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. Jataí 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)

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

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 estudio. 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 jatai 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 jatai 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 phytogeographical characterization of Brazilian honeys (Luz *et al*., 2007), geopropolis (Barth, 2006) and 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 geopropolis (stingless bees) sam-

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

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Ortrud Monika Barth. Ph.D. Researcher. Instituto Oswaldo Cruz (Fiocruz), Brazil. Address: Laboratório de Morfologia e Morfogênese Viral, Pavilhão Hélio e Peggy Pereira, Fiocruz. Avenida Brasil 4365. 21040-900 Rio de Janeiro, RJ, Brazil. e-mail: barth@ioc.fiocruz.br

Alex da Silva de Freitas. Biologist. Collaborator, Universidade Federal Fluminense (UFF), Niteroi, RJ, Brazil.

Graziela L. Sousa. M.Sc. Researcher and Collaborator, Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

Ligia Bicudo Almeida-Muradian. Ph.D. Researcher and Collaborator, Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, USP, São Paulo, SP, Brazil.

ANÁLISE FISICOQUÍMICA E DO PÓLEN DO MEL DE *Apis* Y *Tetragonisca* (APIDAE)

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

RESUMO

Realizaram-se análises físico-químicas e do pólen dos méis 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 gêneros de plantas introduzidas ao Brasil, resultaram ser muito atrativas para as abelhas de mel. A preferência das abelhas jataí esteve orientada a plantas da flora nativa. O conjunto de espécies de plantas visitadas durante o período de floração por Apis e Tetragonisca foi diferente. As análises físico-químicas mostraram diferenças entre todos os parâmetros analisados, exceto a cor. Os méis jataí apresentaram valores mais altos de umidade, acidez, diástase, cinzas, sacarosa e condutividade eléctrica, e os méis de Apis valores de frutose e glucose. Não se observou correspondência entre as propriedades físico-químicas e o mel monofloral.*

ples were presented by Pereira *et al*. (2003).

Trophic 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 and distant 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.

TABLE I
LOCALITIES WHERE HONEY SAMPLES OF *Apis mellifera* AND *Tetragonisca angustula* WERE OBTAINED

Samples / Global position system (GPS)	Localities / beekeepers	Date of collection
Amparo 22°42'04"S 46°45'52"W	Sítio Bela Vista / Amparo-SP / Vicentin	25/08/2006
Itaberaba 23°27'46"S 46°31'58"W	Apiário Capelinha / Estrada de Itaberaba, 7 Guarulhos-SP / Julio de Oliveira	16/04/2007
Lins 21°40'25"S 49°45'23"W	Apacame Lins-SP / Waldemar Monteiro	20/03/2006
Marília 49°56'46"S 23°13'10"W	Marília- BR 153 / Cynthia Anderson de Souza Carvalho Colombo	13/11/2007
Pedreira 22°44'31"S 46°54'05"W	Sítio Caxambu / Pedreira-SP / Luciano Panigassi	06/11/2006
Sto. Antônio de Posse 22°36'22"S 46°55'10"W	Apícola Del Fiore / Marcelo Del Fiore	02/12/2007

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 acetolysis. 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 (Brasil 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 Science School, University of São Paulo. The quality control of *Apis mellifera* honeys moisture (%), acidity ($mEq \cdot kg^{-1}$), 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 $uS \cdot cm^{-1}$ 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 Marí-

TABLE II
RELATIVE FREQUENCY (%) OF NECTARIFEROUS POLLEN TYPES (>3%) RECOGNIZED IN THE HONEY SAMPLES IN THE LOCALITIES OF AMPARO, ITABERABA, LINS, MARÍLIA, PEDREIRA AND SANTO ANTÔNIO DA POSSE, STATE OF SÃO PAULO

Pollen types	Amparo A	Amparo T	Itaberaba A	Itaberaba T	Lins A	Lins T	Marília A	Marília T	Pedreira A	Pedreira T	Posse A	Posse T
Amaranthaceae												
Alternanthera										4.5		
Anacardiaceae				9.9					13.4	4.2	10.3	12.6
Schinus						24.7/AP	33.5/AP					
Arecaceae		4,3	4.0		4.6	27.7/AP			5.9			
∑ Asteraceae		33.3	12.1						5.3	7.2		
Eupatorium	25.2/AP											55.6/PP
Ichthyothere									30.7/AP			
Brassicaceae												
Brassica				4.6				3.9			8.3	
Caesalpiniaceae												
Caesalpinia						7.1						
Caricaceae												
Carica papaya							4.7	76.6/PP				
Fabaceae												
Crotalaria										11.7		
Melilotus alba						15.5				64.8/PP		
Flacourtiaceae												
Casearia						8.4						
Lauraceae												
Persea											6.7	3.6
Mimosaceae												
Leucena											14.7	
Piptadenia				62.8/PP				3.9				
Myrtaceae												
Eucalyptus	64.6/PP	29.3/AP	10.0		92.4/PP		23.7		12.5		11.3	
Myrcia		22.2/AP	4.0				4.7	3.6	13.1			
Rhamnaceae												
Hovenia/Scutia				10.5		9.5			3.7			
Rubiaceae												
Coffea									5.6			
Rutaceae												
Citrus			56.8/PP								26.5/AP	8.7
Not identified	1.4	2.0	3.0	0.5	0.2	0.6	9.7	6.4	1.0	1.6	9.6	11.4
Pollen sum *	365	405	233	1541	419	502	372	423	1255	557	208	437
Nectariferous pollen *	325	351	199	172	394	368	236	415	321	332	204	333

The number of nectariferous pollen grains (100%) was obtained excluding the anemophilous and polliniferous pollen grains from the pollen sum. A: Apis mellifera, T: Tetragonisca angustula, PP: predominant pollen, AP: accessory pollen, * number of counted pollen grains.

lia and Santo Antônio da Posse localities.

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) standed out in addition to pollen types of *Alternanthera*, Anacardiaceae, *Brassica*, *Caesalpinia*, *Casearia*, *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 moistures between 23.40 and 25.60, acidity from 21.65 to 63.85mEq·kg^{-1}, ashes from 0.17 to 0.42%, diastase from 11.01 to 22.45DN, glycose from 20.92 to 25.95%, fructose from 29.77 to 33.57%, saccharose from 8.38 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, represented the nectariferous sources in the two honey samples. *Apis* bees remained preferentially on *Eucalyptus* flowers, and visited in

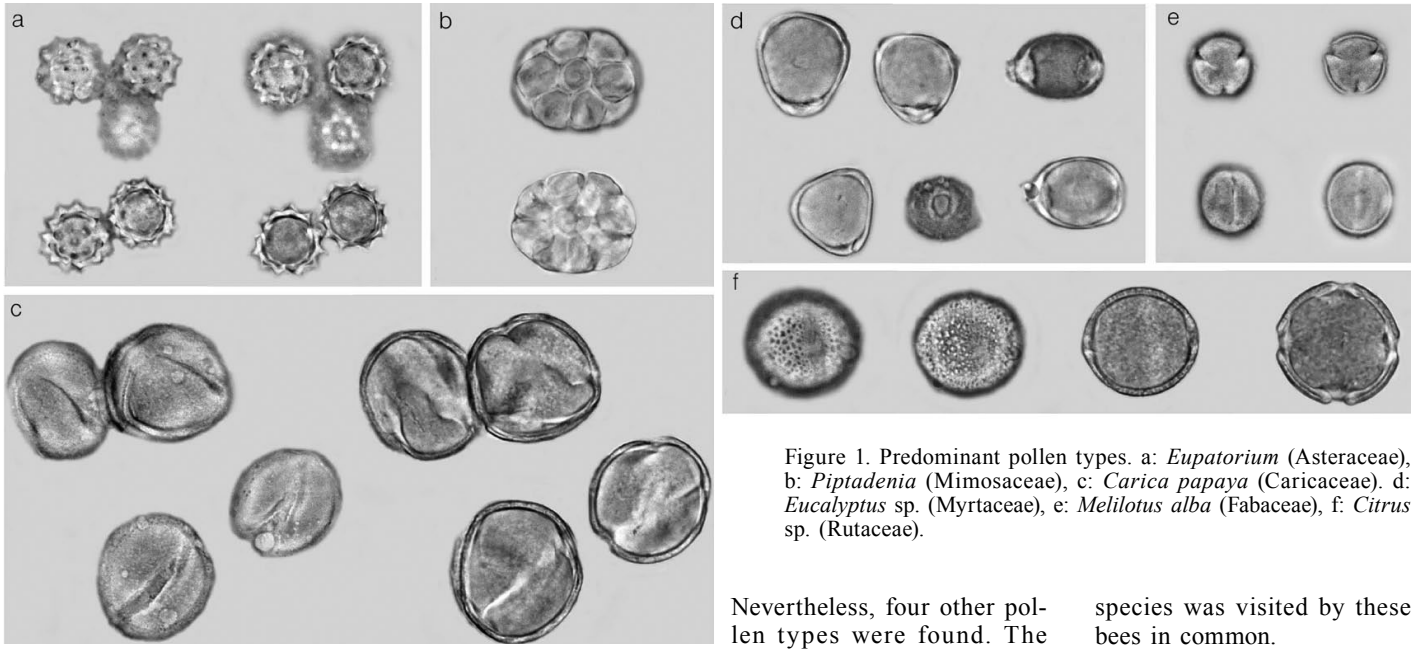


Figure 1. Predominant pollen types. a: *Eupatorium* (Asteraceae), b: *Piptadenia* (Mimosaceae), c: *Carica papaya* (Caricaceae). d: *Eucalyptus* sp. (Myrtaceae), e: *Melilotus alba* (Fabaceae), f: *Citrus* sp. (Rutaceae).

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 visited *Citrus* flowers, and its preference was of a *Piptadenia* species, besides *Schinus* ('aroeira'), *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 palm flowers. No trophic similarity between the two bee species was observed.

Samples from the Marília region. Six pollen types were of

TABLE III
PHYSICOCHEMICAL ANALYSES OF HONEYS OF *Apis mellifera* (A) AND *Tetragonisca angustula* (T) IN SIX LOCALITIES OF THE STATE OF SÃO PAULO*

Chemical analyses	Amparo A	Amparo T	Itaberaba A	Itaberaba T	Lins A	Lins T	Marília A	Marília T	Pedreira A	Pedreira T	Posse A	Posse T
Moisture (%)	17.13 ±0.23	23.40 ±0.23	17.00 ±0.00	24.20 ±0.00	19.00 ±0.00	25.60 ±0.00	17.00 ±0.00	24.80 ±0.00	18.20 ±0.00	23.80 ±0.00	15.40 ±0.00	24.40 ±0.00
Acidity (mEq/kg)	32.47 ±0.58	32.47 ±0.58	27.57 ±0.17	22.38 ±0.36	27.43 ±1.33	48.13 ±0.45	20.74 ±0.87	21.65 ±0.00	27.85 ±0.11	63.85 ±0.35	16.82 ±0.69	27.32 ±0.29
Ashes (%)	0.26 ±0.02	0.25 ±0.00	0.11 ±0.01	0.20 ±0.00	0.25 ±0.01	0.17 ±0.01	0.22 ±0.01	0.40 ±0.02	0.18 ±0.00	0.42 ±0.02	0.16 ±0.00	0.23 ±0.01
Diastase (DN)	9.66 ±0.20	17.19 ±1.56	2.20 ±0.11	22.45 ±0.68	4.40 ±0.19	18.10 ±0.82	11.49 ±0.46	11.01 ±0.21	7.01 ±0.02	18.69 ±0.30	9.16 ±0.29	14.15 ±0.04
Glycose (%)	29.49 ±0.60	23.87 ±1.27	33.93 ±0.33	25.95 ±0.10	37.45 ±1.23	22.75 ±0.98	30.67 ±0.46	22.15 ±0.44	29.97 ±0.81	20.92 ±0.68	30.54 ±0.40	21.08 ±0.71
Fructose (%)	43.44 ±1.70	31.19 ±1.58	47.53 ±0.46	33.57 ±0.32	41.52 ±2.08	33.55 ±1.64	42.84 ±0.15	28.95 ±0.46	42.48 ±0.63	28.59 ±0.49	44.95 ±0.54	29.77 ±0.25
Saccharose (%)	<LD	12.43 ±0.60	1.4 ±0.05	18.20 ±0.25	<LD	8.38 ±0.71	2.67 ±0.24	14.31 ±0.29	0.59 ±0.03	24.00 ±0.27	2.68 ±0.15	18.67 ±0.58
Electrical conductivity ($uS \cdot cm^{-1}$)	546.00 ±0.00	569.00 ±0.00	216.67 ±0.58	466.00 ±1.73	557.00 ±0.00	581.33 ±0.58	471.33 ±0.58	923.00 ±0.00	410.00 ±0.00	931.67 ±1.53	298.00 ±0.00	492.33 ±2.89
Color (mm Pfund)	light ambar	light ambar	ambar	white	light ambar	light ambar	dark ambar	extra light ambar	extra light ambar	light ambar	extra light ambar	light ambar

* 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, Marília, Pedreira and Santo Antônio da Posse).

Superposition at the same time of important trophic resources for *Apis* and *Tetragonisca* 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*, 'gerivá'), 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 Marilia 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* honeys 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.

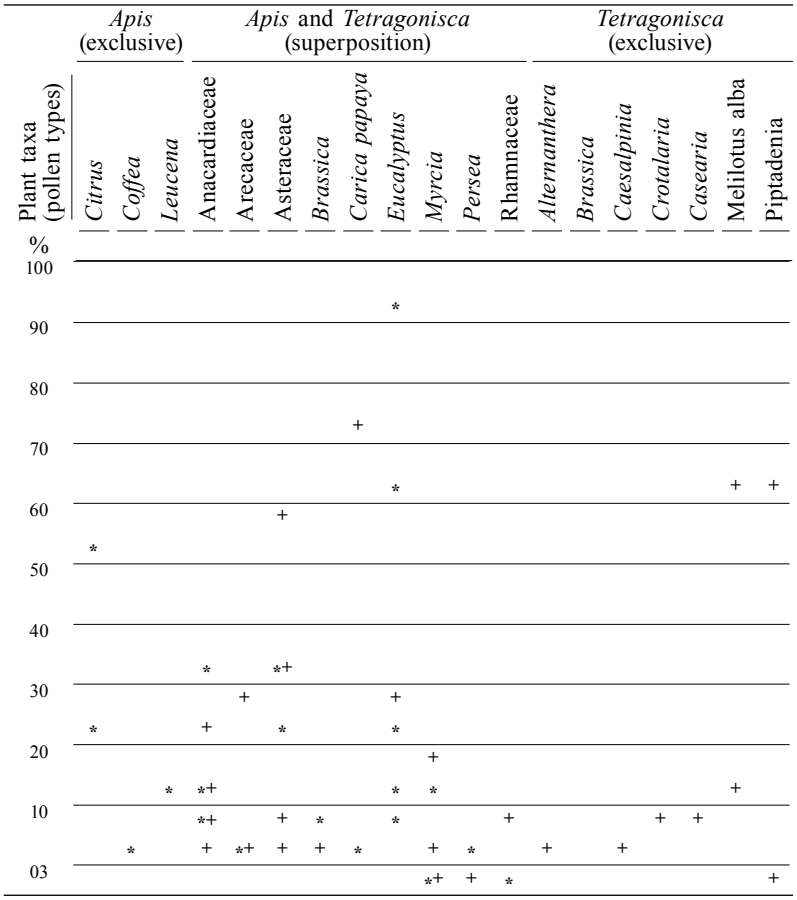
2) *Eucalyptus* and *Citrus*, two introduced plant taxa in Brazil, were very attractive to *Apis* bees, while plant species of the native flora was preferred by jataí bees.

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 apiflora needs more and better detailed local investigation. As a rule, the vegetation around an apiary in a radius of 1,5km must to be very well known.

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

TABLE IV
PAIRED HONEY SAMPLES. TROPHIC RESOURCES USED BY *Apis mellifera* (*) AND *Tetragonisca angustula* (+) BEES‡



‡ Nectariferous plant taxa were identified using pollen analysis, and considered of 100% frequency.

lifera and *T. angustula* was observed.

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