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Pollen spectrum of propolis samples from São Paulo State, Brazil

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ABSTRACT. The pollen spectrum of propolis is a good indicative of the phytogeographical origin of the bee product and the local flora available for bee visiting. The present study aimed to perform pollen analysis of propolis produced by *Apis mellifera* L., in the São Paulo State, Brazil. Propolis samples were directly obtained from beekeepers and slides were prepared for qualitative and quantitative analysis of pollen after propolis extraction. According to the results twenty-eight types of pollen were found and distributed in fifteen botanical families. Pollens from Mimosoideae and Myrtaceae types were found in all samples. In São Paulo State, there is a wide variety of vegetal sources which are components of the final propolis composition.

Keywords: pollen analysis, apiculture, botanical diversity.

Espectro polínico em amostras de própolis do Estado de São Paulo, Brasil

RESUMO. A análise do espectro polínico presente em amostras de própolis poderia ser um bom indicativo da origem fitogeográfica do produto e da flora local disponível para visitação pelas abelhas. O objetivo do presente trabalho foi realizar o levantamento polínico em amostras de própolis produzidas por abelhas *Apis mellifera* L., no Estado de São Paulo, Brasil. As amostras de própolis foram obtidas diretamente de apicultores, processadas e lâminas foram confeccionadas para as análises qualitativas e quantitativas do pólen. De acordo com os resultados, foram encontrados 28 tipos de pólen distribuídos em 15 famílias botânicas identificadas, sendo que o pólen Tipo Mimosoideae e Myrtaceae foi encontrado em todas as amostras. De acordo com os resultados obtidos pode-se concluir Conclui-se que o estado de São Paulo apresenta ampla variedade de fontes vegetais que fazem parte da composição final da própolis.

Palavras-chave: análise polínica, apicultura, diversidade botânica.

Introduction

Propolis, a resinous material, is a complex mixture of substances (30% of bee waxes; 55% of vegetal resins and balsams; 10% of volatile oil and around 5% of pollen) and mechanical impurities. Propolis is prepared by bees from plants exudates (trees, flowers and leaves), pollen and substances secreted by bee glandular metabolism (CASTRO, 2001).

Brazil is an important propolis-producing country due to its great variety of propolis types, and it makes clear the need of scientific knowledge about the botanical and geographical origin of propolis (BARTH, 2004).

The understanding of vegetable species with beekeeping importance through the botanical identification of plants or pollen analysis of bee products can contribute to the distribution of apiaries avoiding saturated areas and beekeepers can also obtain a worth production.

Thus, the pollen spectra of propolis are important indicatives of phytogeographical origin of bee product. Pollen spectrum is consisted of the pollen brought by bees and the anemophilous pollen added to the resin. Furthermore, the pollen grain identification of propolis can describe the local flora available for bee visiting and the period of the year which was produced (BARTH et al., 1999).

The determination of geographical origin and, mainly the botanical origin is important for quality control and standardization of propolis samples for an effective therapeutic use (ALENCAR et al., 2005). Considering the above, the aims of this study were to contribute to the qualitative pollen spectrum information of propolis from ten regions of São Paulo State, Brazil.

Material and methods

Samples were obtained from apiaries located in different political and administrative regions in the São Paulo State, Brazil (Table 1 and Figure 1) from 2008 to 2009, totalizing 32 samples for this study. The identification of botanical species around the apiaries was not feasible because the study comprehended apiaries from distinct regions in the State.

Table 1. Frequency of pollen types in propolis samples from different regions of the state of São Paulo from 2008 to 2009.

	1	2	3	4	5	6	7	8	9	10
Asteraceae	O	I	I	O	O	I	O	I	I	O
Cecropiaceae	I		O							
Caesalpinaceae	I	O	O	I		O				
Convolvulaceae	O	O	I		O	O	O	O	O	O
Euphorbiaceae	I			I	O	O	O	I	O	O
Fabaceae 1								O		
Fabaceae 2	O	O	O		O	O		O	O	O
Lamiaceae	I	O	I	A	O	I		I	O	O
Hyptis umbrosa - Lamiaceae								O		
Mimosoideae	O	A	D	I	D	A	D	A	D	D
Acacia sp. – Mimosoideae	I	O	O	I	O	O	O	I	O	O
Leucaena pallida - Mimosoideae	O	O	O	I	O	O		O	O	
Mimosa caesalpinifolia – Mimosoideae	A		O	I				O	O	O
Myrtaceae	I	A	A	A	I	A	O	I	I	I
Eucalyptus sp. - Myrtaceae			O		O	O		O		O
Panicum sp. – Poaceae	O		O		O			I	O	O
Proteaceae			O	O	O	O		O	O	O
Rutaceae 1	O	O	O		O	O	O	O	O	O
Rutaceae 2			O			O		O		O
Citrus sp. - Rutaceae		O								
Coffea arabica – Rutaceae		O	O		O			O		O
Paullinea carpopodea – Sapindaceae								O		
Serjania sp. - Sapindaceae		O		I	O	O		O	O	O
Scrophulariaceae			O	I	O	O	O	O	O	O
Not Identified Type 1 (ni1)		I	O	O	I	O			O	O
Not Identified Type 2 (ni2)		O	O	O	O	O	O	O	O	O
Not Identified Type 3 (ni3)	I		O		O	O	O	O	O	O
Not Identified Type 4 (ni4)		O		O	O			O		O

(1: Araçatuba; 2. Bauru; 3. Campinas; 4. Franca; 5. Marília; 6. Presidente Prudente; 7. Registro; 8. São José do Rio Preto; 9. São José dos Campos; 10. Sorocaba. D = Dominant pollen [> 45%]; A= Accessory pollen [15 to 45%]; I= Isolated pollen [3 to 14%]; O= Occasional pollen [< 3%]).

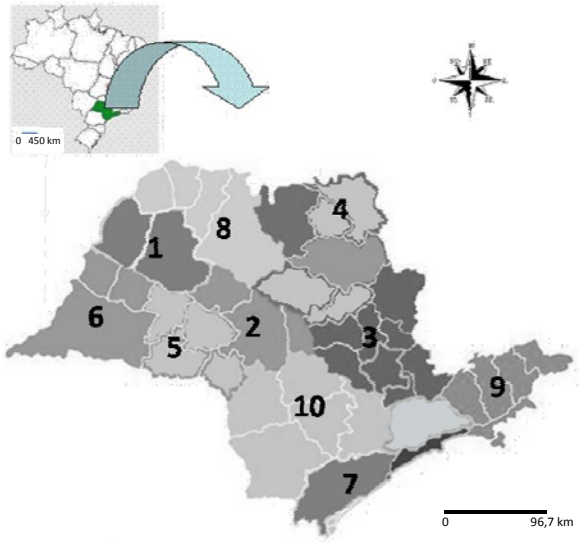

Figure 1. Regions from propolis obtained samples in the São Paulo State of from 2008 to 2009. Regions (cities): 1 – Araçatuba (Guaraçaí); 2 – Bauru (Bauru); 3 – Campinas (Araras, Atibaia and Rio Claro); 4 - Franca (Orlândia); 5 – Marília (Cruzália, Echaporã, Pompeia and Santa Cruz do Rio Pardo); 6 – Presidente Prudente (Dracena, Lucélia, Pirapozinho and Teodoro Sampaio); 7 – Registro (Pariquera-Açu); 8 – São José do Rio Preto (São José do Rio Preto and Macedônia); 9 – São José dos Campos (Cruzeiro, Paraibuna, Redenção da Serra and Santo Antônio do Pinhal); 10 – Sorocaba (Botucatu, Bofete, Capão Bonito, Itaporanga, Itatinga, Rubião Júnior, Sarutaiá and Sorocaba).

All samples were received and stored in identified plastic bags at -20ºC at Núcleo de Estudos, Ciências e Tecnologia em Apicultura Racional (NECTAR) of the Department of Animal Production, School of Veterinary Medicine and Animal Science, UNESP – Univ. Estadual Paulista, Botucatu Campus.

The pollen spectra of propolis samples were analyzed according to the methodology described by Barth et al. (1999). Briefly, 0.5 g of propolis sample was extracted with 15 mL of ethyl alcohol overnight at room temperature. After extraction, the suspension was equally split in two tubes and centrifuged for 10 minutes at 2,500 rpm. The supernatant was discharged; the pellet was resuspended with 13 mL of absolute ethanol and centrifuged for 5 minutes at 2500 rpm.

The supernatant was discarded and the pellet resuspended with 12 mL of 10% potassium hydroxide and kept for 2 minutes in waterbath at 40ºC. Tubes were centrifuged for 5 minutes at 2500 rpm. After centrifugation, the supernatant was discarded and 13 mL of distillated water was added to the tube, after that the suspension was transferred to another tube through a 0.3 mm mesh sieve in order to remove large organic particles.

It was added 5 mL of glacial acetic acid to the tubes and left to stand for 12 hours at room temperature. Tubes were centrifuged and the supernatant was discarded. A solution of acetic anhydride and concentrated sulphuric acid (9:1 v/v) was added to the tubes and the set was kept for 3 minutes in water bath at 40ºC. The supernatant was discarded after the centrifugation, a mixture of glycerine and water 1:1 was added to the pellet and leave to stand for at least 30 min. After 30 minutes, tubes were centrifuged and the supernatant discarded. Slides were prepared with the final sediment and each sample was analyzed in duplicate.

Botanical species (or pollen types) were determined through the qualitative analysis considering morphological aspects of pollen grains compared to the slide database of Banco de Imagens de Pólen de Plantas Apícolas do laboratório de Entomologia da ESALQ (ESALQ, 2009).

Three hundred pollen grains were counted to perform quantitative analysis (BARTH et al., 1999) considering as groups: dominant pollen (D) presence of over 45% of total grains; accessory pollen (A) presence of 15 to 44%; isolated pollen (I) between 3 and 15%; and occasional pollen (O) less than 3%.

Results and discussion

Twenty-eight different pollen types were found in 32 propolis samples from the São Paulo State and distributed in 15 botanical families (Table 1 and 2;

Figure 2). The most frequent pollen types of all propolis-producing regions were Asteraceae, Mimosoideae and Myrtaceae.

Table 2. Frequency and total percentage (%) of pollen types from propolis produced in different regions of the São Paulo State from 2008 to 2009.

	D	A	I	O	%
Asteraceae			5	5	100
Cesalpinaceae			2	3	50
Cecropiaceae			1	1	20
Convolvulaceae			1	8	9
Euphorbiaceae			3	5	80
Fabaceae 1				1	10
Fabaceae 2				8	80
Lamiaceae		1	4	4	90
Hyptis umbrosa - Lamiaceae				1	10
Mimosoideae	5	3	1	1	100
Acacia sp. – Mimosoideae			3	7	100
Leucaena pallida - Mimosoideae			1	7	80
Mimosa caesalpinifolia – Mimosoideae		1	1	4	60
Myrtaceae		4	5	1	100
Eucalyptus sp. - Myrtaceae				5	50
Panicum sp. – Poaceae			1	5	60
Proteaceae				7	70
Rutaceae 1				9	90
Rutaceae 2				4	40
Citrus sp. - Rutaceae				1	10
Coffea arabica – Rutaceae				5	50
Paullinea carpopodea – Sapindaceae				1	10
Serjania sp. - Sapindaceae			1	6	70
Scrophulariaceae			1	7	80
Not Identified Type 1 (ni1)			2	5	70
Not Identified Type 2 (ni2)				9	90
Not Identified Type 3 (ni3)			1	7	80
Not Identified Type 4 (ni4)				5	50

D = Dominant pollen [> 45%]; A = Accessory pollen [15 to 45%]; I = Isolated pollen [3 to 14%]; O= occasional pollen [< 3%].

Asteraceae type was found as isolated pollen or occasional pollen in five regions. Bees use plants from Asteraceae type to collect pollen and nectar. Some studies confirm the presence of *Baccharis dracunculifolia* (Asteraceae) in the composition of green propolis in Franca, São Paulo State (SOUSA et al., 2007) and in Minas Gerais State (BASTOS et al., 2011). However, it was not found pollen of *Baccharis dracunculifolia* in propolis from this study; this is an important finding since this plant grows in dirty pasture and it is commonly visited by bees for resin collection (BARTH et al., 1999).

Acacia sp. (Mimosoidea) was found in ten regions and showed the higher pollen frequency as isolated pollen in three regions and occasional pollen in seven regions. *Mimosa caesalpiniaefolia* pollen was found in six regions as occasional pollen. *Leucaena pallida* was found in eight regions as isolated pollen (one region) and occasional pollen (seven regions).

Pollen from Mimosoideae is commonly found in Brazilian Cerrado and largely used by bees. Cerrado vegetation is disperse in some regions of São Paulo State and serves as bee pasture suggesting that some apiaries of our study are located in these areas (BURMAN, 1991). Freitas and Aires (2001) found that the *M. caesalpiniaefolia* and *M. scabrela* were the most frequent species in the Atlantic watershed of Rio de Janerio State.

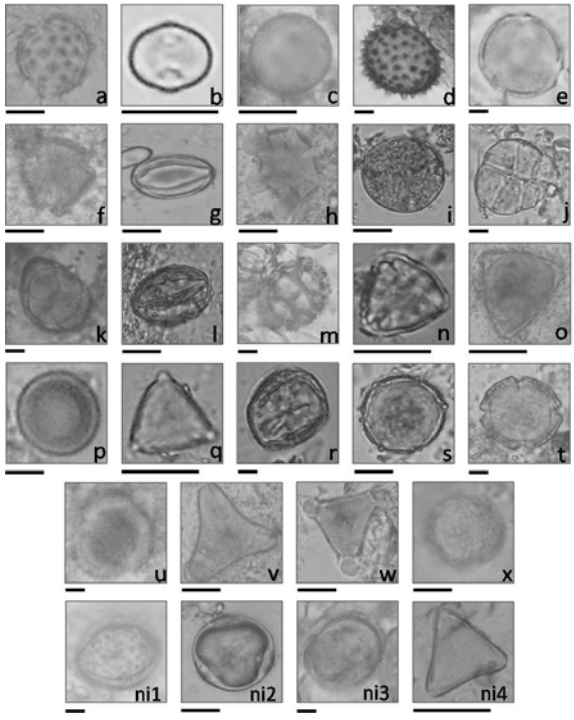

Figure 2. Pollen grains in propolis samples from state of São Paulo from 2008 to 2009. Type of Asteraceae (a), Type of Caesalpinaceae (b), Type of Cecropiaceae (c), Type of Convolvulaceae (d), Type of Euphorbiaceae (e) Type of Fabaceae 1 (f), Type of Fabaceae 2 (g), Type of Lamiaceae (h), *Hyptis umbrosa* – Lamiaceae (i), Type of Mimosoideae (j), *Acacia* sp. – Mimosoideae (k), *Leucaena pallida* - Mimosoideae (l), *Mimosa caesalpinifolia* – Mimosoideae (m), Type of Myrtaceae (n), *Eucalyptus* sp. - Myrtaceae (o), *Panicum* sp. – Poaceae (p), Type of Proteaceae (q), Type of Rutaceae 1 (r), Type of Rutaceae 2 (s), *Citrus* sp. - Rutaceae (t), *Coffea arabica* – Rutaceae (u), *Paullinea carpopodea* – Sapindaceae (v), *Serjania* sp - Sapindaceae (w), Type of Scrophulariaceae (x) and Not Identified Type 1 (ni1), 2 (ni2), 3(ni3) and 4(ni4). Scale: 10 μm.

Due to the large area of eucalyptus crop in São Paulo State the *Eucalyptus* sp. (Myrtaceae) pollen was found in 50% of studied regions. Beekeepers seek eucalyptus crop areas because of its potential for nectar production (KOMATSU et al., 2002). Freitas and Aires (2001) also found Myrtaceae (*Eucaliptus* sp.) pollen as frequent pollen in the Atlantic watershed of Rio de Janerio State.

Others types of pollen were also found: Caesalpinaceae, Cecropiaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Lamiaceae, *Hyptis umbrosa* – Lamiaceae, *Panicum* sp. – Poaceae, Proteaceae, Rutaceae, *Citrus* sp. - Rutaceae, *Coffea arabica* – Rutaceae, *Paullinea carpopodea* – Sapindaceae, *Serjania* sp. - Sapindaceae, Scrophulariaceae and not identified types 1 (ni1), 2 (ni2), 3(ni3) and 4 (ni4).

There are several studies in the scientific literature about the presence of pollen in Brazilian honey. The classification of pollen grains in honey is important for the identification of bee pasture around the apiary (BARTH, 2004; SODRÉ et al., 2008).

There are few studies available in the literature about the presence of pollen in propolis (BARTH; LUZ, 2003; BARTH, 2004, 2006). Barth et al. (1999) found the following pollen types in propolis from states of Rio de Janeiro, Minas Gerais, São Paulo and Rio Grande do Sul: *Eupatorium* sp., *Mimosa caesalpinifolia*, *Cecropia* sp. and *Eucalyptus* sp. as dominant pollen; *Citrus* sp., *Mimosa scabrella*, *Myrcia* sp., *Triumfetta* sp., *Coffea*, *Solanum* and *Palmae* sp. as accessory pollen besides the anemophilous pollen *Brosimum* sp., *Cecropia* sp., *Piper* sp. e *Ambrosia* sp.; and *Antigonon* sp., *Hyptis* sp., *Zanthoxylum* sp., *Anadenanthera* sp., *Borreria* sp., *Elephantopus* sp., *Gochnatia* sp., *Mantanoa* sp. e *Vernonia* sp. as isolated pollen.

The pollen in propolis samples can have different origins. Wind can disperse pollen which adheres to the resin of vegetal exudations, or pollen can be part of propolis as a contaminant. Also, pollen can be adhered to the body when bees are producing in the field or in the hive (BARTH et al., 1999). Bastos et al. (2011) suggest that pollen types with low frequency in propolis samples can be indicative of botanical species that are used by bees to collect resin.

Conclusion

In conclusion, there are wide varieties of vegetal sources which can be found in the final composition of propolis.

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