4. Palynological analysis of Brazilian stingless bee pot-honey

BARTH Ortrud Monika[1*], FREITAS Alex da Silva[1], ALMEIDA-MURADIAN Ligia Bicudo [2], VIT Patricia[3]

[1]Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil.

[2] Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, USP, São Paulo, Brazil.

[3]Food Science Department, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida 5101, Venezuela.

* Corresponding author: Ortrud Monika Barth, Email barth@ioc.fiocruz.br

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Abstract

Scientific investigation of meliponine honey quality provides detailed information on where the bees go for food and also as pollinator agents. Palynological analysis of 22 stingless bee honeys, obtained in several localities of Brazil, showed that ~75% of the samples were monofloral (at least 45% of pollen grains were of one species). Botanical origin was in agreement with regional vegetation, and pollen suggests Meliponini maintain activity on a single plant species when nectar is available.

Key words:
Brazil, honeydew, melissopalynology, pot-honey

Introduction

Native stingless bees are important pollinators of the flora all over the tropical world. Quantitative honey production is relatively small, compared to *Apis mellifera*, and has only recently started to achieve commercial success. Scientific investigation amplifies our knowledge about meliponine honey quality, in order to get detailed information where these bees are really searchinng for food and effecting pollination. Field observations are not completely suitable, and honey, pollen loads and bee bread pollen analysis, as a supplement or surrogate for field work, can reveal which plants are nectar and pollen resources for the stingless bees.

Besides the pollen analysis of honey, a palynological analysis of honey reveals additional elements, such as spores and hyphae of fungi and algae which are indicative of honeydew presence, bacteria, yeast, mould spores, starch, minerals and other impurities (Barth, 1989, 2004). When pollen loads and/or pollen bread harvested inside the hive have to be analyzed with the intention to recognize the bee flora, a different technique than that applied for honey analysis must be used (Barth et al., 2010). Honey quality control, in addition to sensorial and physico-chemical analysis, requires pollen grain isolation and identification. Ideally, the grain's morphological characteristics allow recognizing its taxon. A further diagnosis of a sample quality utilizes all the available data.

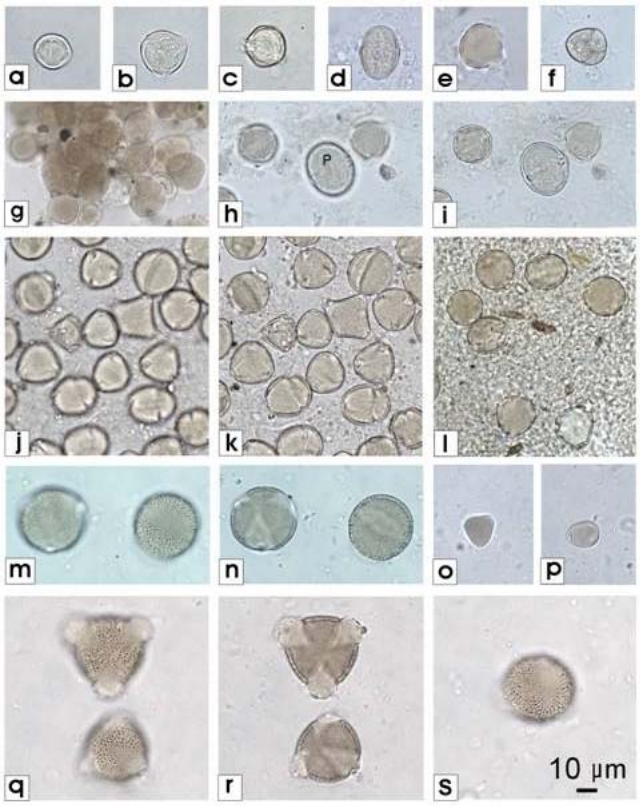
Stingless bees (Meliponini) occur in tropical and subtropical countries all over the world, from Central and South America, Africa, South Asia to Australia (Souza et al., 2006). Melissopalynological analysis of honey has been done, by some examples, in Mexico (Martínez-Hernández et al., 1994), Venezuela (Vit and Ricciardelli D'Albore, 1994; Freitas et al., 2010), Borneo (Leonhardt et al., 2007) and Argentina (Flores and Sánchez, 2010).

Brazil produces a great variety of stingless bee pot-honey, and its production is promoted by official entities. Several states, mainly of the Brazilian Northeast region, are contributors in official programs, getting an additional income to farmers and rural residents.

4.1 Palynological analysis of pot-honey samples

Honey samples were removed from the honey pots in the hives with a pipette, syringe or a vacuum pump. Following the technique introduced by Louveaux et

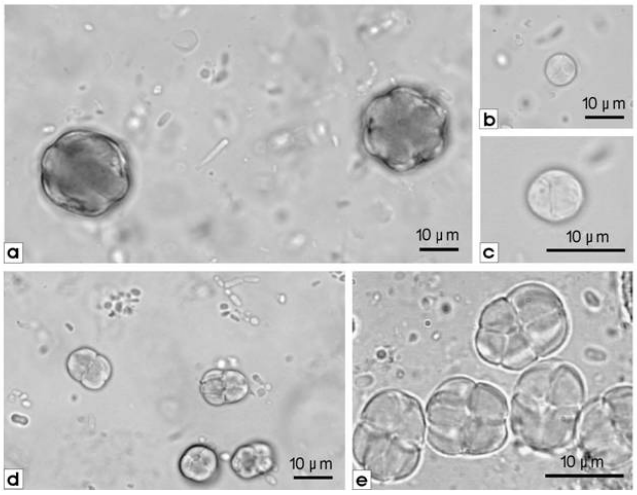
al. (1978), 10 mL of each sample was diluted in 20 mL of water and centrifuged at least at 1,500 rpm; the sediment obtained was suspended into 5 mL of a 1:1 mixture of glycerin and water, left for a half hour, and centrifuged again. Two pollen slides, using non-stained glycerin-gelatin, were prepared and sealed with paraffin. Light microscope observations started with 100x magnification and identification and counting of at least 300 pollen grains per sample at 400x magnification. Photomicrographs were obtained using a Zeiss Axiophot microscope and a Canon digital camera (Figures 1 and 2). Pollen grain frequency classes follow methods of Zander (1935, in Louveaux et al., 1978).



Photography: Ortrud Monika Barth

Figure 1. Most frequent pollen types of some Brazilian stingless bee pot-honeys

a–c = *Solanum* (Solanaceae), pollen grains presenting different positions: a = oblique view showing a typical aspect of one aperture; b = polar view; c = equatorial view. d-e = *Crotalaria* (Fabaceae, Faboideae): d = equatorial view; e = a nearly polar view. f-g = *Mimosa verrucosa* (Fabaceae, Mimosoideae): f = tetrad; g = easily distinguishable tetrads inside a group of agglutinated pollen grains. h-i = two pollen grains of *Tapirira* (Anacardiaceae) and one of *Protium* (P, Burseraceae): h = surface view; i = optical section. j-k = *Hovenia dulcis* (Rhamnaceae), pollen grains presenting different positions: j = surface view; k = optical section. l = *Carica* (Caricaceae): pollen grains presenting different positions. m-n = *Brassica* (Brassicaceae), pollen grains presenting equatorial and polar views: m = surface; n = optical section. o-p = *Myrcia* (Myrtaceae): o = polar view; p = equatorial view. q-s = Fabaceae-Faboideae: q = polar view, surface; r = polar view, optical section; s = equatorial view, surface (all figures are of the same magnification).



Photography: Ortrud Monika Barth

Figure 2. Most frequent pollen types of polliniferous plants

a = Melastomataceae. b-c = *Mimosa scabrella* (Fabaceae, Mimosoideae), tetrads: b = lower magnification; c = higher magnification, another position of view. d-e = *Mimosa caesalpiniaefolia* (Fabaceae, Mimosoideae), ditetrads and yeast cells: d = lower magnification; e = higher magnification.

In this work, the botanical composition of pot-honey was investigated in several counties of the Brazilian Federation using pollen analyisis (Figure 1), mainly in seven states of the north and northeast region (Table 1). In a previous work, pollen analysis of 92 pot-honey samples of the states of Bahia, São Paulo and Rio Grande do Sul was done by Baslen (2000). Twelve botanical families presented a predominant pollen type (>45% of the pollen sum), Anacardiaceae, Asteraceae, Balsaminaceae, Burseraceae, Euphorbiaceae, Fabaceae, Melastomataceae/ Combretaceae, Fabaceae, Mimosoideae, Myrtaceae, Rubiaceae, Sapindaceae and Solanaceae. No plant genus (except *Eucalyptus* sp./Myrtaceae) or species was identified. Pollen grain richness was lowest in the honey *Melipona* stingless bees. Bazlen (2000) detected honeydew elements, like hyphae and spores of fungi, frequently in honey samples of Trigonini (in 18 of 28 samples), but rarely in honeys of *Melipona* and *Apis*.

Table 1. Palynological analysis of meliponine honeys

Locality	Bee species	Common name	Pollen types (crude percentages, >3%)	Pollen types (ammended percentages, >3%)	Palynological evaluation
Manacapuru/Amazonas	*Melipona compressipes manaosensis*	"jupará"	Melastomataceae (66.8%); *Solanum* (22.7%); Gesneriaceae (4.9%); Fabaceae-Faboideae (3.3%)	Solanaceae (69.8%); Gesneriaceae (15.1%); Fabaceae-Faboideae (10.0%); *Brassica* (3.3%)	Monofloral (Solanaceae)
Manacapuru/Amazonas	*Melipona seminigra merrillae*	"jandaíra"	Melastomataceae (52.1%); *Solanum* (24.8%); *Mimosa scabrella* (17.3%); *Cuphea* (5.8%)	Solanaceae (81.1%); *Cuphea* (18.9%)	Monofloral (Solanaceae)
Porangaba/Amazonas	*Melipona seminigra merrillae*	"jandaíra"	Melastomataceae (81.2%); *Protium* (9.8%); Anacardiaceae (4.1%); *Mimosa scabrella* (3.1%)	*Protium* (62.6%); Anacardiaceae (26.4%); *Crudia* (3.3%); Gesneriaceae (3.3%)	Monofloral (*Protium*)
Xingú/Pará	*Scaptotrigona polysticta*	"mijuí"	*Myrcia* (36.9%); *Tapirira* (22.1%); Fabaceae-Faboideae (15.3%); Melastomataceae (13.6%); *Antigonon leptopus* (5.3%); *Eucalyptus* (3.5%)	*Myrcia* (42.4%); *Tapirira* (25.4%); Fabaceae-Faboideae (18.1%); *Antigonon leptopus* (6.1%); *Eucalyptus* (4,0%)	Bifloral (*Myrcia* and *Tapirira*)
Pará	*Melipona quadrifasciata*	"mandaçaia"	*Tapirira* (77.9%); Melastomataceae (7.6%); *Protium* (6.4%); *Myrcia* (3.4%)	*Tapirira* (85.9%); *Protium* (7.2%); *Myrcia* (3.8%);	Monofloral (*Tapirira*)
Pará	*Melipona scutellaris*	"uruçu"	*Tapirira* (32.5%); *Myrcia* (28.0%); *Eucalyptus* (22.7%); *Mimosa verrucosa* (7.0%); *Mimosa invisa* (3.9%)	*Tapirira* (32.6%); *Myrcia* (30.2%); *Eucalyptus* (22.7%); *Mimosa verrucosa* (7.0%); *Mimosa invisa* (3.9%)	Heterofloral
Pará	*Melipona rufiventris*	"uruçu amarela"	*Tapirira* (50.7%); *Mimosa scabrella* (15.0%); Melastomataceae (12.7%); *Paullinia* (5.7%)	*Tapirira* (70.4%); *Cocos* (8.7%); *Myrcia* (3.5%); *Paullinia* (7.9%)	Monofloral (*Tapirira*)
Pará	*Melipona scutellaris*	"uruçu"	Without pollen grains	Without pollen grains	Forged
Moura/Maranhão	*Melipona fasciculata*	"tiúba"	*Brassica* (36.%); Indeterminado (29.0%); Melastomataceae (27.3%);	*Brassica* (86.3%); *Eucalyptus* (4.0%); *Myrcia* (4.0%)	Monofloral (*Brassica*)
Limoeiro/Maranhão	*Melipona fasciculata*	"tiúba"	Fabaceae-Faboideae (37.3%); Combretaceae (22.9%); *Myrcia* (14.4%); Fabaceae *aff. Desmodium* (6.2%); *Citrus* (5.6%); *Brassica* (4.7%)	Fabaceae-Faboideae (52.0%); *Myrcia* (20.1%); Fabaceae *aff. Desmodium* (8.7%); *Citrus* (7.9%); *Brassica* (6.5%), Arecaceae (3.5%)	Monofloral (Fabaceae-Faboideae)

Locality	Bee species	Common name	Pollen types (crude percentages, >3%)	Pollen types (ammended percentages, >3%)	Palynological evaluation
Limoeiro/Maranhão	*Melipona fasciculata*	"tiúba"	*Brassica* (50.5%); Melastomataceae (23.3%); Rhamnaceae (20.6%)	*Brassica* (95.0%); *Myrcia* (3.3%)	Monofloral (*Brassica*)
Moura/Maranhão	*Melipona fasciculata*	"tiúba"	*Carica* (42.5%); Indeterminado (25.2%); *Myrcia* (24.6%);	*Carica* (61.6%); *Myrcia* (3.6%)	Monofloral (*Carica*)
Todos os Santos/ Maranhão	*Melipona fasciculata*	"tiúba"	Combretaceae (40.7%); Fabaceae-Faboideae (25.3%); *Myrcia* (17.0%); Fabaceae *aff*. *Desmodium* (5.5%); *Celtis* (4.3%); Cyperaceae (4.0%)	Fabaceae-Faboideae (51.3%); *Myrcia* (33.7%); Fabaceae *aff*. *Desmodium* (11.0%), Arecaceae (3.7%)	Monofloral (Fabaceae-Faboideae)
Tabocas/ Maranhão	*Melipona fasciculata*	"tiúba"	Few pollen grains only	Few pollen grains only	Extrafloral honey
Natal/Rio Grande do Norte	*Melipona subnitida*	"jandaíra"	*Mimosa caesalpiniaefolia* (95.9%)	*Mimosa caesalpiniaefolia* (95.9%)	Monofloral (*Mimosa caesalpiniaefolia*)
Natal/Rio Grande do Norte	*Melipona subnitida*	"jandaíra"	*Mimosa caesalpiniaefolia* (66.0%); *Crotalaria* (22.8%); *Mimosa verrucosa* (7.7%)	*Crotalaria* (87.6%); *Mimosa verrucosa* (29.5%); *Croton* (5.7%)	Monofloral (*Crotalaria*)
Natal/Rio Grande do Norte	*Melipona subnitida*	"jandaíra"	*Mimosa caesalpiniaefolia* (58.8%); Fabaceae-Faboideae (12.0%); *Croton* (11.5%); *Piptadenia* (10.0%)	Fabaceae-Faboideae (33.0%); *Croton* (32.0%); *Piptadenia* (27.4%); *Mimosa verrucosa* (7.4%); Cactaceae (5.0%)	Heterofloral
Povoado do Cabeço/Rio Grande do Norte	*Melipona subnitida*	"jandaíra"	Few pollen grains only	Few pollen grains only	Extrafloral honey
Patos/Paraíba	*Melipona subnitida*	"jandaíra"	*Mimosa verrucosa* (62.5%); *Crotalaria* (20.8%); *Cassia* (4.2%); *Anadenanthera* (4.2%); *Antigonon leptopus* (4.2%); *Hovenia dulcis* (4.2%)	*Mimosa verrucosa* (62.5%); *Crotalaria* (20.8%); *Cassia* (4.2%); A*nadenanthera* (4.2%); *Antigonon leptopus* (4.2%); *Hovenia dulcis* (4.2%)	Monofloral (*Mimosa verrucosa*)
Patos/Paraíba	*Melipona subnitida*	"jandaíra"	*Mimosa verrucosa* (79.0%); Rubiaceae (7.2%); *Coccoloba* (4.8%); *Myrcia* (3.2%)	*Mimosa verrucosa* (81.7%); Rubiaceae (7.5%); *Coccoloba* (5.0%); *Myrcia* (3.3%)	Monofloral (*Mimosa verrucosa*)
Cruz das Almas/Bahia	*Melipona scutellaris*	"uruçu"	*Mimosa scabrella* (62.8%); *Mimosa verrucosa* (19.4%); *Eucalyptus* (12.0%);	*Mimosa verrucosa* (59.6%); *Eucalyptus* (36.6%)	Monofloral (*Mimosa verrucosa*)
Içara/Santa Catarina	*Tetragonisca angustula*	"jataí"	*Hovenia dulcis* (73.3%); *Brassica* (7.3%); Arecaceae (5.4%); *Echium* (3.3%)	*Hovenia dulcis* (77.0%); *Brassica* (7.6%); Arecaceae (5.7%); *Echium* (3.5%)	Monofloral *(Hovenia dulcis*)

The pollen analysis of fourteen honey samples of Meliponini was basic to investigate its physico-chemical properties and anti-bacterial activity (Cortopassi-Laurino and Gelli, 1991). The samples were obtained in several Brazilian states, and ten of them showed dominant pollen types, such as *Piptadenia*, *Borreria verticillata*, *Mimosa bimucronata* and *Mimosa taimbensis* in Ceará state, *Borreria latifolia* and *Mimosa bimucronata* in Paraíba state, *Eucalyptus* sp. and *Myrcia* pollen type in São Paulo state and *Centella* (Apiaceae) pollen type in Paraná state.

4.1.1 The Northern region

The second scientific experiment (the first was that of Iwama and Melhem, 1979, at São Paulo state) to understand pollen grain identification of nectar collected by stingless bees in Brazil was done by Absy et al. (1980) in Manaus, Amazonas state, considering the bees of *Melipona seminigra merrillae* "uruçu-boca-de-renda" and *M. rufiventris paraensis* "uruçu amarela". Three hundred and two bees were compressed in order to expel the nectar, when coming back into its hives after foraging in the field. Nectar was acetolysed and pollen grains were identified, but not quantified. The most common plant taxa detected all over a year were *Alchornea discolor* (Euphorbiaceae, nectariferous), *Eugenia* sp. (Myrtaceae, nectariferous), *Miconia* sp. (Melastomataceae, nectariferous and polliniferous), *Protium heptaphyllum* (Burseraceae, nectariferous), *Tapirira guianensis* (Anacardiaceae, nectariferous) and *Vismia guianensis* (Clusiaceae, nectariferous).

Many years later, Freitas et al. (2010) analyzed three pot-honey samples obtained in the Amazonas state, two of *Melipona compressipes manaosensis* "jupará" from Manacapuru, both with the same dominant pollen type of Solanaceae, and one of *M. seminigra* "uruçu-boca-de-renda" from Porangaba, with the dominant pollen of *Protium* sp.

Besides the Brazilian stingless bee honeys described above, an additional five samples obtained in the state of Pará (Table 1) are included here. One of a *Melipona scutellaris* "uruçu" sample did not present any pollen grain, the other was heterofloral. Both *Melipona quadrifasciata* "mandaçaia" and *M. rufiventris* "uruçu amarela" produced a monofloral honey of *Tapirira* (Anacardiaceae), probably *T. guianensis*, a very common tree, and *Scaptotrigona polystica* "benjuí" visited *Tapirira* also.

4.1.2 The Northeast region

The Brazilian northeast region was largely investigated for meliponine activities and products. The most common bee in the state of Maranhão is *Melipona compressipes fasciculata* "tiúba" (Kerr et al. 1986/87).

Carvalho et al. (2001) examined honey samples of *Melipona scutellaris* "uruçu" obtained in the Bahia state (Catu municipality, inland of Salvador city). *Eucalyptus* sp. (Myrtaceae) pollen dominated from November just to February, while that of *Psidium* sp. (Myrtaceae) dominanted in September/October.

In the São Gabriel county, Irecê municipality, semi-arid region of Bahia, Alves et al. (2006) analyzed honey samples of *Melipona mandacaia* "mandaçaia" during the main flowering period of April and May. *Piptadenia rigida* (Fabaceae, Mimosoideae) pollen grains dominated in six of eleven samples.

Novais et al. (2006) obtained eight honey samples in the arid caatinga region of Canudos, Bahia state, of *Tetragonisca angustula* "jataí". Dominant pollen types belonged to *Mitracarpus* (Rubiaceae) in October and November, *Ziziphus joazeiro* (Rhamnaceae) in December and *Zornia* sp. (Fabaceae, Faboideae) in October.

Twenty nests of *Trigona spinipes* "irapuá" were followed during month of September occurring in the University Campus, city of São Cristovão, Sergipe state (Oliveira et al. 2008). Two predominant pollen types, one of Euphorbiaceae (51% of honey samples) and one of a Celastraceae (46% of pot samples) could be identified, beside a large number of pollen types.

Breeding of *Melipona scutellaris* "uruçu" in the region of Jequitibá, municipality of Novo Mundo, Bahia state, is a family farming activity. A semideciduous forest presents richness and diversity of flora for honey production. Andrade et al. (2009) analysed 27 honey samples obtained between the months of august to February. Dominant pollen was detected for *Eucalyptus* sp., *Eugenia uniflora*, *Mimosa quadrivalvis*, *M. pudica*, *Solanum paniculatum* and *Solanum* sp.

Freitas et al. (2010) identified the *Mimosa verrucosa* (Fabaceae, Mimosoideae) dominant pollen type in four honey samples analyzed of Patos, Paraíba state. Lopes et al. (2011) studied honey samples of *Melipona compressipes* from several municipalities of the state of Maranhão. Dominant pollen types belonged to *Eichornia* sp. (Ponteriaceae, six samples), *Hyptis* sp. (Lamiaceae, two samples) and to one non-identified plant species (one sample). Recently, Martins et al. (2011) spread a yearly behavior of *Melipona fasciculata* "tiúba", largely known in the lowland counties of the state of Maranhão. Monthly obtained pot-honey samples in

the Palmeirândia municipality revealed its feeding interest. *Pontederia parviflora* (Pontederiaceae) was the most important nectariferous plant during the months from June until October, nevertheless other species of Pontederiaceae occurred in the region. A second important but non-nectariferous plant was *Mimosa caesalpiniaefolia* (Fabaceae-Mimosoideae) which pollen grains predominated during the months of April, May and November. The habitats of these two plant species need to be protected to sustain the apicultural activities in the future.

Besides the Brazilian pot-honeys described above, additional samples obtained in the state of Maranhão (six honeys), Rio Grande do Norte (four honeys) and Bahia (one honey) were (Table 1). All the new samples from Maranhão state came from *Melipona fasciculata* "uruçu-cinzenta" colonies. Dominant pollen types in these honeys were of a non-identified Fabaceae, Faboideae (two honeys), *Brassica* (Brassicaceae, two honeys) and *Carica* (Caricaceae, one honey). One honey with few pollen grains and may be considered from an extra-floral source.

The four samples of the Rio Grande do Norte state (Table 1) were obtained from *Melipona subnitida* "jandaíra". One sample showed an extrafloral origin, one was heterofloral and each of the two resting samples presented dominant pollen types of *Mimosa caesalpiniaefolia* and *Crotalaria*.

One more sample from Bahia state of *Melipona scutellaris* "uruçu" presented *Mimosa verrucosa* as a dominant pollen type also.

4.1.3 The Southeast and Southern regions

Stingless bee activities were less investigated inside these regions. The first of all papers about melissopalynological studies in Brazil was from Iwama and Melhem (1979). Several honey samples of *Tetragonisca angustula* were collected during one year inside two colonies located in the campus of the University of São Paulo. Acetolysis was used, so that, except pollen grains, unfortunately other elements in the honey sediment were lost. Dominant pollen frequency was found of *Alchornea triplinervia* (Euphorbiaceae), *Eucalyptus cinerea*, *E. robusta*, *E.* sp., *Petrosellinum hortense* and *Schinus terebinthifolius*.

Some time later, Ramalho (1990) studied honey sediments of three species of *Scaptotrigona* bees during one year in the campus of the University of São Paulo also, presenting always dominant pollen grains of *Eucalyptus*.

A unique honey sample collected in Içara, Santa Catarina state (Freitas et al. 2010) presented a dominant pollen type of *Hovenia dulcis* (Rhamnaceae).

4.2 Honeydew

Occurrence of honeydew elements in honey samples of Meliponini was rarely found. Certainly the honeydew elements are frequently present in small amounts in the samples from certain bees, such as *Trigona* or *Oxytrigona* which frequently visit such bugs on vegetation, but without a significant contribution of plant sugars. An exception is the honey produced by non-*Melipona* bees (Bazlen, 2000). Field observations about honeydew collection by the bees were not given.

Several investigations related above used the acetolysis methodology to prepare pollen slides. However, the acetolysis mixture destroys important honeydew elements, hyphae, algae, starch, as well as grains of families such as Zingiberaceae and others, and finally some information is thereby lost (Barth, 1989).

4.3 Melissopalynological facts

The palynological analysis of pot-honeys, obtained in several localities of Brazil, showed that ~75% of the samples were monofloral ones. This is a specialized melissopalynological term that only indicates many grains (at least 45% of those counted) were of one species. It cannot claim to represent the entire source of nectar or even the primary nectar source. The botanical origin of honey must be in agreement with the regional vegetation. The pollen analysis method may indicate that Meliponini concentrate foraging activity on a single plant species. Whether this is true depends on field observations to support this assertion. Regarding the continental dimension of the country, insufficient investigation exists. At this statement it is not possible to make wide regional considerations about stingless bee forage preferences. The best way to get more information about quantity and quality of these honeys is a continuing series of detailed local investigation.

The pollen grains of many botanical taxa, beside the dominant pollen types, and those that were detected with a frequency higher than 3%, have to be investigated regarding the possibility of a monofloral honey production. Each region needs a deeper scientific investment, depending upon climate conditions, seasonal flowering, pollen and nectar harvesting, honey sample collecting and processing, laboratorial techniques, and much more in view to achieve comparative diagnosis. Then these little bees,

as it was formerly proposed by Braga et al. (2009), may be useful as bioindicators also.

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