An Acad Bras Cienc (2021) 93(Suppl. 4): e20200591 DOI 10.1590/0001-3765202120200591
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Brazilian peppertree, eucalyptus, and *velame* honeys: does palynology confirm the predominant flower sources indicated by beekeepers?

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Abstract: We investigated ten honey samples from the Discovery Coast of Bahia, Brazil, to confirm the three predominant flower sources indicated by regional beekeepers: Brazilian peppertree – *Schinus terebinthifolia* Raddi; eucalyptus – *Eucalyptus* L'Hér. spp.; and *velame* – *Croton* L. spp.. The honeys were collected in five Southern Bahia municipalities – Belmonte, Eunápolis, Guaratinga, Itabela and Porto Seguro. The samples underwent acetolysis and, after slides mounting, the pollen types were identified and counted to determine the frequency classes. The pollen spectra revealed 31 pollen types in 27 genera and 17 families. The palynological analysis confirmed the predominant eucalyptus flower source indicated for four honey samples from Belmonte, Eunápolis, Itabela and Porto Seguro. Three honey samples previously indicated with predominant eucalyptus and *velame* flower sources had no predominant pollen. Eucalyptus predominated as the main flower source for another three samples previously indicated by beekeepers as Brazilian peppertree and *velame*. We conclude that regional honeys marketed as having a predominant flower source may have distinct botanical origin. This demands further research aimed at reviewing which bee plants provide resources for monofloral, bifloral and plurifloral honeys in the Bahian Discovery Coast.

Key words: *Apis mellifera*, Atlantic Rain Forest, melissopalynology, monofloral honey, pollen analysis, unifloral honey.

INTRODUCTION

Among the consequences caused by the loss of Atlantic Rain Forest areas in Brazil (SOS Mata Atlântica Foundation 2017) is the deficit in ecosystem services, such as pollination, an essential process for the reproduction of most plant species (Varassin et al. 2021). Furthermore, deforestation affects the flora available for bees and other animals that depend on plants for food, shelter, defense, etc. Knowing the bee-exploited flora is essential to plan strategies for biodiversity conservation, and pollen analysis proves to be an effective method employed for this purpose. Also, pollen identification can indicate the geographical and botanical origin of bee products, such as honey, bee pollen, and propolis, since the pollen types found in these products equate to "fingerprints" of bee foraging habits (Jones & Bryant 1996).

Palynological studies subsidize honey classification based on the pollen spectrum. Monofloral or unifloral honeys include above 45% of pollen grains from a single plant species (Louveaux et al. 1978). On the other hand, a multifloral honey sample has no predominant pollen (Ricciardelli-D'Albore 1997). Monofloral honeys are appreciated by consumers due to their flavor, color, aroma, and pharmacological

properties. These honeys have higher prices on both the domestic and foreign market (Andrade et al. 1999) and, therefore, have higher added value. This requires that the information printed on labels or indicated by sellers be verified, in order to know whether such honeys actually meet the criteria necessary to be classified as monofloral. However, classifying the samples as monofloral honeys due to a pollen type with frequency exceeding 45% in the pollen spectrum can lead to misinterpretation, as consumers tend to believe that honey comes from a single floral source. This occurs if we disregard aspects of the floral biology of the plant species associated with the pollen types, as well as the floral diversity of each region. Moreover, "no honey produced by bees flying free is likely to be entirely unifloral" (Molan 1998, p. 79). Therefore, the term "honey from predominant flower sources" seems to be more appropriate than "monofloral honey" for most of the Brazilian honeys.

Pollen grains in monofloral or predominant flower source honeys have been examined by a variety of studies abroad, such as in Algeria (Makhloufi et al. 2015), Argentina (Ciappini et al. 2016, Sánches & Lupo 2017), Austrália (Seijo et al. 2003, Sniderman et al. 2018), Portugual (Seijo et al. 2003), Ethiopia (Belay et al. 2017), Spain (Seijo et al. 2003, 2015). In Brazil, monofloral indicated honeys were palynologically analyzed by Barth (1990), in São Paulo, Maranhão, and Mato Grosso do Sul, Almeida-Muradian et al. (2014) in Ceará, Borges et al. (2014) in Piauí, and Kadri et al. (2016) in Espírito Santo States. However, research on predominant flower source or monofloral honeys in Bahia is lacking.

In southern Bahia, municipalities such as Eunápolis and Guaratinga stand out for their honey production (IBGE 2016), contributing to the Bahian performance in the national production scenario. Predominant flower source or monofloral indicated honeys are commercialized either at regional fairs or directly from producers. This assumed predominant botanical origin still relies on the beekeeper's empirical observations around the apiary. In order to improve this information, palynological analysis can indicate the botanical origin of honeys more accurately, subsidizing regional beekeeping.

In a previous work (Bandeira & Novais 2020), we presented the pollen spectra of 21 honey samples from the Discovery Coast of Bahia. Our current study complements the questions addressed in the previous manuscript by analyzing 10 regional honey samples indicated by beekeepers as coming from three predominant flower sources – Brazilian peppertree (*Schinus terebinthifolia* Raddi), eucalyptus (*Eucalyptus* L'Hér. spp.) and *velame* (*Croton* L. spp.), the main so-called monofloral honeys produced in the study region. We hypothesize that palynological analysis will reveal a multifloral origin for the Discovery Coast's honeys, due to the diversity of regional flora.

MATERIALS AND METHODS

Study site

The study was carried out in five municipalities along the Discovery Coast in the state of Bahia: Belmonte (BL), Eunápolis (EU), Guaratinga (GU), Itabela (IT), and Porto Seguro (PS). Ten samples of *Apis mellifera* L. 1758 (Hymenoptera: Apidae: Apinae) honey indicated to be from predominant flower sources were obtained from local beekeepers (Table I), between November 2017 and August 2018. In general, beekeepers base this indication on empirical observation of flowering plants in the surroundings of apiaries. This vegetation encompasses backyard areas, monocultures of banana, coffee, eucalyptus, pumpkin and pastures (Bandeira & Novais 2019).

Table I. Municipalities of the Discovery Coast (Bahia, NE Brazil), honey sample codes, geographical coordinates of the apiaries, and predominant flower sources indicated by the beekeepers for each honey sample.

Municipalities, honey sample codes and geographical coordinates	Predominant flower source indicated by beekeepers
Belmonte – BL	
BL1 – 16º05.025' S; 39º14.974' W	eucalyptus (*Eucalyptus* L'Hér. spp., Myrtaceae)
Eunápolis – EU	
EU1 – 16º18.657' S; 39º25.728' W	eucalyptus (*Eucalyptus* spp., Myrtaceae)
Guaratinga – GU GU1 – 16º29.128' S; 39º55.926' W	eucalyptus (*Eucalyptus* spp., Myrtaceae)
GU2 – 16º29.042' S; 39º55.836' W	*velame* (*Croton* L. spp., Euphorbiaceae)
GU3 – 16º29.128' S; 39º55.926' W	*velame* (*Croton* spp., Euphorbiaceae)
GU4, GU5 – 16º29.128' S; 39º55.926' W	*velame* (*Croton* spp., Euphorbiaceae)
Itabela – IT	
IT1 – 16º21.423' S; 39º37.430' W	eucalyptus (*Eucalyptus* spp., Myrtaceae)
IT2 – 16º21.423' S; 39º37.430' W	Brazilian peppertree (*Schinus terebinthifolia* Raddi, Anacardiaceae)
Porto Seguro – PS	
PS1 – 16º23.557' S; 39º06.163' W	eucalyptus (*Eucalyptus* spp., Myrtaceae)

Sample processing

All honey samples were prepared using the acetolysis technique (Erdtman 1960), including the initial dilution of 10 grams of honey in warm distilled water (± 40 °C) and ethyl alcohol (Jones & Bryant 1996). At least four slides per sample were prepared, and the pollen sediment was mounted in glycerin gelatin for the microscopic analysis. The pollen slides are deposited in the pollen library at the Federal University of Southern Bahia – palinoFLORAS –, a pollen reference collection associated with the FLORAS Botanical Garden (Novais et al. 2018), based in Porto Seguro, Bahia.

Palynological analysis

The pollen types found on the honey sediment slides were botanically identified by comparison with the reference slides at palinoFLORAS, as well as through specialized literature, such as Roubik & Moreno (1991), Silva et al. (2016), and Lorente et al. (2017). At least 500 pollen grains were counted per sample (Moar 1985). To classify the abundance of pollen grains in each sample, we adopted the frequency classes established by Louveaux et al. (1978): predominant pollen (>45%), secondary pollen (16%–45%), important minor pollen (3%–15%), and minor pollen (<3%). According to Ramalho et al. (1990), pollen sources exceeding 10% can be considered an attractive floral resource for bees. In this work, we classified

honey samples as predominant flower sources if they presented predominant or secondary pollen types, except when these types were related to polliniferous or anemophilous plants. We avoid classifying honeys as monofloral, given the diverse flora of Southern Bahia, and to prevent misinterpretation.

RESULTS

Pollen spectra of the honey samples

We recognized the botanical affinity of 31 pollen types in the honey samples, distributed across 27 genera and 17 families (Table II). The *Eucalyptus* and *Mimosa pudica* pollen types occurred in most of the samples – except for *Eucalyptus* in GU5 and *M. pudica* in EU1 – and stood out in some honeys from Guaratinga (GU1, GU2 and GU4, *M. pudica*), Itabela and Porto Seguro (IT1, IT2 and PS1, *Eucalyptus*). No predominant pollen was found in the samples from Belmonte (BL1) and Eunápolis (EU1), nor in GU3 and GU5 samples from Guaratinga. Five pollen types were within the secondary pollen class: *Cecropia*, *Eucalyptus*, *M. pudica*, *Myrcia 2* and *Solanum 1*.

Honeys from predominant flower sources

According to the beekeepers, eucalyptus (*Eucalyptus* spp., Myrtaceae) was the predominant flower source of the honey samples from Belmonte. We found no predominant pollen (>45%) in that honey. However, the *Eucalyptus* (38.2%) and *Myrcia 2* (29.6%) pollen types appeared as secondary pollen. The pollen types classified as important minor and minor pollen were *Borreria verticillata*, *Cecropia*, *Cuphea flava*, *Desmodium*, *Euterpe*, *Mikania*, *Mimosa caesalpiniifolia*, *Mimosa pudica*, *Myrcia 1*, *Richardia*, *Simira*, and *Vernonia*. The predominant flower source of *Eucalyptus* sp. (Myrtaceae) and *Myrcia* sp. (Myrtaceae) palynologically characterized the honey sample from Belmonte (Table II).

The honey sample from the municipality of Eunápolis had a predominant eucalyptus flower source previously indicated by the local beekeepers. However, no predominant pollen was found. Two pollen types were categorized as secondary pollen: *Cecropia* (16.8%) and *Eucalyptus* (35.8%). The *Borreria verticillata*, *Bougainvilea*, *Brosimum*, *Desmodium*, *Mikania*, *Myrcia* 1, *Piper*, and *Vernonia* pollen types were classified as important minor and minor pollen. Palynologically, *Eucalyptus* spp. (Myrtaceae) predominated as the main floral source for this honey sample, since *Cecropia* sp. (Urticaceae) is predominantly anemophilous (Table II).

Beekeepers have indicated that honeys from Guaratinga had two predominant flower sources: eucalyptus (*Eucalyptus* spp., Myrtaceae) for the sample GU1, and *velame* (*Croton* spp., Euphorbiaceae) for the samples GU2–GU5. The *Mimosa pudica* pollen type predominated in the samples GU1 (86.6%), GU 2 (50%), and GU4 (48,6%). The samples GU3 and GU5 had no predominant pollen. The *Eucalyptus* pollen type was secondary for the samples GU2 (35.6%) and GU4 (30.7%), similar to the *Mimosa pudica* pollen type for GU3 (21.6%) and GU5 (32%), and to the *Solanum 1* pollen type for GU3 (28.3%) and GU5 (28.3%). Therefore, the samples GU1, GU3 and GU5 had no predominant flower source palynologically indicated. The GU2 and GU4 honeys palynologically revealed a predominant eucalyptus (*Eucalyptus* sp., Myrtaceae) flower source, given the polliniferous characteristic of *Mimosa pudica* L. (Fabaceae) flowers (Table II).

The honey samples from Itabela had a predominant eucalyptus (*Eucalyptus* spp., Myrtaceae) (IT1) and Brazilian peppertree – locally known as *aroeira* – (*Schinus terebinthifolia*, Anacardiaceae) (IT2) flower source previously indicated by beekeepers. The

Table II. Pollen spectrum recognized in honey samples of *Apis mellifera* L. from the municipalities along the Discovery Coast (Bahia, NE Brazil): Belmonte (BL), Eunápolis (EU), Guaratinga (GU), Itabela (IT) and Porto Seguro (PS). Frequency classes according to Louveaux et al. (1978).

Honey sample code and number of pollen types	Predominant flower source according to beekeepers	Predominant pollen class (>45%)	Secondary pollen class (16 – 45%)	Important minor (3 – 15%) and minor pollen (<3%) classes	Predominant flower source palynologically indicated
BL1 (n = 14)	eucalyptus (*Eucalyptus* spp., Myrtaceae)	–	*Eucalyptus* (38,2%) and *Myrcia 2* (29.6%)	*Borreria verticillata*, *Cecropia* (14.8%), *Cuphea flava*, *Desmodium*, *Euterpe*, *Mikania*, *Mimosa caesalpiniifolia*, *M. pudica*, *Myrcia 1*, *Richardia*, *Simira*, and *Vernonia*	*Eucalyptus* sp. and *Myrcia* sp.
EU1 (n = 10)	eucalyptus (*Eucalyptus* spp., Myrtaceae)	–	*Cecropia* (16.8%), *Eucalyptus* (35.8%)	*Borreria verticillata*, *Bougainvillea* (13.6%), *Desmodium*, *Brosimum*, *Mikania*, *Myrcia 1*, *Piper* and *Vernonia*	*Eucalyptus* spp.
GU1 (n = 9)	eucalyptus (*Eucalyptus* spp., Myrtaceae)	*Mimosa pudica* (86.6%)	–	*Attalea*, *Cecropia*, *Elaeis guineensis*, *Eucalyptus*, *Miconia*, *Myrcia 1*, Poaceae type, *Vernonia*	No predominant flower source
GU2 (n = 7)	*velame* (*Croton* spp., Euphorbiaceae)	*Mimosa pudica* (50.0%)	*Eucalyptus* (35.6%)	*Brosimum* (11.4%), *Cecropia*, *Elaeis guineensis*, *Myrcia 1* and *Vernonia*	*Eucalyptus* sp.
GU3 (n = 8)	*velame* (*Croton* spp., Euphorbiaceae)	–	*Mimosa pudica* (21.6%) and *Solanum 1* (28.3%)	*Brosimum*, *Cecropia*, *Eucalyptus*, *Mikania*, Poaceae type (15%) and *Vernonia*	No predominant flower source
GU4 (n = 9)	*velame* (*Croton* spp., Euphorbiaceae)	*Mimosa pudica* (48.6%)	*Eucalyptus* (30.7%)	*Cecropia*, *Cuphea*, Lamiaceae type, *Mimosa caesalpiniifolia*, *Myrcia 1*, *Solanum 1*, and *Vernonia*	*Eucalyptus* sp.
GU5 (n = 5)	*velame* (*Croton* spp., Euphorbiaceae)	–	*Mimosa pudica* (32%) and *Solanum 1* (28.3%)	*Brosimum*, *Cecropia* and Poaceae type (15.2%)	No predominant flower source
IT1 (n = 13)	eucalyptus (*Eucalyptus* spp., Myrtaceae)	*Eucalyptus* (79.8%)	–	*Acacia*, *Borreria verticillata, Cordia*, *Elaeis guineensis*, *Hyptis*, *Mimosa caesalpiniifolia*, *M. pudica* (10.8%), *Mormodica*, *Myrcia 1*, Poaceae type, *Solanum 1* and *Vernonia*	*Eucalyptus* spp.
IT2 (n = 6)	Brazilian peppertree (*Schinus terebinthifolia* Raddi, Anacardiaceae)	*Eucalyptus* (53.6%)	*Mimosa pudica* (38.2%)	*Borreria verticillata, Mimosa caesalpiniifolia*, *Myrcia 3* and *Solanum 1*	*Eucalyptus* spp.
PS1 (n = 10)	eucalyptus (*Eucalyptus* spp., Myrtaceae)	*Eucalyptus* (79.2%)	–	*Borreria verticillata, Brosimum* (12%), *Cecropia*, *Mimosa pudica*, *Pilosocereus*, Poaceae type, *Senegalia*, *Solanum 2* and *Vernonia*	*Eucalyptus* spp.

pollen type *Eucalyptus* predominated in the IT1 (79.8%) and IT2 (53.6%) pollen spectra. The IT2 had the *Mimosa pudica* (38.2%) as secondary pollen. Both honey samples were palynologically classified as having a predominant *Eucalyptus* spp. (Myrtaceae) flower source (Table II).

The beekeepers previously indicated eucalyptus (*Eucalyptus* spp., Myrtaceae) as the predominant flower source for the honey from Porto Seguro. We found *Eucalyptus* (79,2%) pollen type as predominant pollen. Therefore, the predominant *Eucalyptus* spp. (Myrtaceae) flower source palynologically characterizes the sample (Table II).

DISCUSSION

The pollen spectrum of some honey samples from the Discovery Coast of Bahia differs from the predominant flower sources previously indicated by regional beekeepers. The beekeepers' empirical knowledge provides substantial information for understanding the pollen spectra of bee products. However, following the bees foraging around apiaries or observing the flowering plant species in the surroundings – how predominant flower source of honey is commonly indicated by beekeepers – is insufficient to reveal the range of floral sources visited by bees throughout a period. Combining the data provided by beekeepers with those derived from the palynological analysis provides a more reliable identification of the floral source of bee products.

Three predominant flower sources had been indicated by local beekeepers for the honey samples: Brazilian peppertree (*Schinus terebinthifolia*), eucalyptus (*Eucalyptus* spp.) and *velame* (*Croton* spp.). However, only the *Eucalyptus* pollen type figured in the honeys. *S. terebinthifolia*, the Brazilian peppertree – locally known as *aroeira, aroeira-da-praia* and *pimenta-rosa* – is widely distributed in Brazil, in different phytogeographic domains (Flora do Brasil 2020), and commonly found in Brazilian monofloral and plurifloral honeys (Barth 1990, Souza et al. 2019). Similarly, *Croton* was absent in the pollen spectra, although the genus has attractive species for bees (Borges et al. 2014, Santos et al. 2018, Souza et al. 2019).

All pollen types identified in honeys from the Discovery Coast figure in an exhaustive list compiled by Souza et al. (2019). This compendium aggregates pollen types reported for Brazilian bee products by melissopalynological studies. In our analysis, the predominant and secondary pollen types found in the honeys were *Cecropia*, *Eucalyptus*, *Mimosa pudica*, *Myrcia 2*, *Solanum 1*, and *Vernonia*. Among them, the *Eucalyptus* types stand out because eucalyptus plantations prevail in the southern Bahia scenario. In according to Marchini et al. (2003), eucalyptus is one of the best and most abundant nectar suppliers for bees. We obtained the honey samples from municipalities that house eucalyptus plantations for cellulose production. The main company maintains a cooperative agreement with beekeeper associations in the region to provide sites – apicultural pastures – for honey production (Veracel 2019). Therefore, beekeepers already expect eucalyptus as the predominant flower source for honeys in some apicultural seasons.

The occurrence of Myrtaceae pollen grains, especially from *Eucalyptus* spp., in honey samples from *Apis mellifera* bees is well known in Brazil, such as in studies by Aires & Freitas (2001) in Ceará, Bastos et al. (2003) in Minas Gerais, and Borsato et al. (2014) in Paraná. They highlight the nectariferous and polleniferous potential of *Eucalyptus* spp. to compound the honeys produced in those states. Oliveira & Santos (2014) reported that pollen types related

to commercial crops, such as eucalyptus, were found at a high frequency in honeys produced in the extreme south of Bahia. These authors also highlighted the *Myrcia* sp. pollen type as a secondary pollen in other regions of Bahia. Similarly, we noticed that *Myrcia* pollen types are frequent in honeys from the Discovery Coast region, although in a lower percentage than *Eucalyptus*. Species- and even genus-level identification of eucalyptus and other Myrtaceae pollen types is often difficult (Sniderman et al. 2018). As the south of Bahia still lacks a comprehensive floristic list, we identified most of the pollen types at a genus-level.

Pollen types related to mostly polliniferous species, such as *Cecropia*, *Mimosa pudica* and *Solanum 1*, figured as predominant or secondary pollen in seven samples. This is common for Brazilian honeys (Barth 2004, Souza et al. 2019). For example, pollen types associated with *Cecropia* Loefl species are reported for the pollen spectrum of dehydrated pollen (Dórea et al. 2010), propolis (Avelino & Santos 2018), and honey (Moreti et al. 2000, Luz et al. 2007, Sodré et al. 2007, Silva & Santos 2014, Nascimento et al. 2015). *Cecropia* is a typical pioneer tree generally recognized as anemophilous, although it also undergoes entomophily (Monjoin & Wandji 2018). The anemophilous pollen is easily dispersed by the wind, and may occasionally enter into the composition of honey. The *Cecropia* pollen type was also recorded as a predominant pollen and a secondary pollen in honeys from the Southern Coast of Bahia (Oliveira & Santos 2014), and in the Discovery Coast (Bandeira & Novais 2020).

Melissopalynological studies in different countries (Ramírez-Arriaga et al. 2011, Dobre et al. 2013, Ponnuchamy 2014), including Brazil (Barth 2004, Freitas & Novais 2014, Souza et al. 2019), report the pollen type related to *Mimosa pudica* (Fabaceae), an invasive plant species that colonize degraded areas (Queiroz 2009). In Bahia, Fabaceae species contribute significantly to the constitution of honey (Oliveira et al. 2010, Novais et al. 2009, Nascimento et al. 2015, Oliveira & Santos 2014, Bandeira & Novais 2020). Many Fabaceae species have high apicultural potential due to their widespread distribution across many geographical regions, and their supply of floral resources for bees (Carvalho & Marchini 1999, Carvalho et al. 2001, Lorenzon et al. 2003).

Finally, we highlight that the plantation and deforestation corridors on the Bahian Discovery Coast strongly mark the anthropic action in the areas where the honeys come from. This explains why the pollen spectra reveals many pollen types related to cultivated or widely distributed plant species, commonly found in anthropized areas. We confirmed the predominant botanical origin (*Eucalyptus* spp.) indicated by beekeepers for 40% of the honey samples from the Discovery Coast investigated in the present study. Similarly, Horn (1997) and Borges et al. (2014) studied monofloral honeys, and also noticed a discrepancy between the predominant flower source indicated by the seller or beekeeper and that established through pollen analysis. This reinforces how palynological studies can validate and complement the data obtained by beekeepers in the apiary's daily routine.

Acknowledgments

We thank to the beekeepers for providing the honey samples, and the coordinators of the Plant Micromorphology Laboratory at the Universidade Estadual de Feira de Santana (UEFS), especially Dr PP Oliveira, for methodological advices. We are also indebted to the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for a MSc grant to MSFB (BOL2041/2017) and for financial supporting this research project (APP011/2016), and to the Universidade Federal do Sul da Bahia and the Instituto Federal da Bahia (IFBA) for logistical support to field work.

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How to cite

BANDEIRA MSF & NOVAIS JS. 2021. Brazilian peppertree, eucalyptus, and *velame* honeys: does palynology confirm the predominant flower sources indicated by beekeepers?. An Acad Bras Cienc 93: e20200591. DOI 10.1590/0001-3765202120200591.

Manuscript received on April 20, 2020;
accepted for publication on July 21, 2021

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Both authors conceived the main research question, designed the field and laboratorial procedures, read and approved the final text submitted for publication. MSF Bandeira collected and processed the samples, analyzed the data, and wrote the manuscript original draft. JS Novais was the academic advisor and project supervisor, acquired the research funding, supervised data collecting and analysis, revised and edited the final version of the manuscript.