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Pollen analysis of honey samples produced in the counties of Santa Helena and Terra Roxa, Western Region of Paraná, Southern Brazil

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Abstract

Melissopalynology was used to determine the botanical origin of honey, which is important for its traceability. Forty samples of honey from the counties of Santa Helena (20) and Terra Roxa (20), the western region of Paraná, Southern Brazil, were analyzed according to the pollen types and comparisons with pollen catalogs and specialized literature. Regarding analytics quality, 300 grains of pollen per sample were identified. In the samples from Santa Helena, 71 pollen types of 24 families were found, classified as *Hovenia dulcis* monofloral honey (8) and the remainder as polyfloral (12). In the samples from Terra Roxa, 64 pollen types belonging to 29 families were classified as monofloral *Glycine max* (7), *Mimosa scabrella* Benth (2), *Mimosa caesalpiniifolia* Benth (1), *Mimosa verrucosa* Benth (1), *Mikania* (1), and Senecio (1) and as polyfloral (7). The two locations have a high similarity index; however, the predominance of some pollen types indicates the botanical specificity of the locality. For Santa Helena, a greater significance was observed for *H. dulcis* pollen, *Eucalyptus*, *Parapiptadenia rigida*, and *Leucaena leucocephala*; in Terra Roxa, the pollen types *G. max*, *M. scabrella*, and *Eucalyptus* had more incidents. Despite the similarity index, the indicators show differences between the producing areas. The samples of honey from Santa Helena presented higher pollen diversity in relation to the samples of Terra Roxa, reflecting the predominant vegetative cover of riparian forests and agricultural crops, respectively, in each county.

Introduction

Honey production in Brazil has greatly evolved in recent years, with a 91% increase between the years 2000 and 2013 (Paula et al., 2015). According to Abemel (2018), Brazil is currently the ninth largest exporter of honey at approximately 37,000 tonnes. The activity is concentrated mainly in the southern region, with Paraná being one of the largest producers, being the western region responsible for 706 tonnes (IBGE, 2016), concentrating most of the production in the counties along Itaipu Lake, on the Paraná River.

The quality of honey depends on the botanical source foraging by the bees, geographical characteristics of its place of origin, climatic conditions throughout the production process, as well as processing and storage conditions. Since the Brazilian flora is highly diversified due to the country's territorial extension and climatic (edaphoclimatic) variability, it is possible to produce honey all year long with different compositions and characteristics (Marchini et al., 2004).

Some researches aim claims differ in honey according to its geographic origin. Kaškonienė and Venskutonis (2010) concluded that it is rather difficult to find reliable chemical markers for the discrimination of collected honey from different floral sources, because the chemical composition of honey also depends on several other factors, such as geographic origin, collection season, mode of storage and bee species.

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In the state of Paraná, Brazil, De Andrade et al. (2014) evaluated the relationship between geographical origin and contents of Pb, Cd, and Cr in honey samples, with a chemometric approach, associated with environmental pollution. Kuchla et al. (2015) classified samples of wild honey, by Principal Component Analysis (PCA). The parameters pH, electrical conductivity and HMF were determinant for the identification of the geographic origin, on Central South, Southeast and West mesoregions. The results were attributed to the differences between the producing regions.

Bees collecting nectar to produce honey are contaminated with pollen grains, which are transferred to this product and identified through melissopalynology, indicating the botanical and geographical origin of the honey and allowing greater traceability of the product (Fagúndez et al., 2015).

In the Western region of Paraná, studies carried out with physical-chemical and pollen analyses of honey by Sekine et al. (2013), Camargo et al. (2014), Moraes et al. (2014) and Sekine et al. (2019) have characterized the region's honey. For the implementation of the Geographic Information System (GIS) by Camargo et al. (2014), 383 apiaries of 126 beekeepers were mapped in eight counties of the micro-region of Toledo, characterizing the activity composed of small, medium, and large producers (46%, 29%, and 25%, respectively), with a production representativity of 9%, 20%, and 71%, respectively.

This region presents peculiar edaphoclimatic and botanical characteristics, due to the construction of the Itaipu Binational Hydroelectric Power Plant, which caused a large area of flooding, covering 18 counties (Ostrovski, 2014). This area, called Paraná Hydrographic Basin 3 (BP3), is part of Cultivating Good Water Programme, which, so far, has promoted the planting of 44 million native and exotic seedlings; recovered Permanent Preservation Areas (Itaipu, 2017).

Since 2006, the State University of the West of Paraná (UNIOESTE) has been conducting research with characterization of the honeys of the region, in partnership with COOFAMEL - Solidarity Cooperative of Beekeepers of the West of Paraná, which collaborated for the right to use of the Indication of Provenance Seal, by the small beekeepers of the region (INPI, 2017), adding value to the honey produced by them. The demand of producers now, is for obtaining a seal of Denomination of Origin for the product, which will differentiate it by its intrinsic characteristics, related to the phytogeographic characteristics of its region of origin.

Considering that the Western region of Paraná has specific phytogeographic characteristics and representative honey production, this research aimed the melissopalynological characterization and calculation of similarity among *Apis mellifera* L., 1758 honey samples from counties of Santa Helena and Terra Roxa, located in this same region.

Material and Methods

We analyzed 40 samples of *A. mellifera* honey from the 2008/2009 harvest, provided by beekeepers associated with the Beekeeping Cooperative of the West of Paraná (COOFAMEL), including 20 samples from Santa Helena and 20 samples from Terra Roxa.

Both studied counties are part of the Paraná basin, located in the Western mesoregion of Paraná, between latitudes 24º 01' S and 25º 35' S and longitudes 53º 26' W and 54º 37' W (Fig 1). The Paraná River runs through the damming region of the Itaipu hydroelectric power plant. A reforestation

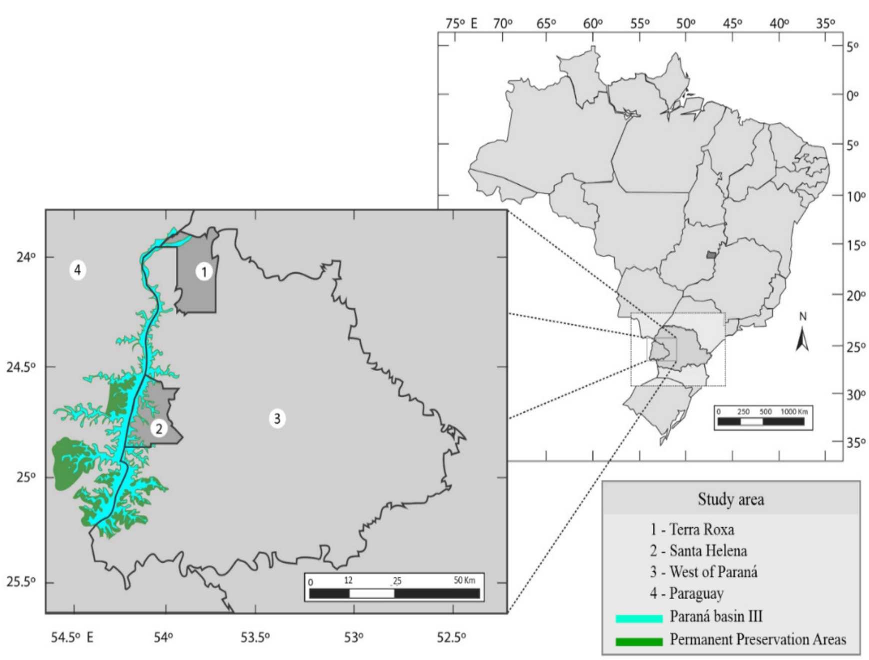

Fig 1. Location map showing the study area. (right) map showing the position of Paraná State in Brazil, (Left) map showing the sampling counties in the region west of Paraná State, as well as the Paraná River Basin III, which borders Paraguay.

area, along the lake, is called the green belt, due to its 200-m width (Ostrovski, 2014; Itaipu, 2017).

Santa Helena is located on the banks of Itaipu Lake, covers 184 km² of Itaipu Lake area, of which approximately 80 km is riparian forest; it has altitude of 258 m, latitude 24º 51' 37'' S, longitude 54º 19' 58" W-GR, and total area of 754.7 km².

The county of Terra Roxa is located in a straight line, 18.27 km from Itaipu Lake, with an altitude of 260 m and a total area of 845.38 km² (latitude 24° 09' 40'' S and longitude 54° 06' 30" W-GR). The county does not border Itaipu Lake, so the vegetation characteristics are distinct in relation to Santa Helena. The county is characterized by agriculture centered on the wheat and soybean, highly mechanized and with use of inputs (Willers et al., 2010).

According to Köppen (1948), the climate of the region is classified as Cfa: humid subtropical mesothermic, hot summers with a tendency for rainfall concentration, winter with infrequent frost (average temperature below 18 °C), and no defined dry season. The average annual temperature is approximately 22 °C, with the highest monthly average values concentrated in January and February at around 28 °C. The colder periods are concentrated in the months of June, July, and August, with average monthly minimum temperatures around 18 °C.

The average annual rainfall is 1.700 mm, with the lowest values in June, July, and August, with an average of 287 mm. The rainy months are December, January, and February, with average precipitation of 450 mm. The soil from the region is composed of eutrophic purple latosol (45%), eutrophic structured purple earth (45%), and eutrophic lithographic solos soils (10%) (Embrapa, 2006).

Melissopalynological analyses

For the identification and quantification of the pollen grains present in honey samples, we used the methodology of acetolysis (Erdtman, 1960), recommended by the International Commission for the Botanical Determination of Honey (Louveaux et al., 1978), which allows its identification with more precision (Martins et al., 2011; Osterkamp & Jasper, 2013).

To determine the degree of importance of the botanical origin in the honey constitution, we used the classification given by the count of 300 pollen grains per sample (Behm et al., 1996). The pollen types were classified in four classes of frequency (Louveaux et al., 1978, Barth, 1989): dominant pollen (D) (> 45% of the total grains in the sample), accessory pollen (A) (16–45%), important isolated pollen (I) (3–15%), occasional isolated pollen (O) (<3%), and unidentified (NI) pollen. Samples with pollen grain frequencies of a given plant, above 45% in the samples, were considered as monofloral.

The identification of the pollen grains was carried out by consulting the specialized literature and comparing them with pollen collection slides from the Department of Entomology and Acarology of the College of Agriculture Luiz de Queiroz (ESALQ/USP), with the help of Augusta C.C.C. Moreti.

Fig 2. Principal pollen types found in honey samples of *A. mellifera* from Santa Helena and Terra Roxa, PR, 2008/2009 harvest: 1- *Parapiptadenia rigida* (Leguminosae-Caesalpinioideae); 2- *Myrcia* type (Myrtaceace); 3- *Leucaena leucocep*hala (Leguminosae-Caesalpinioideae); 4- *Hovenia dulcis* (Rhamnaceae); 5- *Eucalyptus* (Myrtaceae); 6- *Mimosa verrucosa* (Leguminosae-Caesalpinioideae); 7- *Mimosa scabrella* (Leguminosae-Caesalpinioideae); 8- *Mimosa caesalpiniifolia*; 9- *Glycine max* (Leguminosae); 10- *Mikania* (Asteraceae); 11- *Senecio* (Asteraceae).

Statistical analyses

According to the frequency of the pollen types found in the samples of the two studied localities, Sorensen's similarity coefficient (Legendre & Legendre, 1984) was calculated using the following formula: ISS = 2c / (a + b + c) x 100, where: a) number of species restricted to area a; b) number of species restricted to area b; c) number of species common to areas a and b. This coefficient indicates the pollen types common to both localities.

The mean, expressed as the percentage of pollen types identified in total samples, for each county, was calculated, indicating which are the most representative (Table 3). With the frequencies of the pollen types found in each sample of each county, a bipartite interaction network was created between the pollen types and the honey samples, using the bipartite software package R (2014), observed in Figure 3. This software generated the calculation of the Shannon Specialization Index (H2') according to the following formula:

$$H_2' = \frac{H_{2max} - H_2}{H_{2max} - H_{2min}} H_2' = \frac{H_{2max} - H_2}{H_{2max} - H_{2min}} .$$

The result varies between 0 (minimum specialization or maximum generalization) and 1 (maximum specialization or minimum generalization). The pollen frequency in each sample was used to determine other ecological parameters (Table 4).

The Shannon-Weaver Diversity Index (H'), to determine the diversity in each sample, was obtained by the following formula: $H' = \sum_{i=1}^{s} p_i \ln p_i$,

where H': Shannon–Weaver Diversity Index; *S*: number of species (species richness); and *pi*: ratio species *i*, estimated as ni/N, where in is the number of individuals, N is the total number of individuals (pollen grains), and *ln* is the natural logarithm (Shannon & Weaver, 1949; Song et al., 2012).

Table 1. Pollen types and frequency classes of: Santa Helena honey samples, where: D: dominant pollen (> 45%); A: accessory pollen (15–45%); I: important isolated pollen (3–5%); O: occasional isolated pollen (<3%); NI: not identified.

Pollen types	Honey sample number from Santa Helena																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Amaranthaceae																				
Alternanthera tenella															O				O	
Amaranthus					O									O	O					
Chamssoa																	O			
Tapirira					O			I		I					O	I				
Manguifera índica									I											
Schinus		I				O			O		O	I		I			O			I
NI															O	I				
Apocynaceae																				
NI					O				O		I									
Araceae																				
NI		O							I	O			O			O		O		
Anthurium															O					
Arecaceae																				
Astrocaryum																O				
Palmae		O	O	O					O	O		O		O	O	I		A	I	O
Ambrosia															O				O	
Bidens		I					O									O	I		I	
Helianthus annuus											O			O						O
Asteraceae																				
Mikania														O						
Pluchea															O					
Senecio	A				O			O	O						A		I			O
Sonchus													O				O			O
Tithonia																	O			
Vernonia															O		I		I	O
Bignoniaceae																				
NI														O						
Bignoniaceae																				
Tabebuia													O							
Boraginaceae																				
Cordia Trichotoma														O						
Brassicaceae																				
Raphanus																			O	
Raphanus sativus					I	O		I	O		O				O	O				O
Caesalpiniaceae																				
NI			O																	
Bauhinia														O						
Delonix regia				I										O				O		O
Peltophoroides														O						
Euphorbiaceae																				
NI									I	I		I								
Alchornea						I				I		I		O						
Croton											O				O				O	
Leguminosae																				
NI 1		O		I	I		A	I					I			O				

Table 1. Pollen types and frequency classes of: Santa Helena honey samples, where: D: dominant pollen (> 45%); A: accessory pollen (15–45%); I: important isolated pollen (3–5%); O: occasional isolated pollen (<3%); NI: not identified. (Continuation)

Pollen types	Honey sample number from Santa Helena																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Leguminosae (Continuation)																				
NI 2															I					O
NI 3															O					
Calopogonium					O															
Desmodium																				I
Glycine max	I	I			A			A	O				O	I	I		O	O		I
Machaerium																			I	
Senna									O											
Stenolobium													I							
Lamiaceae																				
Leonurus																	O			
Leguminosae-Caesalpinioideae																				
Tipo *Acacia*					O		I	O			I		O	I	O	O		I		I
Entada															O				O	
Leucaena.																O	O			
Leucaena leucocephala	I	O	I	O	O	O		O	I	I	O	A	A	I	O	I	A	O	O	
Mimosa caesalpiniifolia	I								O		O			O						
Mimosa scabrella																	O			
Leguminosae-Caesalpinioideae																			I	
Parapiptadenia rigida	A	I	I	I	I	I	I	A	I	O	I	O	O	I	O	I	O	O	I	I
Morus alba					O				O	O				O	O	O				
Malvaceae																				
Malvastrum		O																		
Melastomataceae																				
Miconia																O				O
Tibouchina					I															
Myrtaceae																				
Eucalyptus	O	I	I	O	A	A	A	I	I	I	A	I	I	A	A	O	I	O	I	I
Eucalyptus																	O			
type *Myrcia*		I		A	O	I	I	O	I	O	I	O	I	O	I	O	O	O		O
Oleaceae																				
Ligustrum		O																		
Piperaceae																				
Piper																	O	O		
Polygonaceae																				
Antigonon cordatum																	O			
Polygonum																			O	
Polypodiaceae																				
NI				O																
Rhamnaceae																				
Hovenia dulcis	I	D	D	D	I	D	O	A	A	D	A	A	A	I		D	A	D	A	D
Rutaceae																				
Citrus					O										O					
Zanthoxylum															A	I				
Sapindaceae																				
Paullinia	I																			
Serjania										O							O			
Solanaceae																				
NI					I			I		I	I	I	O			O				
NI	O						O													

In order to verify the degree of uniformity of types pollen, according to the distribution of the number of pollen grains of each pollen type, in each sample, we used the Pielou Evenness Index (J'). Mathematically it is defined as a diversity index, with the following formula: J' = H' (observed)/H' Maximum, where H' Maximum is the maximum possible diversity that can be observed if all species (pollen types) have equal abundance, and H' Maximum = log S, where S = total number of pollen types (richness). The value of J' ranges from 0 to 1, according to the heterogeneous or homogeneous presence of the pollen types (Pielou, 1966; 1975). It would be 1, if the numbers of pollen grains were the same for all pollen types found, and 0 if there was a single dominant pollen type. Data were analyzed using the statistical program *Paleontological Statistics* (PAST), version 1.89b (Hammer et al., 2008).

Results

A total of 69 pollen types belonging to 27 families, and two unidentified ones, were characterized in the 20 samples of *A. mellifera* honey analyzed in Santa Helena. The families with the highest numbers of pollen types represented in the honey samples were Asteraceae (9), Leguminosae (9), and Leguminosae-Caesalpinioideae (8). The predominant pollen types in the samples were *P. rigida* (Leguminosae-Caesalpinioideae) and *Eucalyptus*. (Myrtaceae), which were present in all samples; *H. dulcis* (Rhamnaceae) and *L. leucocephala* (Leguminosae-Caesalpinioideae), present in 19 and 18 samples, respectively; and *Myrcia* (Myrtaceae), found in 17 samples.

Considering the individual samples, *H. dulcis* (Rhamnaceae) appears as the dominant pollen type (D) in 40% (8) of the samples, which were classified as monofloral;

Table 2. Pollen types and frequency classes of: Terra Roxa honey sampless, where: D: dominant pollen (> 45%); A: accessory pollen (15–45%); I: important isolated pollen (3–5%); O: occasional isolated pollen (<3%); NI: not identified.

Pollen types	Honey sample number from Terra Roxa																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Amaranthaceae																				
Alternanthera tenella									O										O	
Amaranthus	O													O					I	O
Anacardiaceae																				
Manguifera indica															O				O	
Schinus										O			O			O				O
Tapirira		O													O					
Apocynaceae																				
NI												O								
Araceae																				
NI												O	O						O	O
Anthurium											O			O					O	
Didymopanax								O												
Palmae	O			O	O	O			I			I		O	A	I	I			I
Syagrus	O																			
Bidens							O							O						
Helianthus annuus	O											O							O	I
Mikania		O			I		O		I	I	I	O	A	D		I	O	O	O	
Senecio	A			O		O	O	I	D	O	I	I	O	O		I			I	I
Sonchus																O			O	
Vernonia														O						
Bignoniaceae																				
NI																	O			
Brassicaceae																				
Raphanus sativus	O												O	I	O				I	I
Caricaceae																				
Carica					I															
Caesalpiniaceae																				
NI											O	O								
Arcoa	O																			
Bauhinia														O						
Delonix regia				O												O				
Euphorbiaceae																				
NI																		O		
Croton								O						O				O		

Table 2. Pollen types and frequency classes of: Terra Roxa honey sampless, where: D: dominant pollen (> 45%); A: accessory pollen (15–45%); I: important isolated pollen (3–5%); O: occasional isolated pollen (<3%); NI: not identified. (Continuation)

Pollen types	Honey sample number from Terra Roxa																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Guttiferae																				
NI									O											
Leguminosae																				
NI																O				
Desmodium																			O	
Glycine max	A	D	O	D	D	D	A	D	I	A	O	D	A	I	A	I	I	O	A	D
Leguminosae-Caesalpiniaideae																				
Tipo Acacia	O			O	O															
Leucaena leucocephala				O							O			I		O		O	O	I
Mimosa caesalpiniifolia		O				O	D				I			O		A			I	
Mimosa scabrella	A	I	D	I	O			A	O	A	I		I	O			A	D		I
Mimosa verrucosa																	D	A		
Inga									O											
Parapiptadenia rigida	O			O	O	O			A	O			O							I
Lamiaceae																				
Yptis											O									O
Malphiguiaceae																				
NI												O								O
Malvaceae																				
Chorisia												O								
Moraceae																				
Morus alba	O										O								O	
Myrtaceae																				
Eucalyptus	I	O	O	O	O	I	I	O	I	I	A	I	I	I	I	A	O	O	I	I
Myrcia	O	I			O					I	O	O	O			O		O		
Oleceae																				
Ligustrum								O		O										
Polygonaceae																				
NI						I														
Antigonon cordatum	O				O					O			O	O					O	
Rhamnaceae																				
Hovenia dulcis											O	O			O					O
Rubiaceae																				
NI							O													
Borreria						O								O	O				O	
Guettarda																		I		
Rutaceae																				
Zanthoxylum												O					O			
Salicaceae																				
Casearia											I	O								
Sapindaceae																				
Serjania											O				O					
Solanaceae																				
NI											I	I		I				O		I
Verbenaceae																				
NI												O								
NI 1																				
NI	O																			
NI 2																				
NI									O											
NI 3																				
NI						I														

the others (60%) were classified as polyfloral. Accessory pollen grains (A) were found for *Senecio* (Asteraceae), *P. rigida* (Leguminosae-Caesalpinioideae), *Myrcia* type (Myrtaceae), *Eucalyptus*. (Myrtaceae), *G. max* (Leguminosae), *L. leucocephala* (Leguminosae-Caesalpinioideae), *Zanthoxylum* (Rutaceae), and *Palmae* type (Arecaceae). There were 92 occurrences classified as important isolated pollen (I), belonging to 31 pollen types, and 147 occurrences of occasional isolated pollen (O), derived from 61 pollen types.

In the 20 samples from Terra Roxa, 57 pollen types belonging to 29 plant families and four unidentified pollen types were found (Table 2). The families Leguminosae-Caesalpinioideae (7), Asteraceae (6), and Leguminosae (5) represented the highest percentage in the samples. The most frequent pollen types were *G. max* (Leguminosae) and *Eucalyptus* (Myrtaceae), which were present in the 20 samples, followed by *Senecio*. (Asteraceae) and *M. scabrella* (Leguminosae-Caesalpinioideae), found in 14 samples, and *Myrcia* type (Myrtaceae) and *Mikania* (Asteraceae) in 13 samples.

Considering the samples individually, *G. max* (Leguminosae) was present as the dominant pollen type (D) in seven samples (35%); *M. scabrella* (Leguminosae-Caesalpinioideae) in two; and *M. caesalpiniifolia*, *Mimosa verrucosa* (Leguminosae-Caesalpinioideae), *Mikania* (Asteraceae), and *Senecio*. (Asteraceae) in one. These samples are classified as monofloral, and the remaining seven are classified as polyfloral. We observed 62 important isolated pollen types (I), belonging to 19 pollen types, and 136 occurrences of isolated pollen (O), derived from 53 pollen types.

The presence of the different pollen grains in each sample from each county and the importance of each botanical species in the honey samples of these counties, can be observed in Figure 3, through the network bipartite interaction between pollen types present in each sample from Santa Helena and Terra Roxa. The percentage of pollen types identified for each county are showed in Table 3.

Table 3. Percentage of the main pollen types occurring in total samples of honey from Santa Helena and Terra Roxa (PR), 2008/2009 harvest.

Pollen Type	**County**	
	Santa Helena (%)	**Terra Roxa (%)**
Hovenia dulcis	38.68	0.10
Eucalyptus	12.55	7.74
Parapiptadenia rigida	8.00	1.54
Leucaena leucocephala	6.99	0.85
Glycine max	4.20	36.55
Mimosa caesalpiniifolia	0.31	5.14
Mikania	0.12	7.04
Mimosa scabrella	0.02	15.46
Others	29.13	25.58

The values of the specialization index (H2') of the two split networks (A and B) were 0.33 and 0.46, respectively. The Sorensen Similarity Coeficient (ISS), calculated among the honey samples of the counties in relation to the present flora, was 87.23%. The richness (S) and diversity (H'), and evenness (J') indexes calculated for the pollen types found in the honey samples of the studied counties can be observed in Table 4.

Table 4. Number or richness (S), and diversity (H '), and evenness (J') indexes of the pollen types found in each honey sample from the counties from Santa Helena and Terra Roxa, PR.

Sample number	**Santa Helena**			**Terra Roxa**		
	(*S*)	(*H'*)	(*J'*)	(*S*)	(*H'*)	(*J'*)
1	9	1.77	0.81	11	1.67	0.69
2	13	1.85	0.72	6	0.61	0.34
3	6	0.83	0.46	3	0.11	0.10
4	9	1.32	0.60	8	0.72	0.34
5	18	2.38	0.82	10	0.95	0.41
6	7	1.02	0.52	9	0.93	0.42
7	8	1.57	0.75	5	1.00	0.62
8	12	2.12	0.85	7	0.89	0.46
9	17	2.41	0.85	10	1.55	0.67
10	11	1.80	0.75	10	1.65	0.71
11	13	1.94	0.75	16	2.25	0.81
12	10	1.67	0.73	17	1.76	0.62
13	12	1.85	0.74	11	1.69	0.70
14	20	2.24	0.75	18	1.97	0.68
15	14	2.01	0.75	9	1.78	0.81
16	13	1.63	0.63	12	1.66	0.72
17	18	1.88	0.65	8	1.26	0.60
18	10	1.33	0.57	11	1.16	0.48
19	15	2.16	0.79	17	2.01	0.71
20	15	1.78	0.65	16	1.95	0.70
Average	12.5	1.78	0.71	10.7	1.38	0.58

Discussion

The present study provides new insights into the pollen composition of honey samples produced by *A. mellifera* bees in the counties of Santa Helena and Terra Roxa, in Western region of Paraná.

The profile of the pollen spectrum determined in the study indicated predominance of the families Asteraceae, Leguminosae, and Leguminosae-Caesalpinioideae, presenting a great diversity of botanical species. Sekine et al. (2013) also observed the predominance of the Asteraceae in honey samples from others counties of the Western region of Parana, as well as of the Myrtaceae, and Solanaceae.

In this study, it was observed that 40% of the Santa Helena honey samples presented *H. dulcis* (Rhamnaceae) as dominant pollen and this pollen type was present in 95% of the samples. In 35% of the Terra Roxa samples *G. max* showed as dominant pollen, and was present in all samples. The pollen type *Eucalyptus* was observed in all samples of both counties (Table 1 and 2). Sekine et al. (2013) observed in the analyzed honey samples from two predominantly agricultural counties, that 47% of the samples present dominant pollen *G. max*, *Eucalyptus*, *Machaerium stipitaum*, *Brassicaceae*, *Melia azedorach* and *P. rigida*. However, more than 50% of the pollen types found were of native species, such as *Schinus terebinthifolius*, *Baccharis*, *P. rigida*, *Hexaclamys edulis*, and *Serjania.*

The use of bipartite matrices (Figure 3) demonstrated the importance of certain pollen types, allowing visualization and evaluation of the distribution of the different species in each honey sample. The results indicated that the type pollen of *H. dulcis*, *Eucalyptus*, *P. rigida*, and *L. leucocephala* were more frequent in the Santa Helena samples, while the Terra

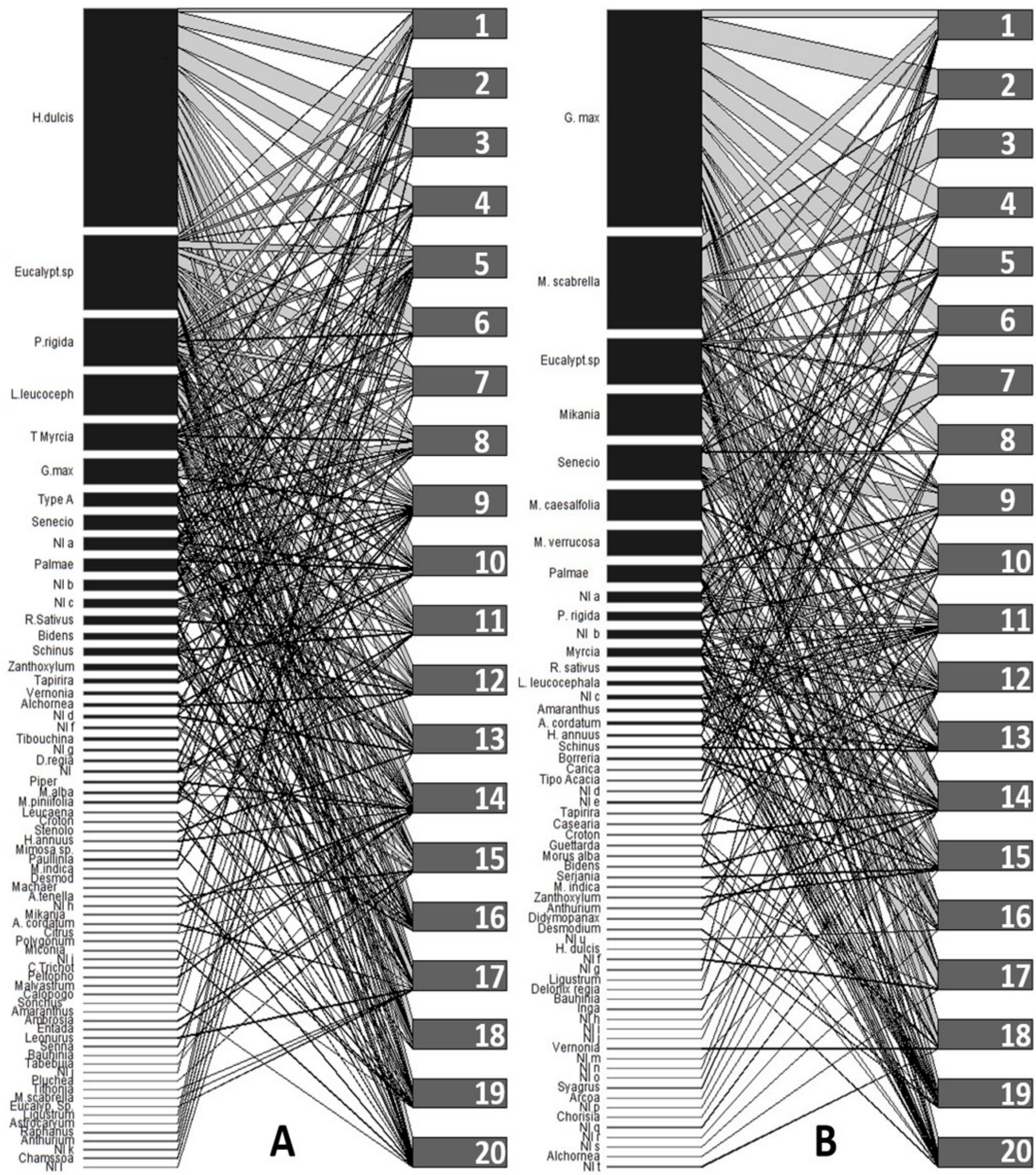

Fig 3. Quantitative bipartite network among pollen types identified in different honey samples. The binary matrix (A) represents the honey samples from Santa Helena and (B) samples of honey collected in Terra Roxa. Each black rectangle represents species of plants (left) and samples of honey from the counties (right). The lines between the columns indicate the number of pollen types in each sample, and the width of each rectangle on the left indicates the pollen frequency in all samples.

Roxa ones showed a higher amount of type pollen of *G. max*, *M. scabrella*, *Eucalyptus*, and *Mikania* (Table 3). Apparently, the matrices behave in equal ways, with the diversity of some botanical species and predominance of others, according to the predominant type of vegetation cover of each study area, riparian forests or agriculture.

Qualitative analyses of the pollen types in the Santa Helena and Terra Roxa honey samples, revealed 69 and 57 pollen types (richness), respectively (Table 1). The presence of a greater number of pollen types in the samples of honey from Santa Helena, confirms a greater diversity of apicultural plants in this county in relation to Terra Roxa (Figure 3).

Ramírez-Arriaga et al. (2011) observed great variation in the diversity of pollen types in samples of honey collected in four districts of the State of Oaxaca, Mexico. Estevinho et al. (2016) carried out an association of multivariate statistical techniques with physico-chemical, melissopalynological and phenolic components analyzes of 112 samples of monofloral honey from *Lavandula* from different regions of Portugal. They concluded that the presence of different pollen types, in small quantities, influenced the amount of phenolic compounds in the samples.

The relatively higher value of the Shannon–Weaver Diversity Index (H': 1.78 and 1.38) of pollen types in Santa Helena samples in relation to Terra Roxa samples (Table 4), respectively, also suggests a great diversity of species used in the reforestation of riparian forests, predominant in Santa Helena. However, the Shannon-Weaver values observed in this study were lower than those presented by Sekine et al. (2013), which evaluated three apiaries (3.89, 3.77 and 3.60), in the counties of Ubiratã and Nova Aurora, in region Northwest and Western of Parana State, which indicates greater diversity of pollen types in these samples (80 types of pollen in honey samples collected monthly), although, only three apiaries were considered.

In the samples from Terra Roxa, the relatively smaller diversity (23%) compared to those of Santa Helena, can be explained by the presence of large areas of agricultural crops, such as soybean, since, according to Newbold et al. (2015), agricultural areas have on average 30% less biodiversity of species. Although soybeans are not a honey plant (Garibaldi et al., 2016), it appeared as dominant pollen in 35% of these samples and were present in 100% from them. This predominance in the honey samples may have occurred due to the lack of other nectar suppliers in the period evaluated in this county.

The relatively lower value of the Shannon Specialization Index (H2': 0.33 and 0.46) also indicated this greater species variety in Santa Helena. This same trend can also be confirmed when considering the proportion of monofloral honey samples (40% for Santa Helena and 60% for Terra Roxa). However, this index also reflecting the peculiar vegetal cover of each county, also observed in Figure 3, that some botanical species were of great importance for the constitution of honey sampled in each county: pollen type *H. dulcis* (grape from Japan) and type pollen *G. max* (soybean), presents (95% and 100%) and dominants pollen (40% and 35%), considered monofloral honey, in the samples of Santa Helena and Terra Roxa, respectively. Sereia et al. (2011) evaluated 11 samples of organic and six nonorganic honey from the region of the state between the states of Mato Grosso, São Paulo and Paraná and classified them as 41.20% of monofloral origin and the remainder polyfloral.

The results of the evenness index (J') also suggest that there is heterogeneity of the floral resources used by the bees in each study area, since they were greater than 0.5. The samples from Terra Roxa presented lower average value of evenness (0.58) in pollen types than those from Santa Helena (0.71), possibly because the plant species with many individuals, such as soy, contributed to the reduction of this value (Table 4). This information corroborating the diversity indexes (H') and authors discussed above.

The relatively high Sorensen Similarity Index (83.7), for the frequency of the pollen types found in the samples of the two studied localities, can be explained by the relative proximity between the counties, about 80.46 km in a straight line. Sekine et al. (2013) recorded 87% similarity from the pollen types present in samples of honey from Ubiratã and Nova Aurora, also in Western Paraná, at 27.74 km distance from each other. These results are similar to those found in the present study. Marchini et al. (2001), studying honey samples from the counties of Piracicaba and Pindamonhangaba, both in the State of São Paulo, at 225.56 km distance in a straight line, found a similarity index among the families of 62.68%.

Another important point to be considered, regarding the similarity observed in the botanical composition between both counties, is that both are part of the reforestation programs of the Paraná River Basin 3. This botanical constitution expresses the phytogeographic characteristics of the region, since, for the restoration of the riparian forest on the banks of Lake Itaipu (Paraná River) and its tributaries, about 44 million seedlings were distributed over several years, creating an ecosystem with peculiar vegetation (Itaipu, 2017), with an abundance of grapes of Japan (*H. dulcis*) and Leucena (*L. leucocephala*).

The presence of these two pollen types and of *P. rigida* (native species), was representative in the samples from Santa Helena, and *M. scabrella* was important pollen types in the samples of Terra Roxa, honey bee species plant widely propagated by beekeepers in region. In relation to Eucalyptus, whose pollen was detected in significant quantities in the honey samples of both counties, it has several species of beekeeping importance, used in the reforestations in the region.

This melissopalynological information confirms ones of Camargo et al. (2014), that found, in floristic survey, greater plant diversity and greater presence of apicultural plants in Santa Helena than in Marechal Cândido Rondon, county with predominance of agriculture. The authors discussed that this greater floristic diversity allowed a greater honey production of the first county, although there was a greater overlap of the georeferenced apiaries areas.

According to Mensah et al. (2017), the bee plant richness in natural forests would produce diverse nutritious resources to bee colonies, at different times of the bees' foraging activities. This trend may also reflect the amount of honey produced by the counties involved in this study, 80,380 kg by Santa Helena and 8,500 kg produced by Terra Roxa (IBGE, 2015).

Sodré et al. (2008) analyzed 35 samples of honey and observed great variability, in county of Picos, in state of Piaui, region Northeast of Brazil, that has stood out in the last decade for the high production of honey. They found 36 types of pollen, distributed in 18 botanical families, and *Piptadenia*, *M. caesalpiniiaefolia*, and *M. verrucosa* (all Leguminosae-Caesalpinioideae) and *Croton urucurana* (Euphorbiaceae) as dominant pollen types.

The information obtained and discussed in this study, referring to pollen specificity and diversity, in the honey of the region, will be important for the conquest of the Denomination of Origin seal of this product by beekeepers, after further research to prove this correlation between the composition of the product and its origin phytogeographic. The importance of some plants for beekeeping in the region was discussed, so that beekeepers can conserve this flora and better plan the implantation of their apiaries.

Author Contributions

All authors were involved in designing and performing the study. All authors except F. J. wrote an initial draft of the manuscript, which D. G. then revised and finalized. F. J. assisted with data analyses and writing of the manuscript.

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