POLLEN AND PHYSICOCHEMICAL ANALYSIS OF Apis AND Tetragonisca (APIDAE) HONEY

Ortrud M. Barth, Alex S. Freitas, Graziela L. Sousa and Ligia B. Almeida-Muradian

SUMMARY

Pollen and physicochemical analyses of honey were carried out in order to distinguish between trophic resources and nutritional preferences of Apis mellifera (honeybees) and Tetragonisca angustula (‘jataí’ bees). Honey samples from both bee species were obtained on the same day in each apiary. Six apiaries, localized in different regions of the State of São Paulo, Brazil, contributed, in different days, to the experiment. No bee species was more generalist than the other. Eucalyptus and Citrus, two introduced plant genera in Brazil, were very attractive to honeybees. The preference of jataí bees was directed to plants of the native flora. The assemblage of plant species visited during the flowering period by Apis and Tetragonisca was not the same. Physicochemical analyses showed differences between all analyzed parameters, except for color determination. Jatal honeys presented higher values of humidity, acidity, diastase, ashes, sucrose and electric conductivity, and Apis honeys of fructose and glucose. No correspondence between physicochemical and monofloral honey properties was observed.

ANÁLISIS FISICOQUÍMICO Y DEL POLEN DE MIELES DE Apis Y Tetragonisca (APIDAE)

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RESUMEN

Se llevaron a cabo análisis fisicoquímicos y del polen de las mieles a fin de distinguir entre los recursos tróficos y las preferencias nutricionales de Apis mellifera (abejas de miel) y Tetragonisca angustula (abejas ‘jataí’). Muestras de miel de ambas abejas fueron obtenidas el mismo día en cada apiario. Seis apiarios localizados en diferentes regiones del estado de Sao Paulo, Brasil, contribuyeron, en días diferentes, al experimento. Ninguna de las especies fue más generalista que la otra. Eucalyptus y Citrus, dos géneros de plantas introducidas al Brasil, resultaron ser muy atractivas para las abejas de miel. La preferencia de las abejas jataí estuvo dirigida a plantas de la flora nativa. El conjunto de especies de plantas visitadas durante el periodo de floración por Apis y Tetragonisca fue diferente. Los análisis fisicoquímicos mostraron diferencias entre todos los parámetros analizados, excepto el color. Las mieles jataí presentaron valores más altos de humedad, acidez, diastasa, cenizas, sacarosa y conductividad eléctrica, y las mieles de Apis valores de fructosa y glucosa. No se observó correspondencia entre las propiedades fisicoquímicas y la miel monofloral.

Introduction

European bees (Apis sp.) were introduced into the southern region of Brazil since 1839 by colonists (Wiese, 1984) intending commercial activities. The native stingless bees have had no value for honey production. At present, the natural bee flora has been drastically reduced and food competition increased between bees, requiring investigation. The former melissopalynological knowledge in Brazil was reviewed by Barth (2004). Trophic preferences of bees and the phytophagous characterization of Brazilian honeys (Luz et al., 2007), royal jelly (Barth, 2005; Morgado and Barth, 2011) were later studied using pollen analysis as well.

Both palynological and physicochemical analyses of pollen loads (Bastos et al., 2004; Almeida-Muradian et al., 2005) and honeys (Barth et al., 2005; Almeida-Muradian et al., 2007; Luz et al., 2007) were applied to honeybee products only. Pollen spectra of stingless bee honeys were presented by several authors (Vit and Ricciardelli D’Albore, 1994; Bazlen, 2000; Carvalho et al., 2001; Alves et al., 2006). Comparative physicochemical analyses between propolis (honeybees) and propolis (stingless bees) sam-

KEYWORDS / Apis mellifera / Honey / Meliponinae / Physicochemical Analysis / Pollen Analysis / Tetragonisca angustula / Trophic Resources /

Received: 05/04/2012. Modified: 04/01/2013. Accepted: 04/08/2013.
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RESUMO
Realizaram-se análises físico-químicas e do pólen dos meios com o fim de distinguir entre os recursos tróficos e as preferências nutricionais de Apis mellifera (abelhas de mel) e Tetragonisca angustula (abelhas ‘jataí amarela’). Mostras de mel de ambas as abelhas foram obtidas no mesmo dia em cada apiário. Seis apiários localizados em diferentes regiões do estado de São Paulo, Brasil, contribuíram, em dias diferentes, ao estudo. Nenhuma das espécies foi mais generalista que a outra. Eucalyptus e Citrus, dois géneros de plantas introduzidas ao Brasil, resultaram ser muito atrativas para as abelhas de mel.

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RESUMO
Realizaram-se análises físico-químicas e do pólen dos meios com o fim de distinguir entre os recursos tróficos e as preferências nutricionais de Apis mellifera (abelhas de mel) e Tetragonisca angustula (abelhas ‘jataí amarela’). Mostras de mel de ambas as abelhas foram obtidas no mesmo dia em cada apiário. Seis apiários localizados em diferentes regiões do estado de São Paulo, Brasil, contribuíram, em dias diferentes, ao estudo. Nenhuma das espécies foi mais generalista que a outra. Eucalyptus e Citrus, dois géneros de plantas introduzidas ao Brasil, resultaram ser muito atrativas para as abelhas de mel.

Material e Métodos

Os recursos tróficos usados por abelhas de mel e abelhas ‘jataí amarela’ foram considerados em relação a pólenes e mel de diferentes regiões. No total, seis apiários localizados em diferentes regiões do estado de São Paulo, Brasil, contribuíram, em dias diferentes, ao estudo. Nenhuma das espécies foi mais generalista que a outra. Eucalyptus e Citrus, dois géneros de plantas introduzidas ao Brasil, resultaram ser muito atrativas para as abelhas de mel.

Análise Físico-química e Pólen do Mel de Apis mellifera e Tetragonisca angustula

Trocic resources used by honeybees and stingless bees were considered in relation to pollen loads also (Carvalho and Marchini, 1999; Souza et al. 2002; Ramalho et al., 2007; Morgado et al., 2011). No available palynological data is known for Brazilian honey samples obtained in a same day from both species of bees living in a same apiary (designated ‘paired honeys’). When honeybee and stingless bee combs were located closely in an apiary, competition of foraging activities must be studied. Considered sometimes as specialists and sometimes as generalists, these bees may have some trophic resources in common. Palynological and chemical analysis of these honeys (Vit et al., 2006) may help to get a better knowledge of the apiflora and, consequently, a better honey quality, income and profit to beekeepers.

Therefore, we analyzed paired honey samples of Apis mellifera and Tetragonisca angustula, and identified the respective plants visited by these bees at the same time in the same locality, using palynological and physicochemical methods, in order to get information about the competition for nectar resources, nutritional preferences and honey quality.

Material and Methods

The paired honey samples proceeded from six distinct localities (Table I) in the State of São Paulo, Brazil: Amparo, Itaberaba, Lins, Marília, Pedreira and Santo Antônio da Posse. The apiaries maintained combs of the honeybee Apis mellifera (Apidae), and the stingless bee Tetragonisca angustula (Meliponinae, ‘jataí’). The honey samples were obtained from both species in each apiary and in the same day by the beekeepers.

Six honey samples from A. mellifera (A) and six from T. angustula (T) were analyzed, starting with 10g of well mixed honey, and using the European palynological methodology (Louveaux et al., 1978) to obtain the honey sediments without the use of acetylation. Available palynological literature (Barth, 1989; Roubik and Moreno, 1991; Moreti et al., 2002) and the pollen slide collection of the Laboratory of Palynology, Federal University of Rio de Janeiro, enabled pollen grain identification.

Standards of Apis mellifera honeys for commercialization used the Normative Instruction 11, of October 20th 2000 (Brazil 2000), based upon the European legislation. It only attends the characteristics of the honeybees and does not include the honey of native stingless bees of Brazil, which presents differences in some physicochemical parameters (Azeredo et al., 2000).

Physicochemical analyses were carried out in the Laboratory of Food Analyses of the Department of Foods and Experimental Nutrition, Pharmaceutical School, University of São Paulo. The quality control of Apis mellifera honeys moisture (%), acidity (mEq·kg⁻¹), ashes (%) and diastase number followed the methodology of the AOAC (1990), Brasil (1981) and CAC (1989). Sugars were analyzed by HPLC: glucose (%), fructose (%) and sucrose (%) following the methodology in Bogdanov et al. (1997); the electric conductivity in uS·cm⁻¹ was determined following the methodology in Stefanini (1984). The color determination (mm Pfund) followed the methodology in Brasil (1981). Standard values were presented (Marchini et al., 2004, 2005; Barth et al., 2005; Almeida-Muradian and Bera, 2008; Souza, 2008).

Results

The data obtained are presented in Table II, comprising 20 pollen types of nectariferous plants, whose frequency is ≥3% of pollen grains counted per sample (sum of grains). Fourteen plant taxa were visited by Apis mellifera and 18 by Tetragonisca angustula. The percentage of unidentified pollen grains remained <3%, except in samples from Mar-
Predominant pollen grains (PP, comprising more than 45% of the nectariferous pollen grains counted) belonged to Eucalyptus in two samples of Apis (Amparo-A and Lins-A) and one of Citrus (Itaberaba-A). Samples of Tetragonisca presented dominant pollen types of Carica papaya (Marília-T), Eupatorium (Posse-T), Melilotus alba (Pedreira-T) and Piptadenia (Itaberaba-T), characterizing monofloral honeys (Figure 1 a-f).

Accessory pollen types (AP, comprising 15 to 45% of the nectariferous pollen grains counted) included pollen types of Arecaceae, Eucalyptus, Myrcia and Schinus in samples of Tetragonisca (Amparo-T, Lins-T), characterizing bifloral honeys when two of these taxa occurred simultaneously. Heterofloral honeys contained in addition accessory pollen grains of Citrus, Eupatorium and Ichthyothere in several samples of Apis.

The isolated pollen taxa (IP, comprising 3 to 15% of the nectariferous pollen grains counted) stood out in addition to pollen types of Alternanthera, Anacardiaceae, Brassica, Caesalpinia, Cassaria, Coffea, Crotalaria, Hovenia/Scutia, Leucena and Persea in several samples of both the bees.

Physicochemical analyses (Table III) of Apis honeys showed moisture variation between 15.40 and 19.00%, acidity from 16.82 to 32.47mEq·kg⁻¹, ashes from 0.11 to 0.26%, diastase from 2.20 to 11.49DN, glycose from 29.49 to 37.45%, fructose from 41.52 to 47.53%, saccharose from below the limit of detection to 2.68%, and electric conductivity from 216.67 to 557.00uS·cm⁻¹. The colors of honey samples varied from extra light to dark ambar.

Physicochemical analyses of Tetragonisca honeys showed moisture variation between 23.40 and 25.60, acidity from 21.65 to 24.00% and electric conductivity from 466.00 to 931.67uS·cm⁻¹. The honey color varied from white to light ambar.

**Discussion**

Samples from the Amparo region. Five pollen types only with frequencies over 3% of all counted nectariferous pollen grains counted were present in the two honey samples. Apis bees remained preferentially on Eucalyptus flowers, and visited in...
addition a wild Asteraceae species like *Eupatorium*, *Tetragonisca* used *Eucalyptus* nectar also, but equally a Myrtaceae species like *Myrcia*, a palm tree and several species of Asteraceae. Therefore, it seems that the nectar resources were not so abundant for jataí preferences. *Eucalyptus* pollen was abundant in all honey samples of *Apis*, but occurred only in the Amparo sample of *Tetragonisca* honey.

*Samples from the Itaberaba region.* Plants represented by nine pollen types were visited significantly. The preference of *Apis* was for *Citrus* nectar. Nevertheless, four other pollen types were found. The sub-representation of orange pollen grains in honeys indicates a higher nectar contribution of this plant than indicated by its pollen grains counted. The jataí bees did not visit *Citrus* flowers, and its preference was of a *Piptadenia* species, besides *Schinus* ('aroehir'), *Brassica* ('mostarda') and a Rhamnaceae species. Therefore, no plant species was visited by these bees in common.

*Samples from the Lins region.* Seven significant pollen types indicated plants visited by the bees. *Apis* remained on *Eucalyptus* flowers. *Tetragonisca* showed preferences mainly of *Schinus* and palms flowers. No trophic similarity between the two bee species was observed. *Samples from the Marília region.* Six pollen types were of

<table>
<thead>
<tr>
<th>Chemical analyses</th>
<th>Amparo A</th>
<th>Amparo T</th>
<th>Itaberaba A</th>
<th>Itaberaba T</th>
<th>Lins A</th>
<th>Lins T</th>
<th>Marília A</th>
<th>Marília T</th>
<th>Pedreira A</th>
<th>Pedreira T</th>
<th>Posse A</th>
<th>Posse T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>17.13 ±0.23</td>
<td>23.40 ±0.23</td>
<td>17.00 ±0.00</td>
<td>24.20 ±0.00</td>
<td>19.00 ±0.00</td>
<td>25.60 ±0.00</td>
<td>17.00 ±0.00</td>
<td>24.80 ±0.00</td>
<td>18.20 ±0.00</td>
<td>23.80 ±0.00</td>
<td>15.40 ±0.00</td>
<td>24.40 ±0.00</td>
</tr>
<tr>
<td>Acidity (mEq/kg)</td>
<td>32.47 ±0.58</td>
<td>32.47 ±0.58</td>
<td>27.57 ±0.17</td>
<td>22.38 ±0.36</td>
<td>27.43 ±1.33</td>
<td>48.13 ±0.45</td>
<td>20.74 ±0.87</td>
<td>21.65 ±0.00</td>
<td>27.85 ±0.11</td>
<td>63.65 ±0.35</td>
<td>16.82 ±0.69</td>
<td>27.32 ±0.29</td>
</tr>
<tr>
<td>Ashes (%)</td>
<td>0.26 ±0.02</td>
<td>0.25 ±0.00</td>
<td>0.11 ±0.01</td>
<td>0.20 ±0.00</td>
<td>0.25 ±0.01</td>
<td>0.17 ±0.01</td>
<td>0.22 ±0.01</td>
<td>0.40 ±0.02</td>
<td>0.18 ±0.00</td>
<td>0.42 ±0.02</td>
<td>0.16 ±0.00</td>
<td>0.23 ±0.01</td>
</tr>
<tr>
<td>Diastase (DN)</td>
<td>9.66 ±0.20</td>
<td>17.19 ±1.56</td>
<td>2.20 ±0.11</td>
<td>22.45 ±0.68</td>
<td>4.40 ±0.19</td>
<td>18.10 ±0.82</td>
<td>11.49 ±0.46</td>
<td>11.01 ±0.21</td>
<td>7.01 ±0.02</td>
<td>18.69 ±0.30</td>
<td>9.16 ±0.29</td>
<td>14.15 ±0.04</td>
</tr>
<tr>
<td>Fructose (%)</td>
<td>43.44 ±1.70</td>
<td>31.19 ±1.58</td>
<td>47.53 ±0.46</td>
<td>33.57 ±0.32</td>
<td>41.52 ±0.08</td>
<td>33.55 ±1.64</td>
<td>42.84 ±0.15</td>
<td>28.95 ±0.46</td>
<td>42.48 ±0.63</td>
<td>28.59 ±0.49</td>
<td>44.95 ±0.54</td>
<td>29.77 ±0.25</td>
</tr>
<tr>
<td>Saccharose (%)</td>
<td>&lt;LD</td>
<td>12.43 ±0.60</td>
<td>1.4 ±0.05</td>
<td>18.20 ±0.25</td>
<td>&lt;LD</td>
<td>8.38 ±0.71</td>
<td>2.67 ±0.24</td>
<td>14.31 ±0.29</td>
<td>0.59 ±0.03</td>
<td>24.00 ±0.27</td>
<td>2.68 ±0.15</td>
<td>18.67 ±0.58</td>
</tr>
<tr>
<td>Electrical conductivity (μS·cm⁻¹)</td>
<td>546.00 ±0.00</td>
<td>569.00 ±0.00</td>
<td>216.67 ±0.58</td>
<td>466.00 ±1.73</td>
<td>557.00 ±0.00</td>
<td>581.33 ±0.58</td>
<td>471.33 ±0.58</td>
<td>923.00 ±0.00</td>
<td>410.00 ±0.00</td>
<td>931.67 ±1.53</td>
<td>298.00 ±0.00</td>
<td>492.33 ±2.89</td>
</tr>
<tr>
<td>Color (mm Pfund)</td>
<td>light ambar</td>
<td>light ambar</td>
<td>ambar</td>
<td>white</td>
<td>light ambar</td>
<td>light ambar</td>
<td>dark ambar</td>
<td>extra light ambar</td>
<td>extra light ambar</td>
<td>light ambar</td>
<td>extra light ambar</td>
<td>light ambar</td>
</tr>
</tbody>
</table>

* Each value represents the mean ±standard diversion of three analyses. <LD: below the limit of detection, considered as zero by the t Student test or its non-parametric equivalent Mann-Whitney.
interest to the bees, but two only were visited by both. From these, *Carica papaya* was dominant for jataí only, while *Apis* visited *Schinus* and *Eucalyptus* flowers. Two pollen types (*C. papaya* and *Myrcia*) were found in both.

**Samples from the Pedreira region.** Eleven pollen types were significant in these honey samples. Nevertheless, *Apis* bees produced a heterofloral honey with a predominant participation of a wild Asteraceae; *Tetragonisca* remained on Fabaceae flowers, like *Melilotus alba*. Two pollen types (*Schinus* and *Eupatorium*) were found in both species.

**Samples from the Santo Antônio da Posse region.** Seven significant pollen types indicated plants visited by the bees, six of them by *Apis*, mainly *Citrus*. This means that flowering of orange shrubs was not satisfactory for honey production, and that wild plant species were visited in addition. *Tetragonisca* visited *Citrus* also, but the wild flowers of *Eupatorium* were of greater interest to them. This observation demonstrates an inversion of the bee trophic preferences.

Taking all honey samples into account, *Apis* produced monofloral honeys of *Eucalyptus* and *Citrus* in three localities (Amparo, Itaberaba, Lins). *Tetragonisca* produced monofloral honeys of *Piptadenia, Carica papaya* pollen type, *Fabaceae* (*Melilotus alba* pollen type) and *Eupatorium* in four localities of the state of São Paulo (Itaberaba, Marilia, Pedreira and Santo Antônio da Posse).

Superposition at the same time of important trophic resources for *Apis* and *Tetragonisa* bees (considering preponderant?? and accessory pollen types) occurred only once in Amparo over *Eucalyptus* trees (Table IV). This behavior may be explained by deficient wild flower nectar resources for the jataí bees, as it was observed by Luz et al. (2007) for honeybees also. *Apis* visited *Eucalyptus* flowers in all the localities studied. When sufficient flowering of *Eucalyptus* occurred in Lins, *Apis* bees remained on this tree, while *Tetragonisca* visited mainly the native flora of *Schinus* and a palm tree (like *Syagrus*, ‘geriva’), showing clearly its trophic preference.

*Tetragonisca* bees showed an affinity to species of the Fabaceae family as observed in samples of Lins and Pedreira. Similar behaviour of *T. angustula* was emphasized by Carvalho et al. (1999).

Honey samples of *Apis mellifera* were distinct in regard to all physicochemical parameters presented when compared with honey samples of *T. angustula*, except for determination of its color in mm Pfund. The physicochemical analyses showed the differences between the honey samples of *Apis* and *Tetragonisca* at each locality. The honeys of *Apis* contained always a higher concentration of glucose and fructose, while the honeys of *Tetragonisca* were the richest of sucrose and presented a high electric conductivity. The humidity, acidity (except the Itaberaba sample), ashes (except Amparo and Lins samples) and diastase number (except Marília sample) presented higher values in the *Tetragonisca* honeys.

Considering the monofloral honeys of *Apis*, the *Citrus* honey sample showed the highest content of fructose and the lowest of ashes. The two monofloral *Eucalyptus* honey samples showed the highest values of ashes and electric conductivity. In relation to the monofloral honeys of *Tetragonisca*, that from *Piptadenia* was the sole white colored honey and presented the highest values of diastase number, glucose and fructose. Honey of the *Carica papaya* pollen type had the lowest acidity value and that of *Melilotus alba* pollen type presented the highest values of ashes, sucrose and electric conductivity. No special data were recorded for *Eupatorium* honey.

Considering all the data obtained using palynological and physicochemical analyses of the paired honey samples of *A. mellifera* and *T. angustula*, the following observations could be established:

1) No bee species was more generalist than the other according to the honey samples analyzed. Both bees produced monofloral, bifloral and heterofloral honeys.

2) *Eucalyptus* and *Citrus*, two introduced plant taxa in Brazil, were very attractive to *Apis* bees, while plant species of the native flora was preferred by jataí bees.

3) The assemblage of nectariferous plant species visited during the flowering period by *Apis* and *Tetragonisca* bees was not the same. Lack of food from native plant species may probably induce the jataí bees to visit introduced plant species.

4) Several pollen types could be determined at the specific, generic or family level. This demonstrated that our knowledge about the main nectar producing api flora needs more and better detailed local investigation. As a rule, the vegetation around an apiary in a radius of 1.5 km must to be very well known.

5) No correspondence between the physicochemical properties and the monofloral pollen spectra of honey samples of *A. mel-
lifera and T. angustula was observed.

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support and scholarship. Thanks are due to Adriana Matuda from Centro Tecnológico de Análise de Alimentos (CETAL) for sugars analysis.

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