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Pollen analysis of the post-emergence residue of *Melipona (Melikerria) interrupta* Latreille (Hymenoptera: Apidae) bred in the central Amazon region

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ABSTRACT

We applied an "adapted" protocol for collecting and processing pollen grains in the pollen analysis of the post-emergence residue of *Melipona (Melikerria) interrupta* Latreille. The study was conducted at the Sant'Ana honey farm, located on the banks of the Solimões River, in the municipality of Manacapuru, in the state of Amazonas, Brazil, where a colony was monitored in October and November 2010. From that colony, 10 samples of post-emergence residue were collected. Unlike in the acetolysis method, there was no need to expose pollen grains to an acidic medium, because pollen loses its content during the larval digestive process. We identified 32 pollen types, from 19 botanical families, plus three undetermined pollen types. The most representative family was Fabaceae (Mimosoideae), with eight pollen types, *Mimosa guilandinae* being the most common species. Only the pollen of *Miconia* (Melastomataceae), with 74.10%, was classified as a common pollen. We also found that the pollen of *Mimosa pudica* (Fabaceae: Mimosoideae) retained its content, indicating that not all resources furnished by workers are utilized by the larvae. The protocol applied here, despite omitting the acetolysis process, was efficient, providing full details of pollen contained in post-emergence residue.

Key words: stingless bees, palynology, pollen, floral resources

Introduction

The relationships between pollen-collecting bees and plants can be analyzed in an indirect and practical manner by analysis of the pollen load carried by foraging bees, thus allowing the spectrum of floral resources and its relative attractiveness to the colonies to be determined for a given period or habitat (Imperatriz-Fonseca *et al.* 1993). Likewise, knowledge of plant species based on pollen and nectar sources contributes to the characterization and origin of the resource used (Carvalho *et al.* 2001). When performed on a monthly basis, such analyses also provide valuable floral calendars for further studies (Luz *et al.* 2007) and contribute to maintaining bee pasture.

Recently, the palynological analysis of post-emergence residue has been successfully applied in studies of trophic resources accessed by solitary bees of the Centridini tribe (Dórea *et al.* 2009; Dórea *et al.* 2010). However, a number of studies have evaluated pollen collected by stingless bees in the central Amazon region. Those studies have involved the use of classical protocols for the collection of pollen foraged by bees, such from pollen pots (Absy *et al.* 1984; Rech & Absy 2011a; Rech & Absy 2011b), from nectar and honey samples (Absy *et al.* 1980) or, more commonly, from the pollen baskets of worker bees (Absy & Kerr 1977; Marques-Souza *et al.* 1995; Marques-Souza 1996; Marques-Souza *et al.* 1996; Marques-Souza *et al.* 2002; Oliveira *et al.* 2009). In this context, palynology has become an important tool, supporting the processes related to the analysis and identification of pollen grains. Regardless of the method of collection, the most widely adopted chemical process is acetolysis, as described by Erdtman (1960), which exposes pollen grains to an acidic medium (acetic anhydride and sulfuric acid), destroying its content, allowing a better assessment of the morphological characteristics of the walls of pollen grains and consequently their identification. Other, simpler methods, as proposed by Wodehouse (1935), using only the preparation of fresh pollen grains without eliminating its content, provide rapid morphological results. However, the use of such methods hampers the visualization of exine details, which is necessary for the palynological identification.

The aim of this study was to apply an "adapted" protocol of collecting and preparing pollen contained in the post-emergence residue of the stingless bee *Melipona*

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interrupta (Melikerria) Latreille, in order to enable detailed analyses of the pollen grains, in order to make rapid and accurate identifications of the plant species in the diet of this bee.

Material and methods

The study was conducted at the Sant'Ana honey farm, located on the banks of the Solimões River (03°17'08"S; 60°27'54"W), in the municipality of Manacapuru, in the state of Amazonas, Brazil. We used a colony of *Melipona interrupta*, popularly known as jupará, widely distributed and kept in honey farms in the region, the honey of which is nationally renowned for its excellent flavor.

Sampling occurred during October and November 2010, when a colony was monitored, from the inside, and observed until the emergence of new bees. Then, with the aid of tweezers, we withdrew, from the bottom of the cell, the pellet containing the feces of newly emerged bees. We obtained ten samples of this post-emergence residue, which, in this species, is retained for only a matter of minutes, before it could be discarded by the colony. The ten samples were designated A1 through A10, respectively.

Each residue sample was placed in a separate Eppendorf tube and taken to the Palynology Laboratory of the Brazilian *Instituto Nacional de Pesquisas da Amazônia* (INPA, National Institute for Amazonian Research). Glacial acetic acid was added, and the samples were allowed to sit for at least 24 h. Then, with the aid of a glass rod, we macerated the samples and strained them with a copper micrometric screen, separating the wax content from the pollen, and packed the former into test tubes. Thereafter, unlike the acetolysis method described by Erdtman (1960), the process followed three steps prior to assembly of the slides:

1. The content was centrifuged at 2500 rpm for 5 min, and the supernatant was put off.
2. Distilled water (3 ml) and absolute alcohol (two drops) were added, after which the contents were stirred and again centrifuged at 2500 rpm for 5 min, and the supernatant was put off.
3. Glycerin water (3 ml, 1:1) was added, the content was stirred and again centrifuged at 2500 rpm for 5 min. After the supernatant had been discarded, the tubes were overturned on filter paper and sat for at least 30 min before slide mounting.

The slides were mounted using Kisser glycerin gelatin (Salgado-Labouriau 1973), then paraffinized, three permanent slides being prepared for each sample.

We measured and photographed pollen grains using a light microscope (Primo Star; Carl Zeiss Microimaging, Jena, Germany), with a micrometric eyepiece, coupled to a digital camera (PowerShot A650 IS; Canon U.S.A., Inc., Lake Success, NY, USA). We identified pollen types by making comparisons with specimens in the reference pollen collection of the INPA Laboratory of Palynology, as well as by consulting reference materials (Roubik & Moreno 1991; Punt *et al.* 2007).

For quantitative analysis, we counted 500 pollen grains from each sample and the frequency of pollen grains, considering only the simple presence or absence of a pollen type in any of the samples, was classified in accordance with Novais *et al.* (2009), who established the following categories of frequency: very common (> 75%); common (> 50% to ≤ 75%); uncommon (> 25% to ≤ 50%); rare (≥ 5% to ≤ 25%); and very rare (< 5%).

Results

In the residue samples evaluated, we identified 32 pollen types, representing 19 botanical families, and three pollen types were undetermined. The most representative family in the study was Fabaceae (Mimosoideae), with eight pollen types, pollen of the species *Mimosa guilandinae* being present in all samples. Other pollen types, such as those of *Alchornea* (Euphorbiaceae), *Schizolobium amazonicum* (Fabaceae: Caesalpinioideae), *Miconia* (Melastomataceae) and *Solanum* type (Solanaceae), were also present in all samples (Tab. 1).

None of the pollen types were classified as very common. Overall, the most common pollen types were from *Miconia* species, with 74.10%, the only genus whose pollen was classified as common, followed by those of *Schizolobium amazonicum*, with 11.98%, and *Vismia* species, with 6.30%, both of which were classified as rare, and *Alchornea* species, with 3.72%, whose pollen, although classified as very rare, was present in nearly all samples. Some botanical families, such as Anacardiaceae, Aquifoliaceae, Arecaceae, Boraginaceae, Burseraceae, Malpighiaceae and Passifloraceae, were also well represented, although their pollen was classified as very rare, as were all remaining pollen types (Tab. 1, Fig. 1).

Of the pollen grains evaluated in this study, only three were indeterminate. Those grains were characterized as small to medium in size and 3-colporate, with exines ranging from psilate to perforated, collectively accounting for only 0.38% of the pollen evaluated, being also classified as a very rare pollen type (Tab. 1).

At the family level (Fig. 1), the greatest contributions to the composition of the residue samples were made by Melastomataceae (74%), Fabaceae (13.42%), Hypericaceae (6.3%), Euphorbiaceae (3.72%) and Solanaceae (0.94%), indicating that these are main components of the *Melipona interrupta* trophic niche.

One interesting detail was observed in the pollen of *Mimosa pudica*, which was the only pollen considered "very small". In most of the samples, *M. pudica* pollen retained its content, because, unlike the other pollen types, it often passed through the digestive tract of the larvae without being digested (Fig. 2).

Table 1. Presence and frequency of pollen types in samples of post-emergence residue of *Melipona interrupta* Latreille. October-November 2010, state of Amazonas, Brazil.

FAMILY Species/pollen type	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Total (%)
ANACARDIACEAE											
Tapirira guianensis	x	x	x								
AQUIFOLIACEAE											
Ilex type	x										
ARECACEAE											
Elaeis type			x					x			
ASTERACEAE											
Mikania type	x	x	x				x	x	x	x	0.10
BIGNONIACEAE											
Tecoma type	x	x	x	x	x		x	x	x	x	0.18
BORAGINACEAE											
Cordia type			x	x	x			x	x		
BURSERACEAE											
Protium type		x	x							x	
EUPHORBIACEAE											
Alchornea type	x	x	x	x	x	x	x	x	x	x	3.72
FABACEAE/CAESALPINIOIDEAE											
Hymenaea type	x	x	x		x	x	x				0.36
Schizolobium amazonicum	x	x	x	x	x	x	x	x	x	x	11.98
FABACEAE/MIMOSOIDEAE											
Inga type					x						
Mimosa invisa		x			x	x					0.08
Mimosa guilandinae	x	x	x	x	x	x	x	x	x	x	0.42
Mimosa asperata		x								x	0.30
Mimosa pudica		x		x				x		x	0.26
Mimosa spruceana		x					x	x		x	0.02
Piptadenia type				x				x	x	x	
Stryphnodendron guianense	x		x		x	x	x	x			
HYPERICACEAE											
Vismia type	x	x	x	x	x	x	x	x	x	x	6.30
MALPIGHIACEAE											
Byrsonima type					x						
MALVACEAE											
Bombax munguba	x	x	x	x	x	x	x	x	x	x	0.40
MELASTOMATACEAE											
Miconia type	x	x	x	x	x	x	x	x	x	x	74.1
MYRTACEAE											
Eugenia type		x	x								
Myrtaceae type	x								x		0.02
PASSIFLORACEAE											
Passiflora type	x						x			x	
POLYGONACEAE											
Triplaris type	x	x	x	x	x	x	x	x	x	x	0.38
SAPINDACEAE											
Cupania type		x	x	x	x	x	x	x			0.06
Serjania type										x	
SOLANACEAE											
Solanum type	x	x	x	x	x	x	x	x	x	x	0.94
INDETERMINATE											
Type 1		x				x	x				0.28
Type 2				x							0.06
Type 3							x				0.04

*Grains small in size, 3-colporate; exine perforate.
**Grains intermediate in size, 3-colporate; exine perforate.
***Grains intermediate in size, 3-colporate; exine psilate.

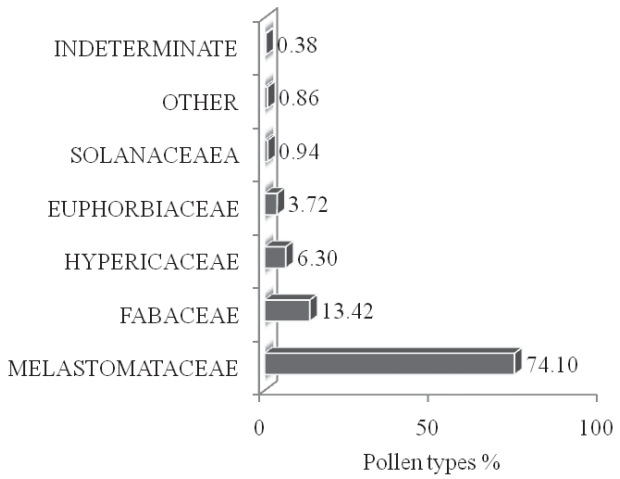

Figure 1. Distribution (%) of pollen types, by family, in samples of post-emergence residue of the stingless bee *Melipona interrupta* Latreille. October-November 2010, state of Amazonas, Brazil.

Discussion

Despite the short sample collection period, this modified protocol showed great viability, producing rapid and conclusive results for the pollen collected from the post-emergence residue of *Melipona interrupta*, bypassing some of the steps of the acetolysis method proposed by Erdtman (1960), which are paramount to visualizing detailed characteristics of the pollen grain wall. The exposure of the pollen grain to an acidic medium was not necessary in this case, because it already undergoes a similar process in the digestive system of the larvae.

Many protocols applied, for studies and identification of pollen collected by bees, work separately, collecting only the pollen from the pollen basket (Oliveira *et al* 2009; Ferreira *et al* 2010), in regurgitated nectar, in pollen pots or in honey (Rech & Absy 2011a; Rech & Absy 2011b). In two separate studies, Dórea *et al.* (2009; 2010) studied the post-emergence residue of *Centris tarsata* Smith. In the protocol applied by those authors, the pollen had to be separated from the sediment. In the present study, we separated only pollen from wax, making it possible to quickly and accurately identify the pollen types contained in the post-emergence residue of *Melipona interrupta*.

The efficiency of our "adapted" protocol was evidenced by the low frequency of indeterminate pollen in the samples. In addition, we demonstrated that certain types of pollen are not digested by the larvae, as was the case for the pollen grains of *Mimosa pudica*, which is often foraged by workers of this species, as previously shown (Marques-Souza 1996). This might be related to the extremely small size of the *M. pudica* pollen grain (Erdtman 1952), coupled with the low frequency in the studied samples. However, in order to fully understand this phenomenon, further studies, involving longer sample collection periods and larger numbers of samples, are needed, especially for other bee species.

Our study shows the importance of the Fabaceae family (Mimosoideae), which had a high number of attractive species in times of flowering, thus expanding the trophic niche of the bee. Other species, such as *Alchornea* (Euphorbiaceae), *Schizolobium amazonicum* (Fabaceae: Caesalpinioideae), *Miconia* (Melastomataceae), *Solanum* type (Solanaceae) and *Vismia* (Hypericaceae), were present

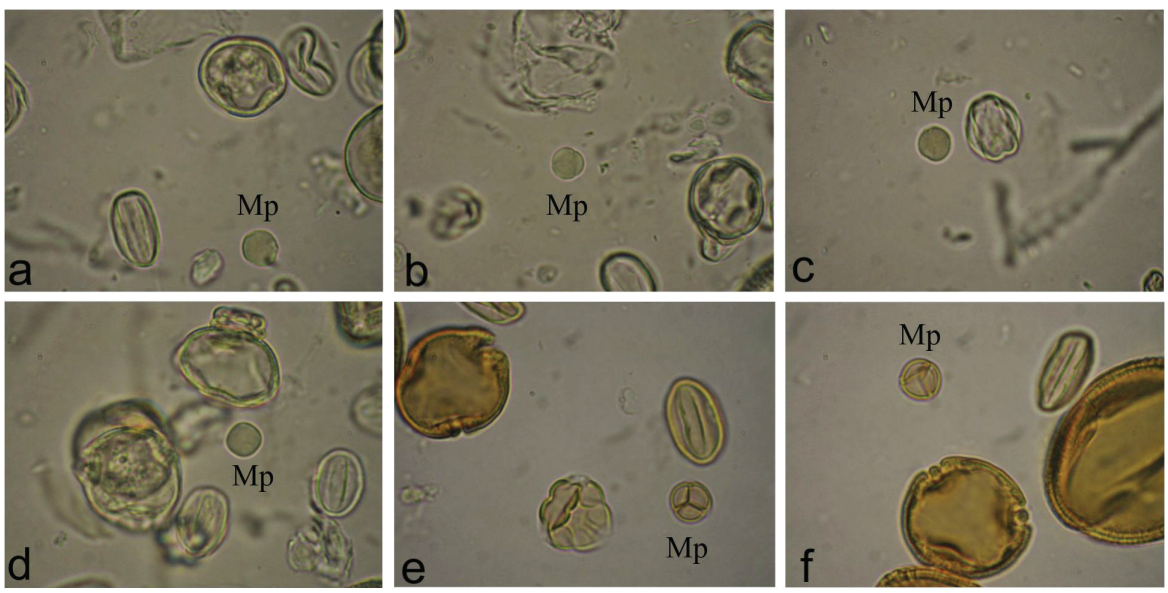

Figure 2. Photomicrographs of pollen grains obtained from the post-emergence residue of *Melipona interrupta* Latreille, detailing pollen grains of *Mimosa pudica* (Mp), which retained its content (a-d), in slides prepared in accordance with the modified protocol (a-d) and slides prepared by the acetolysis method (e,f). Scale = 10 µm.

in several samples, underscoring their importance for the maintenance of the colony. Overall, the Melastomataceae family was the most representative in terms of pollen types. Melastomataceae is widely distributed in the region and is well represented in numerous studies of stingless bees in the Amazon (Marques-Souza *et al.* 1995; Marques-Souza 1996; Marques-Souza *et al.* 1996; Marques-Souza *et al.* 2002; Oliveira *et al.* 2009).

Stingless bees are widely distributed in the Amazon, where many species are bred by beekeepers, who can use knowledge of the floral resources used by these bees for the maintenance of colonies and the improvement of stingless bee pasture. The protocol evaluated here enables the identification of the pollen collected, stored and digested by these bees, providing accurate information about the origin and use of trophic resources, in a timely manner.

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