinert-Giovannini & Imperatriz-Fonseca [12] and Ramalho et al. [14] in the Campus of the University of São Paulo, São Paulo city, where patches of forest were maintained, besides ornamental plant species and grassland. A similar observation was made by Carvalho & Marchini [7] studying forage resources of Meliponinae in the Campus of the University in Piracicaba (ESALQ-USP), state of São Paulo. It was observed also by Ramalho et al. [15] that different species of *Melipona* used different floral resources within a same community, as in our study.

Similar to earlier palynological studies on Brazilian propolis samples from *Apis* collected in different regions and apiaries [2, 5] and geopropolis samples [4], the six samples of geopropolis analysed in the present paper reflected the vegetation and the environmental conditions around the apiary. The results, indicating the importance of pollen from some anemophilous plant species, as *Cecropia*, *Piper* and *Typha*, indicate that these pollen grains stick to resins before being introduced in geopropolis production by the bees. On the other hand, the different pollen spectra of geopropolis samples collected in a same apiary from different species of Meliponinae, indicate that the trophic preferences are bee species dependent. This was also demonstrated by a pollen analysis of intranidal pollen storage [7,8,10,11,12,14,15]. Nevertheless, certain plant species were visited by all species of this study.

Acknowledgments

I am greatful indebt to Dr. Lionel Segui Gonçalves and his student Ms. Geusa of the Department of Genetics, Faculdade de Medicina de Ribeirão Preto,Universidade de São Paulo, for the obtaining of samples, to Dr. M. J. Sommeijer (Utrecht University, The Nederlands) for critical and helpfull suggestions, and to the Conselho Nacional de Desenvolvimento Técnico e Científico (CNPq) for financial support.

References

[1] Barth O.M., O Pólem no Mel Brasileiro. Editora Luxor, Rio de Janeiro, 1989.

[2] Barth O.M., Pollen analysis of Brazilian propolis. Grana 37 (1998), 97-101.

[3] Barth O.M., Melissopalynology in Brazil: a review of pollen analysis of honeys, propolis and pollen loads of bees. Scientia Agrícola 61 (2004), 342-350.

[4] Barth O.M., Luz CFP, Palynological analysis of Brazilian geopropolis samples. Grana 42 (2003), 121-127.

[5] Barth O.M., Dutra V.M.L., Justo R.L., Análise polínica de algumas amostras de própolis do Brasil Meridional. Ciência Rural, Santa Maria 29 (1999), 663-667.

[6] Camargo J.M.F., Mazucato M., Inventário da apifauna e flora apícola de Ribeirão Preto, SP, Brasil. Dusenia 14 (1984), 55-87.

[7] Carvalho C.A.L., Marchini L.C., Tipos polínicos coletados por Nannotrigona testaceicornis e Tetragonisca angustula (Hymenoptera, Apidae, Meliponinae). Scientia Agrícola 56 (1999), 717-722.

[8] Cortopassi-Laurino M., Ramalho M., Pollen harvest by africanized Apis mellifera and Trigona spinipes in São Paulo: botanical and ecological views. Apidologie 19 (1988), 1-24.

[9] Cure J.R., Bastos-Filho G.S., Oliveira M.J.F., Silveira F.A., Levantamento de abelhas silvestres na Zona da Mata de Minas Gerais. I – Pastagem na região de Viçosa (Hymenoptera, Apoidea). Revista Ceres 40 (1993), 131-161.

[10] Guibu L.S., Ramalho M., Kleinert-Giovannini A., Imperatriz-Fonseca V.L., Exploração dos recursos florais por colônias de Melípona quadrifasciata (Apidae, Meliponinae). Revista Brasileira de Biologia 48 (1988), 299-305.

[11] Kerr W.E., Absy M.L., Marques-Souza A.C., Espécies nectaríferas e poliníferas utilizadas pela abelha *elípona crassipes fasciculata* (Meliponinae, Apidae) no Maranhão. Acta Amazônica16/17 (1986/87), 145-156.

[12] Kleinert-Giovannini A., Imperatriz-Fonseca V.L.,. Aspects of the trophic niche of Melípona marginata marginata Lepeletier (Apidae, Meliponinae). Apidologie 18 (1987), 69-100.

[13] Louveaux J., Maurizio A., Vorwohl G., Methods of melissopalynology. Bee World 59 (1978), 139-157.

[14] Ramalho M., Giannini T.C., Malagodi-Braga K.S., Imperatriz-Fonseca V.L., Pollen harvest by stingless bee foragers (Hymenoptera, Apidae, Meliponinae). Grana 33 (1994), 239-244.

[15] Ramalho M., Kleinert-Giovannini A., Imperatriz-Fonseca V.L., Utilization of floral resources by species of Melípona (Apidae, Meliponinae): floral preferences. Apidologie 20 (1989), 185-195.

[16] Ricciardelli D'Albore G., L'origine géographique de la propolis. Apidologie 10 (1979), 241-267.

[17] Silveira F.A., Rocha L.B., Cure J.R., Oliveira M.J.F., Abelhas silvestres (Hymenoptera, Apoidea) da Zona da Mata de Minas Gerais. II. Diversidade, abundância e fontes de alimento em uma pastagem abandonada em Ponte Nova. Revista Brasileira de Entomologia 37 (1993), 595-610.