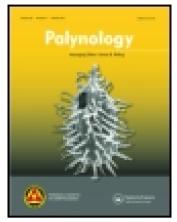
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Palynology

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/tpal20

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Published online: 14 Aug 2015.

To cite this article: Guillermina Fagúndez (2015): Botanical and geographical characterisation of honeys in Diamante, Entre Ríos, Argentina, Palynology, DOI: 10.1080/01916122.2015.1045994

To link to this article: http://dx.doi.org/10.1080/01916122.2015.1045994

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Botanical and geographical characterisation of honeys in Diamante, Entre Ríos, Argentina

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Seventy-five samples of honey produced by honeybees from the Department Diamante (Entre Rios, Argentina) were analysed for pollen content. One hundred and forty-two morphological types were identified, belonging to 62 botanical families. Unifloral honeys were predominant (59%). The main type of honey produced was from *Glycine max* (21%), *Lotus corniculatus* (15%), 'clovers' (11%), *Ammi* spp. (5%), *Melilotus albus, Salix humboldtiana* (3%) and *Medicago sativa* (1%). By means of quantitative analysis, 85% of the honeys were classified as Classes I and II. Foraging behaviour and pollen diversity were analysed in honey samples. Geographical markers are proposed for these honeys. The expected differentiation of samples attributable to sub-environments and harvest dates is discussed.

Keywords: honeybee; nectariferous plants; honey; Argentina; melissopalynology; pollen diversity; geographical markers

1. Introduction

The variability of honey types produced in a region depends on the diversity of the nectar resources available in the area. Argentina, with latitudes ranging from 22 to 55 degrees south, presents a wide biogeographic diversity suitable for apiculture. But not all areas are being fully exploited or studied. Argentinean apiculture is the most important and developed in the southern hemisphere and one of the biggest worldwide. It is the third largest producer in the world with 6% of the total amount harvested, and 70% in South America accounting for 25% of production across the continent (Ferrari et al. 2011). By means of melissopalynologic studies, the types of honeys produced and the resources exploited in a region can be determined. In the Entre Ríos province, these are the Delta del Paraná (Basilio & Romero 1996, 2002; Basilio 1998; Basilio et al. 2010; Caccavari & Fagúndez 2010), the central region (Fagundez & Caccavari 2003b, 2006) and the departments of Colón (Fagúndez et al. 2012), Gualeguaychú and Ibicuy islands (Fagúndez 2010). The Entre Ríos province is the second most important producer in the country (Ferrari et al. 2011), and the Diamante Department is one of the 17 political—administrative divisions of the province. It is situated in the southwest corner of the province, bordering the Paraná River on the west (Figure 1). The total area of the Diamante Department is 253,600 hectares, and it consists of two markedly contrasting sectors with regard to its

physical, botanical and productive characteristics. It consists of an insular area of approximately 45% of the total territory comprising the upper region of the 'Complejo Litoral del Río Paraná' (Aceñolaza et al. 2008) and the remaining mainland (Figure 1). It has a humid subtropical climate with water stress and moderate rainfall (1250–1000 mm per year; average annual temperature 18.4°C).

The aim of the present study was to provide information on the botanical characterisation of Argentinean honeys from the Diamante Department of the Entre Ríos administrative province in order to determine the main types of honeys as well as the resources that are being exploited by *Apis mellifera*. Moreover, this bee's foraging behaviour is analysed, as well as the pollen diversity from honey samples. Geographical markers are proposed for these honeys.

2. Methods

2.1. Vegetation of the study area

Three phytogeographic regions are represented in the Diamante Department: the Pampean Province, the Paranaense Province and Del Espinal Province (Cabrera 1976). The first one is characterised by the predominance of subtropical gramineous plants, among which usually appear subshrubs or small bushes like *Baccharis*, *Eupatorium*, *Heimia* and *Vernonia* genera.

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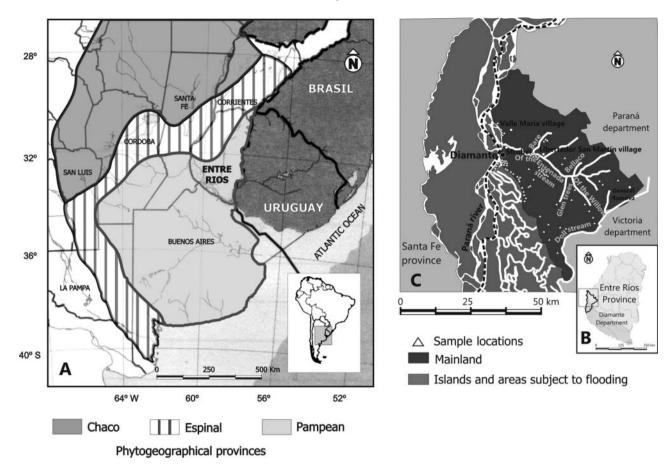


Figure 1. Location of the study area. A. Phytogeographical provinces. B. Location of the Diamante Department in the province administrative of Entre Ríos. C. Sectors of the Diamante Department.

The Paranaense Province consists of narrow galleries along the Paraná River and their tributaries, and it is characterised by hydrophilic species. Along the Paraná River, typical botanical composition can be observed, but human activity has altered or reduced natural vegetation along the streams where high shrubs and herbaceous species are found. Lastly, the Del Espinal Province is characterised by the dominance of semixerophilous arboreal species, in the form of intrusions from the northern area where they are characteristic (Figure 1). The species from these formations are not dominant but are found together with hydrophilous trees, mainly along the roads. On the west side of the Department, the different vegetation units are distributed following the topographic gradient. Many apiaries are located on the western fringe and on the islands due to the existence of natural vegetation which is, in many cases, attractive for honeybees.

2.2. Land usage

The natural vegetation of the Diamante Department has been greatly modified and reduced by agricultural activities, and only some gallery forests along the streams remain (Zamboni 2008). The Diamante Department is one of the most important agricultural areas within the province (considering its mainland surface), but cattle and dairy farming are also present in the area. The most important crops are 'soybean' Glycine max (L.) Merill, 'wheat' Triticum aestivum L., 'corn' Zea mays L. and, on a lesser scale, 'sunflower' Helianthus annuus L., 'sorghum' Sorghum caffrorum (Thunb.) P. Beauv. and 'flax' Linum ussitatisumum L. (Bolsa de Cereales de Entre Ríos 2014). The main foraging species are Medicago sativa L., Melilotus albus Desr., Trifolium repens L. and T. pratense L. Extensive cattle farming and apiculture are the main activities in the island region (Figure 1).

2.3. Honey analyses

The pollen content of 75 honey samples produced by *A. mellifera* was studied. The samples, obtained by centrifugation, were provided by beekeepers between the years 1999 and 2008. Qualitative analysis of the samples was carried out following the method described by Louveaux et al. (1978), slightly modified. A sub-sample of 10 g of honey was dissolved in 100 mL of distilled

water, centrifuged for 10 minutes, washed once with distilled water, centrifuged again and acetolysed. Pollen sediment was mounted in glycerine gelatine or glycerine and sealed with paraffin. The samples were centrifuged at 1000 g (2500 rpm) in a Rolco CM 2036 centrifuge, with a radius from the centre of the rotor to the sample during centrifugations of 15.4 cm (Pendleton 2006). Louveaux's method was followed during the whole sampling period to enable the comparison among results. To determine frequency classes (Louveaux et al. 1978), 1200 pollen grains were counted. Pollen types were identified by comparing them with a reference collection, and specific literature was also consulted. These slides form part of the Laboratory of Palynology of CICyTTP-CONICET/ Modern FCyT-UADER. The pollen types were identified to species whenever possible, and otherwise to genus, tribe or family ranks. Pollen types were classified into five categories: D, predominant pollen (>45%); S, secondary pollen (16–45%); M, important minor pollen (>3-15%); m, minor pollen (>1-<3%) and (+), present pollen (<1%) (Louveaux et al. 1978). When one pollen type represented > 45% of the total number of pollen grains, the sample was classified as a monofloral honey (Louveaux et al. 1978). The samples from Medicago sativa were considered monofloral when the presence of pollen was in a proportion higher than 20% (Maurizio and Louveaux 1961; Serra & Cañas 1988). We considered 'clover' honeys those in which the composition was found to be > 45% pollen of Trifolium spp., Medicago sativa, Melilotus spp. and Lotus spp., according to Resolution 274/95 (SAGPyA 1995).

Quantitative analysis followed Moar's methods (1985) in using tablets of Lycopodium clavatum L. spores (Stockmarr 1971). A separately processed subsample of 10 g of honey was dissolved in distilled water, and two L. clavatum tablets (dissolved in 5 mL of 10% hydrochloric acid) were added, containing approximately 10,679 spores per tablet. The sediment was concentrated by repeated centrifugation at 1000 g (2500 rpm) for 10 minutes, mounted in glycerine gelatin or glycerine and sealed with paraffin. The count of grains of pollen and spores was continued until the predetermined total standard error was <7%, according to the equation established in Stockmarr (1971). Honey was classified according to Maurizio's classes (1939). The honeydew index (HDE/P), the ratio of honeydew elements (HDE) to pollen grains (P) of nectariferous plants, was calculated (Louveaux et al. 1978).

2.4. Statistical analysis

By means of qualitative pollen analysis, it was possible to determine the similarity among the 75 samples. An association matrix was calculated using the Zcekanowsky index. For this analysis, pollen types with values of frequency >5% were considered. A dendogram was designed from the matrix. Samples were analysed using principal components to determine the pollen types that best explain segregation among samples. PC-ORD version 4 statistical software was used.

2.5. Taxonomy

The update of taxa was based on the *Catalogue of Vas*cular Plants of Argentina (Zuloaga et al 1994; Zuloaga & Morrone 1996, 1999). The TROPICOS® data base was used for exotic species.

3. Results

From the total number of samples, 142 pollen types were identified; 89% were entomophilous species and 75% native species. All pollen types found belonged to 62 botanical families (Table 1). Taking into consideration the number of species, the families best represented were Asteraceae and Fabaceae, with 24 and 23 pollen types respectively. Unifloral honeys were predominant (59%; Figure 2). With the exception of Salix humboltiana, taxa that generated monofloral honeys were exclusively exotic. The main type of monofloral honey was from Glycine max (21%), followed by Lotus corniculatus (15%), 'clovers' (11%), Ammi spp. (5%), Melilotus albus and Salix humboldtiana (3%), and Medicago sativa (1%). The most frequent pollen types were Ammi spp., type Baccharis, Brassicaceae, type Eucalyptus, Ervngium spp., G. max, L. corniculatus, and M. albus and they were found in more than 80% of the samples (Figure 3; Table 1). The number of pollen types identified in each sample varied from 19 to 65. The average number of pollen types per sample was 33 ± 10. Considering pollen types per sample, S. humboldtiana (28 \pm 12) and G. max (29 \pm 6) monofloral honeys had the lowest diversity. In honeys of L. corniculatus 32 \pm 13 pollen types, were identified; in M. albus 34 \pm 11; in 'clovers' 35 \pm 10; in Ammi spp. 38 \pm and in M. sativa 50 pollen types., 34 ± 11 in M. albus, 35 ± 10 in 'clovers', 38 ± 9 in *Ammi* spp. and 50 in *M*.

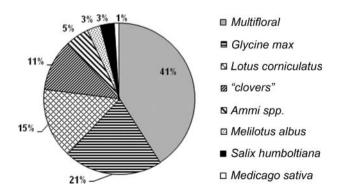


Figure 2. Botanical origin of the honey samples analysed from the Diamante Department (Entre Ríos, Argentina).

Table 1. Frequency distribution classes and frequency of occurrence of pollen types found in the 75 honey samples studied from the Diamante Department.

Botanical families D S M M + FOPollen types Acanthaceae Dicliptera sp.* 4 Alismataceae Echinodorus sp.* 5 Sagittaria montevidensis* 3 5 8 32 64 Alternanthera spp.*a 11 Amaranthaceae 8 1 39 t. Amaranthus*a 2 56 t. Gomphrena perennis*a 9 12 1 25 Anacardiaceae Schinus sp.* 35 Apiaceae Ammi majus 7 31 20 10 93 8 21 19 21 92 Ammi visnaga t. Conium 2 15 23 Eryngium spp.* 4 14 13 33 85 27 Foeniculum vulgare 1 37 Hydrocotyle sp.3 2 14 21 Arecaceae Trithrinax campestris* 6 8 Asteraceae t. Ambrosia*a 36 49 Anthemidae* 20 28 1 Artemisia sp.ª 3 4 2 6 10 31 65 Asterea* 14 21 14 17 t. Baccharis* t. Bidens* 4 7 36 63 8 49 84 Carduus spp. Carthamus lanatus 2 3 Centaurea sp. 11 16 Centaurea chilensis 3 4 Cichorium intybus 2 23 33 Cirsium vulgare 1 24 33 Gaillardia megapotamica* 2 26 37 5 Gymnocoronis spilanthoides* 1 2 Grindelia pulchella* 1 1 17 27 Helianthus annuus 2 23 35 Holocheilus hieracioides* 12 17 39 Mutiseae* 12 21 4 Plagiocheilus tanacetoides* 1 4 2 9 14 19 Senecio sp.* Solidago chilensis* 5 11 13 41 t. Sonchus 5 7 8 18 49 Tessaria integrifolia* Vernonia sp.* 1 1 Berberidaceae Berberis sp.* 1 1 Bignoniaceae Dolichandra cynanchoides* 1 2 4 Jacaranda mimosifolia* 1 1 7 Tabebuia sp.* 1 11 Tecoma stans* 2 3 5 46 75 Boraginaceae Echium plantagineum Borrago officinalis 2 3 Heliotropium sp.* 1 1 8 34 26 91 Brassicaceae Brassicaceae 9 Hydrocleis nymphoides* 2 1 16 Butomaceae 2 Cactaceae Cereus sp.* 3 Caliceraceae Acicarpha tribuloides* 4 18 31 Caparaceae Capparis tweediana* 3 4 Cleome sp.* 1 1 Cariophyllaceae Stellaria sp. 1 1

Table 1. (Continued)

Table 1. (Continueu)								
Botanical families	Pollen types	D	S	M	M	+	FO	
Casuarinaceae	Casuarina cunninghamiana ^a					1	1	
Celastraceae	Maytenus sp.*					2	3	
Celtidaceae	Celtis sp.*a			2	4	26	43	
Convolvulaceae	t. Convolvulus*					3	4	
Cucurbitaceae	Cayaponia sp.*					1	1	
	Cucurbita sp.*			1		7	11	
	Sicyos polyacanthus*				1		1	
Cyperaceae	Cyperaceae*a				1	13	19	
Ephedraceae	Ephedra sp.*a					4	5	
Euphorbiaceae	t. Croton*					8	11	
1	Sapium haematospermum*				2	30	43	
	Sebastiania sp.*				_	1	1	
Gentianaceae	Centaureum pulchellum				1	11	16	
Geraniaceae	Erodium sp.				1	1	1	
Iridaceae	t. Sisyrinchium*				2	9	15	
Lamiaceae				3	5	22	40	
Lamiaceae	Hyptis sp.*			3	3	9		
	t. Cunila *			1		9	12	
	Salvia sp.*			1	2	21	1	
	t. Scutellaria racemosa*			1	2	21	32	
	Teucrium sp.*			1	3	20	32	
Leguminosae	Acacia bonariensis*				3	10	17	
	Acacia caven*					3	4	
	Adesmia sp.*					16	21	
	Albizia inundata*					9	12	
	Bauhinia sp.*					1	1	
	Desmodium sp.*			3	5	19	49	
	Erythrina crista-galli*					9	12	
	Glycine max	16	17	12	14	12	95	
	Lotus corniculatus	11	19	20	9	11	93	
	Medicago lupulina				2	4	8	
	Medicago sativa	1		8	14	34	76	
	Melilotus albus	2	12	19	20	15	91	
	Mimosa pigra *				2	11	17	
	Mimosoideae*					1	1	
	Papilionoideae					2	3	
	Parkinsonia aculeata*					12	16	
	Prosopis sp.*			1	1	18	27	
	Rhynchosia sp.*					1	1	
	Sesbania sp.*					2	3	
	Trifolium sp.				1	20	28	
	Trifolium pratense				9	31	53	
	Trifolium repens			4	5	34	57	
	Vigna sp.*			7	J	2	3	
Lentibulariaceae	Utricularia sp.*					1	1	
	•					1	1	
Lythraceae	Cuphea sp.*						-	
T4h	Lagerstroemia indica					3	4	
Loranthaceae	Ligaria cuneifolia*					1	1	
Malpighiaceae	Heteropteris sp.*					1	1	
Malvaceae	Abutilon sp.*					7	9	
Martiniaceae	Ibicella lutea *					1	1	
Meliaceae	Melia azedarach					1	1	
Menianthaceae	Nymphoides indica*				3	14	23	
Myrtaceae	t. Eucalyptus			3	18	43	85	
Nictaginaceae	Boerhavia diffusa*					2	3	
Oleaceae	Ligustrum spp.			1	2	28	41	

(continued)

Table 1. (Continued)

Botanical families	Pollen types	D	S	M	M	+	FO
Onagraceae	t. Ludwigia peploides*				1	17	24
Papaveraceae	Argemone sp.*a					2	3
Passifloraceae	t. Passiflora coerulea*					1	1
Phytolaccaceae	Phytolacca dioica*			1		10	15
•	Rivina humilis*					1	1
Pinaceae	t. Pinus ^a					1	1
Plantaginaceae	Plantago sp.*a					4	5
Poaceae	Poaceae*a				7	47	72
	Zea mays ^a					16	21
Polygonaceae	t. Polygonum hydropiperoides*			1	5	17	31
	Rupretchia sp.*					1	1
Pontederiaceae	Eichhornia spp.*			3	2	20	33
	Pontederia sp.*		1	2	2	11	21
Ranunculaceae	Clematis sp. *					17	23
Rosaceae	Prunus sp.					5	7
Rubiaceae	Borreria sp.*				2	12	19
Rutaceae	Citrus sp.					3	4
Salicaceae	Salix humboldtiana*	2	7	16	6	28	79
Sapindaceae	t. Serjania*					16	21
	Dodonea viscosa*					1	1
	Pouteria sp.*					3	4
Scrophulariaceae	Agalinis communis*					11	15
	Scoparia montevidensis*					9	12
Simarubaceae	Castella tweedii*				1	18	25
Solanaceae	Cestrum parqui*				1	13	19
	Lycium sp.*					9	12
	Petunia sp.*					2	3
	Solanum glaucophyllum*		1	1	1	7	13
Sterculiaceae	Byttneria sp.*					1	1
Typhaceae	Thypha angustifolia*a					1	1
	Thypha latifolia*a					1	1
Verbenaceae	Phyla sp.*					3	4
	t. Aloysia grattissima*				2	27	39
Vitaceae	Cissus sp.*					2	3
Indeterminate				1		17	24

Frequency classes: D (predominant pollen >45%), S (secondary pollen 16-45%), M (important minor pollen >3-15%), m (minor pollen >1-53%), + (present pollen 51%). The values indicate the numbers of samples in which the pollen type appears in each class. Frequency of occurrence (FO): percentage of samples in which the pollen type appears. t.: pollen type; *native plant; ane-morphilous plant.

sativa honeys. The mean value for multifloral honeys was 35 ± 9 pollen types per sample.

Quantitative analysis of honeys revealed that the 57% of samples corresponded to Class I, 28% to Class II, 12% to Class III and only 3% to Class V (Tables 2 and 3). There are no samples in Class IV. The pollen richness of samples was low; 85% of samples belonged to Classes I and II. *M. sativa*, 'clovers' and *Ammi* spp. honeys showed the lowest pollen content. *M. albus* honey samples belonged to Classes I and II, while *G. max* and *L. corniculatus* honeys corresponded to Classes I to III. *S. humboldtiana* honeys presented the

highest pollen content, corresponding to Class V. Most multifloral honey samples varied from Class I to III, being predominantly the ones with low pollen content. Honeydew indicators were scarce or absent, as the HDE/P ratio was < 1 in all the samples.

By means of cluster analysis, samples were separated into seven groups (Figure 4). Group 1 (n = 3) is composed of honeys with a moderate to high percentage of S. humboldtiana (37-84%); two of the three samples were produced on the island and considered monofloral (a500 and a79), and the remaining one was produced in the riverbank area (a ravine forest on the Paraná River). Their pollen spectrum is represented almost exclusively by aquatic and marshy species, making them completely different to the other groups. Other important pollen types found in these samples were: Eichhornia spp., Sagittaria montevidensis, Solanum glaucophyllum and Tessaria integrifolia. Group 2 (n = 2) includes honeys with moderate contribution of Pontederiaceae (Eichhornia spp. and Pontederia sp.; 27–33%). Their pollen spectrum presented abundant aquatic and marshy species. One of the samples (a92) was from the island and the other (a116) from the riverbank area. Other important pollen types were Eryngium spp. (10 and 24% respectively) and Plagiocheilus tanacetoides Haenke ex DC. (24 and 9%, respectively). Group 3 (n = 24) includes honey with moderate to high values of G. max (12-84%). Other important pollen types were L. corniculatus, M. albus, Solidago chilensis, type Baccharis, Meyen and Astereae. It was possible to identified three subgroups. The lowest one (n = 15), characterised by a greater homogeneity among the samples, consists of monofloral honey samples with the exception of one (a65: 43%). The middle subgroup (n = 4) shows both monofloral and mixed honey samples, with secondary pollen values of type Baccharis (32–44%) and slightly lower L. corniculatus and M. albus (pollen category: important minor pollen). And the top subgroup (n = 5) consists of mixed samples alone with a mean percentage of type Baccharis, S. humboldtiana and Tessaria integrifolia. Samples were obtained from agricultural areas with the exception of T6, 104, T21 and T5 whose apiaries were located in ravine forests. Group 4 (n = 7) contain mixed honeys rich in type Baccharis, (19–43%) and accompanying Asteraceae (Solidago chilensis; type Bidens and Astereae) with lower values. Other species such as Erygium spp., Sagittaria montevidensis and S. humboldtiana were also found with important values. Samples from this group were obtained from ravine forests, with the exception of sample (a501) produced on islands. Group 5 (n = 1) comprises sample a407, mixed honey with 33% of Acicarpha tribuloides Juss. This species is very abundant in sites which have not been ploughed for long periods of time, used for

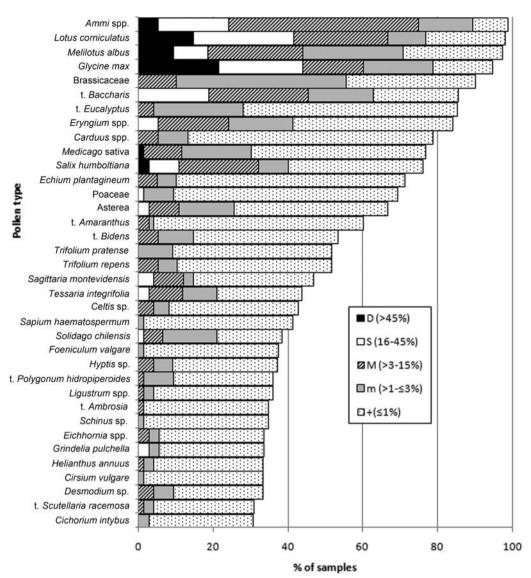


Figure 3. Frequency of occurence and frequency classes (FC) of the main pollen types present in the 75 honey samples analysed. FC: D (predominant pollen >45%), S (secondary pollen 16-45%), M (important minor pollen >3-15%), m (minor pollen >1 $\le 3\%$), + (present pollen $\le 1\%$).

pasture or grazing. Group 6 (n = 28) consists of 'clover' honeys in general as well as monofloral honey from L. corniculatus and M. albus. It is divided into three subgroups; the lower one contained samples with a high percentage of M. albus (26-48%), with the exception of one sample (T16) which has only 3%. Only two of the samples were monofloral (a93, T25). All samples have L. corniculatus pollen, with values ranging from low to medium (4-34%), and *Ammi* spp. (6-38%). The top subgroup presents greater homogeneity among the samples and comprises monofloral honeys from L. corniculatus, with the exception of one sample (a95: 33%). And the intermediate subgroup (n = 10) comprises 'clover' honeys with dominant and variable percentages of L. corniculatus and M. albus; one of the samples is monofloral of L. corniculatus

(a102), and the rest mixed. Group 7 (n = 10) contain samples with moderate to high values of *Ammi* spp. (9-84%). Other accompanying pollens are *S. humboldtiana*, type *Baccharis* and *Eryngium* spp. Four of the samples were from apiaries located in the ravine forests (T20, T4, T2 and T1), the remaining samples were from agricultural and livestock production areas.

The biplot shown in Figure 5 is the result of the analysis of main coordinates. It shows a marked distancing between a499, a500 and a79 samples with a strong presence of *Salix humboldtiana*, and a92 and a116 samples with abundant Pontederiaceae. These results agree with the dendogram. From the second main coordinate, a distancing can be seen between samples from Group 2 of the dendogram (hardly altered riverbank and/or island environments) and

Table 2. Results of quantitative analysis, discriminated for number of sample and botanical origin.

No. pollen Pollen grains + concentration Sample spores (grains/10 g Maurizio's Botanic class origin no. counted honey) T1 386 + 51613,901 Multifloral T2 182 + 7724381 I Ammi spp. T3 220 + 12583250 Ι Multifloral T4 289 + 8686187 Multifloral T T5 296 + 17663115 Multifloral T T6 317 + 10725495 T Glycine max T7 296 + 9595736 I Medicago sativa T8 299 + 14593808 Glycine max T9 1855 + 254135,714 Ш Lotus corniculatus T10 264 + 22272203 Multifloral I T11 289 + 1568Multifloral 3425 I T12 240 + 24081852 Clovers T13 324 + 11635177 I Multifloral T14 266 + 16692962 I Multifloral T15 310 + 6948301 Lotus corniculatus 467 + 456T16 19,031 Clovers T17 462 + 46118,623 Clovers T18 489 + 45719,884 Clovers T19 352 + 51812,628 Clovers T20 419 + 73210,637 Ammi spp. T21 406 + 73710,237 Multifloral T22 289 + 10695024 Clovers T23 282 + 8316306 Glycine max T24 429 + 67511,811 Glycine max T25 357 + 6999491 Melilotus albus Glycine max T26 210 + 14612671 I T27 286 + 12434276 Ι Multifloral A14 1898 + 273149,042 Ш Lotus corniculatus A19 595 + 19306400 I Ammi spp. A44 456 + 55317,616 I Clovers A46 832 + 23027240 Ι Clovers 431 + 9419784 A47 T Lotus corniculatus 1004 + 585Π A48 43,761 Lotus corniculatus A49 2570 + 246221,613 Ш Lotus corniculatus A50 3040 + 353184,775 Ш Lotus corniculatus A51 1416 + 504Π 60,536 Lotus corniculatus 1570 + 335II A52 98,832 Lotus corniculatus A53 804 + 33252,279 Π Glycine max A58 912 + 65829,540 П Multifloral A59 537 + 43126,688 Π Multifloral A60 332 + 57812,331 I Glycine max A61 435 + 25453611 I Multifloral A62 1176 + 73434,284 Π Glycine max 980 + 631A63 33,374 Π Glycine max A64 632 + 39034.542 Π Multifloral 739 + 34745,340 Π Multifloral A65 808 + 362Π 47,657 A66 Lotus corniculatus Π A67 1090 + 35166,320 Glycine max A68 804 + 30356,671 II Glycine max 995 + 34362,005 Π Glycine max A69 Π A70 719 + 32747,029 Glycine max 460 + 52518,701 Multifloral A71 I A76 396 + 59814,263 Multifloral

Table 2. (Continued)

Sample no.	No. pollen grains + spores counted	Pollen concentration (grains/10 g honey)	Maurizio's class	Botanic origin
A77	569 + 581	20,931	II	Multifloral
A78	252 + 1202	4480	I	Multifloral
A79	6099 + 68	3,591,532	V	Salix humboldtiana
A89	1004 + 357	61,457	II	Multifloral
A91	474 + 553	18,459	I	Ammi spp.
A92	446 + 563	16,953	I	Multifloral
A93	1020 + 335	67,398	II	Melilotus albus
A94	410 + 266	103,352	III	Lotus corniculatus
A95	384 + 576	15,866	I	Multifloral
A102	2695 + 558	14,650	I	Lotus corniculatus
A103	612 + 240	239,757	III	Glycine max
A104	324 + 474	27,614	II	Multifloral
A107	331 + 1234	5602	I	Glycine max
A116	410 + 764	9237	I	Multifloral
A229	383 + 467	17,516	II	Multifloral
A407	19852 + 502	19,852	II	Multifloral
A408	255 + 1300	4189	I	Multifloral
A409	272 + 1179	4927	I	Glycine max
A499	1309 + 237	102,638	III	Multifloral
A500	3492 + 214	1,516,164	V	Salix humboldtiana
A501	3494 + 286	227,024	III	Multiflora
A502	1866 + 247	140,388	III	Multiflora

Maurizio's classes: I (<20,000), II (20,000—100,000), III (100,000—500,000), IV (500,000—1,000,000), V (>1,000,000).

those from Groups 3 and 5 (anthropic environments characterised by honeys with high contents of 'soybean' and 'clover' pollen).

4. Discussion

4.1. Resources used

The pollen spectrum of the samples studied shows the diversity of nectar resources exploited by *Apis melli-fera*, highlighting its pollectic behaviour that is

Table 3. Classification of samples for their pollen content, according to Maurizio's classes.

Botanical origin		% samples/classes				
of honey	No. samples	I	II	III	V	
Medicago sativa	1	100				
'Clovers'	8	100				
Ammi spp.	4	100				
Melilotus albus	2	50	50			
Glycine max	16	50	43.75	6.25		
Lotus corniculatus	11	27.3	36.35	36.35		
Salix humboldtiana	2				100	
Multifloral	31	59	28	13		

(continued)

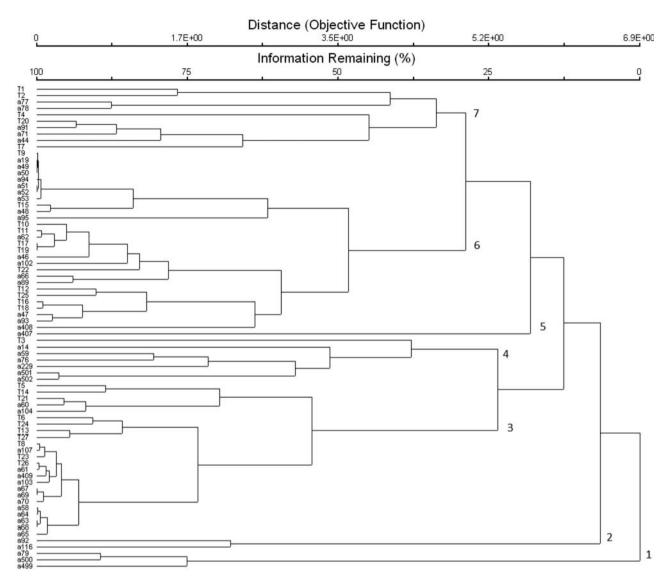


Figure 4. Agglomerative hierarchical dendrogram of the resulting cluster pollen count of the 75 honey samples studied from the Diamante Department.

characteristic of social bees (Ricciardelli D'Albore & Intoppa 2000). Although the diversity of resources exploited is high, it remains limited when compared with the great number available in the area (Fagúndez 2011), in agreement with what has already been expressed by other authors (Andrada et al. 2004).

A. mellifera makes use of a variety of native vegetation which dominates in number of species over the exotic ones. Due to the fact that native species are scarcely represented on the mainland, they are not the most intensively exploited resources. The intensively foraged species are almost exclusively exotic. They are either cultivated plants or widely distributed weeds, mainly herbaceous. This shows the high degree of environmental disturbance on the mainland of this department. But, on the other hand, this highlights the

capacity of *A. mellifera* to take advantage of patches of resources (Eickwort & Ginsberg 1980).

The families best represented in the studied samples (Asteraceae and Fabaceae) are also mentioned in other apiculture regions in the world (Louveaux 1985; Bilisik et al. 2008) as well as in Argentina (Forcone et al. 2005; Fagúndez & Caccavari 2006).

Most monofloral honeys studied are produced from Fabaceae Papilionoideae nectar, herbaceous exotic species known as 'clovers' (30%). Among these species are Lotus corniculatus, Medicago sativa and Melilotus albus, mainly grown to be used as forage for cattle. The last two are adventitious species and widely distributed in the area, especially M. albus. Monofloral honeys from 'soybean', the main crop in the department, are mentioned here for the first time for the country and in

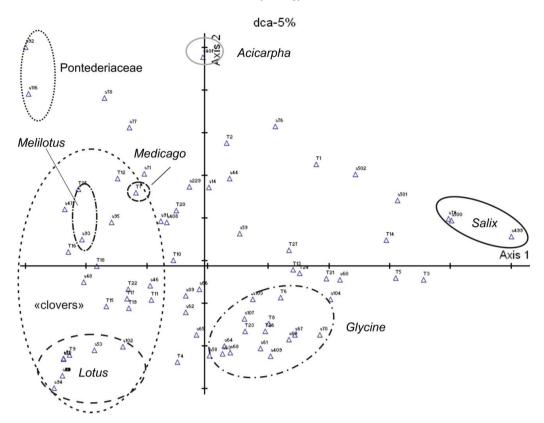


Figure 5. Distribution of the 75 samples of honey from the Diamante Department in terms of the first and second principal components.

the context of this study (Fagundez & Caccavari 2003a, They have also been mentioned other regions of the Entre Ríos province, namely Gualeguaychú (Fagúndez 2010) and Colón (Fagúndez et al. 2012) departments. However, the presence of Glycine is mentioned as a taxon of eventual appearance that characterises the Argentinean honeys associated with clovers (Persano Oddo et al. 2007). Ammi spp. honeys have only been recorded in Argentina in the province of Entre Ríos (Fagúndez & Caccavari 2003b, 2006), and worldwide in Egypt (Ricciardelli D'Albore 1997) and Morocco (Terrab et al. 1998). The spring--summer annual exotic weeds A. visnaga (L.) Lam. and A. majus L. are widely distributed in Diamante Department and are mainly associated with long fallows or abandoned sites.

In the present study, the percentage of monofloral samples was high. The abundance of homogeneous flowerings in this area would allow for the production of monofloral honeys without specific management. Possibly, an adequate productive management focusing on harvesting times and location of apiaries could help to increase the volume of these types of honeys.

Honeys from the island region were poorly addressed, as only four samples were analysed. The study of these honeys must be deepened, considering that monofloral botanical origins from native species typical of these environments could be detected, such as those mentioned by Basilio & Romero (1996, 2002), Basilio (1998) and Caccavari & Fagúndez (2010) for southern sectors of the Paraná Delta region.

4.2. Palynological characterisation of the honeys

The number of pollen types per sample identified in this study were high and only comparable, in Argentina, with that of other honeys produced in the Entre Ríos province (Fagúndez & Caccavari 2006; Caccavari & Fagúndez 2010; Fagúndez et al. 2012). From a botanical point of view, this is a diverse region as it receives flora contributions from all four cardinal points (Ibañez 1962). The Diamante Department, where three phytogeographic provinces converge, also presents a great floristic diversity with tropical or subtropical elements carried by the Paraná River. In accordance with the statement by Louveaux & Vergeron (1964), monofloral honeys presented lower pollen diversity. It is worth noting that honeys with an overrepresented dominant pollen type such as *Salix* sp. and Lotus sp. (Ricciardelli D'Albore 1997) showed lower values than those with an underrepresented pollen type such as *M. sativa* (Ricciardelli D'Albore 1997).

Pollen richness in honey samples studied was low, resulting in 85% of samples being allocated to Classes I and II. Demianowics (1964) has expressed that the highest values of absolute pollen are frequently recorded for plants with small pollen grains. In the present study, results recorded for L. corniculatus and S. humboldtiana support that hypothesis. Ricciardelli D'Albore (1997) mentions *Salix* as a pollen type that shows difficulties in being represented in honeys due to the fact that it is a dioic species. He also indicates that pollen representation will be decreased when female flowers are visited. Nevertheless, due to the small size of its pollen grains, it is considered overrepresented when found in honeys. The value mentioned is > 70%(Ricciardelli D'Albore 1997). On the other hand, it should also be noted that Salix presents mixed pollination or ambophily (Peeters & Totland 1999), i.e. entomophily and anemophily simultaneously. Adaptation to anemophilous pollination would be responsible for the high production of pollen in species from this genus (Faegri & van der Pijl 1979). Regarding Lotus, Ricciardelli D'Albore & Persano Oddo (1981) and Ricciardelli D'Albore (1997) state that percentages higher than 80% and 90%, respectively, must be present to typify honeys of this taxon. In Argentina, studies carried out by Fagúndez & Caccavari (2003b) which include basic sensorial analysis indicate that 45% would not be enough to typify such honeys. Further studies are needed to determine the representativeness of these pollen types in honeys.

4.3. Foraging behaviour of A. mellifera

Considering the species used, it is evident that Apis *mellifera* is a highly polyphagous and generalistic species showing an opportunistic foraging strategy in all habitats, as has already been expressed by many authors (Köppler et al. 2007). The reasons for this are related to the life strategy of this eusocial honeybee whose colonies survive several years. They show highly developed communication skills and sense of direction (Ruttner 1988), which facilitates an effective exploration of food resources during the developing period of the plants, avoiding, therefore, specialising on one pollen or nectar resource. The foraging behaviour of honeybees depends on several factors such as distribution of resources and the presence of other colonies. Generally, they forage within 1 km of the hive (Gary et al. 1977), but they can fly for more than 14 km if necessary (Eckert 1933). Some bees from each colony generally derive nearby floral resources to exploit other plant species (Gary et al. 1977). This increases the diversity of flowers used by a colony and may have evolved as an adaptation to ensure nutritionally adequate pollen and nectar (Eickwort & Ginsberg 1980).

Regarding the density of the resource, *A. mellifera* specialises in large clusters of productive flowers. The large size of its colonies requires an abundant supply of pollen and nectar for their maintenance and growth, encouraging the exploitation of large patches of resources. The ability of honeybees to communicate with nest mates and guide them to the resource promotes this specialisation (Eickwort & Ginsberg 1980). The main resources used in the samples analysed as well as their areal distribution pattern support this theory.

4.4. Differentiation of samples according to sub-environments and production dates

By means of multivariate analysis, it was not possible to arrive at a conclusive differentiation of samples according to origin and/or date of harvest (phenology of species). The current extensive agricultural area of the department minimises the expected differentiation of honeys attributable to different sub-environments within the department. This is because of the superlative way it disturbs the native and/or naturalised vegetation, providing exotic pollen in almost all the evaluated honeys. Samples from the island or riverbank areas (ravine forests) were divided almost exclusively into three groups with little similarity among them (Groups 1, 2 and 4). Group 1 was clearly separated from all others. The determinant of the difference among the three groups was the proportion of each species determined by its phenology. Honeys in Group 1, with a dominance of S. humboldtiana, were produced early in the season (spring), followed by those in Group 2 with dominance of Pontederiaceae, which flowers up to summer and autumn. Group 4, with abundant t. Baccharis, was produced towards the end of the season (late summer to mid autumn).

Samples from the riverbank region were also included in Group 3; they were characterised by the dominance of *Glycine max*. The dominant species in Groups 3 and 4 (*G. max* and type *Baccharis*, respectively) presented simultaneous flowering periods, which explains the greater similarity between them over other groups.

The absence of a more homogeneous group among island or riverbank honeys, and, in turn, different to those of other regions, could be related to agricultural—pastoral areas extending to almost the edge of the ravine, so that the strip of vegetation called 'ravine forest' (riparian areas) does not exceed 100 m in width in many cases. Therefore, different environments (riverbank, island and crops) are found within the foraging radius of apiaries in the area. Additionally, aquatic and marshy species are not exclusive of island and riverbank areas (with the exception of *Solanum*

glaucophyllum) but they are scattered in the department through its extensive hydrographic network.

Considering the strong presence of clovers, it could be said that samples from Group 6 were from apiaries located in livestock production areas. However, the high anthropisation of the department prevents its characterisation by type of region — agricultural or livestock production areas — because parcels intended for one or the other activity are atomised without a geographic pattern, sharing accompanying species.

Lastly, beekeeping management practices also hinder the separation of honeys produced. In the study region, honeys are harvested 2 or 3 times per beekeeping season depending on weather conditions. In the case of three harvests, samples from the first and third (spring and autumn/ or late summer) could differ from the botanical point of view, but not samples from the second as it will have botanical elements from one of the others (Fagundez 2011). Not all beekeepers harvest honey after major flowering, but add honey supers during the beekeeping season, decreasing the number of harvests to be performed in each apiary. Moreover, frames from different periods (blooms) or origins are not always separated by the beekeeper in the extraction rooms, because this does not receive additional payment that justifies it, or they do not have a market request for honeys with certain characteristics.

4.5. Geographical characterisation of honeys

Honeys from the Diamante Department are characterised by the association of pollen from native and exotic species: Ammi spp., Asteraceae Asteroideae, Glycine max, Lotus corniculatus, Melilotus albus, (mainly natives such as type Baccharis, type Bidens, Grindelia pulchella and/or Solidago chilensis Dunal) and Salix humboldtiana. They also present abundant aquatic and marsh taxa such as type Ludwigia peploides (Kunth) Raven, Nymphoides indica (L.) Kuntze, type Polygonum hydropiperoides Eichhornia spp., Pontederia sp., Sagittaria montevidensis, Sapium haematospermum, type Serjania, and Tessaria integrifolia, found on the coast and islands of the Paraná River and a large hydrographic network inland. High values of frequency of occurrence and frequency classes recorded are associated to the areas where these species are found (ravine forests, stream edges and islands). These areas are ideal for the location of apiaries as they provide resources, shade, shelter and water for hives.

Compared with other regions of Entre Rios province, they are characterised by the scarcity of typical arboreal elements of the Nandubay District of the Espinal Phytogeographical Province, such as *Acacia* spp., *Mimosa* spp., *Prosopis* spp., *Schinus* sp., and the absence of others like *Scutia buxifolia* and *Trithrinax*

campestris (Burmeist.) Drude & Griseb. Reissek (Fagúndez & Caccavari 2003b, 2006). The shortage of these arboreal elements is due to the intensive agricultural activity of Diamante Department. This activity is also responsible for the low frequency occurrence of herbaceous species such as *Acicarpha tribuloides* and *Eryngium* spp. These taxa are associated with natural or no-till areas which are well represented in honeys from the central region of Entre Ríos (Fagúndez & Caccavari 2003b, 2006).

Although honeys from both areas (the central region of Entre Rios and Diamante Department) share exotic taxa such as *Ammi* spp., *Glycine max*, *Lotus corniculatus* and *Melilotus albus* with high values of frequency of occurrence, the frequency classes recorded for the last two pollen types are higher in the Diamante honeys.

The presence of 'clovers' is characteristic of the humid Pampas region of Argentina; however, this, together with the shortage of *Echium plantagineum* spp. and *Eucalyptus* L. in the samples analysed in the present study, made it possible to differentiate them. At the same time, the high frequency of occurrence and abundance of *Ammi* spp. are distinguishing features of honeys from Diamante Department and the Entre Ríos mainland (Fagúndez & Caccavari 2003b, 2006). Another important difference is the abundance of native Asteraceae in Entre Ríos honeys, while the ones from the Pampa region are dominated by adventitious exotics of the Cardueae tribe (*Carduus* spp., *Centaurea* spp., *Cirsium* sp.; Tellería 2009).

Honeys from other regions of Espinal share with those of this study taxa such as *Prosopis* sp. and *Schi*nus sp. (Costa de Bringas et al. 1995; Andrada 2001; Naab et al. 2001; Andrada & Tellería 2002; Valle et al. 2007). Nevertheless, the presence of Condalia microphylla Cav., Larrea sp. and Lycium sp., which is not found in the latter, enables the differentiation. To the north of Caldenal (Costa de Bringas et al. 1995), Capparis atamisquea Kuntze and various Lamiaceae and Verbenaceae are present, some of which are also found in Diamante Department (Hyptis sp. and type Scutellaria racemosa). In the central and southern Caldenal, the presence of *Discaria* sp. is important, as are exotic species such as Centaurea spp., Eucalyptus spp. and Helianthus annuus. In the southern Caldenal (Andrada 2001) Prosopidastrum globosum (Gillies ex Hook. & Arn.) Burkart and Vicia sp. are also characteristic. These taxa differentiate honeys produced in subregions of Espinal.

Honeys in the present study share taxa such as *Acacia*, *Mimosa* and *Prosopis* with those produced in northern regions such as the Phytogeographic Chaco Province (Figure 1; Salgado & Pire 1998, 1999; Basilio & Noetinger 2002; Cabrera 2006; Salgado 2006);

however, they differ in the absence of Astronium balansae Engl. and Citrus spp. taxa that are present in the honey produced in the east of the region, corresponding to the administrative province of Corrientes (Salgado & Pire 1998, 1999). Honeys of the present study are further differentiated by the absence of Bulnesia sarmientoi Lorentz ex Griseb., Capparis spp., Cercidium praecox, Schinopsis spp., Zizyphus mistol Griseb. (Ruiz & Pav. ex Hook.) Harms, taxa present in honey produced in the western sector, corresponding to the administrative provinces of Chaco and Formosa (Basilio & Noetinger 2002; Cabrera 2006, 2007; Salgado 2006; Cabrera & Andrada 2009).

5. Conclusions

- (1) In the honeys from the Diamante Department (Entre Ríos, Argentina) I identified 142 pollen types, belonging to 62 botanical families.
- (2) Unifloral honeys were predominant (59%). The main type of honey produced was from *Glycine max* (21%), *Lotus corniculatus* (15%), 'clovers' (11%), *Ammi* spp. (5%), *Melilotus albus* and *Salix humboldtiana* (3% each) and *Medicago sativa* (1%).
- (3) The intensively foraged species are almost exclusively exotic. They are either cultivated plants or widely distributed weeds, mainly herbaceous. This shows the high degree of environmental disturbance on the mainland of this department. But, on the other hand, it highlights the capacity of *A. mellifera* to take advantage of patches of resources.
- (4) The pollen diversity per samples was high, varying from 19 to 65. This is related to the great floristic diversity of the region.
- (5) Pollen richness in honey samples studied was low. Quantitative analysis of honeys revealed that 57% of samples corresponded to Class I, 28% to Class II, 12% to Class III and only 3% to Class V.
- (6) According to the resources exploited in this region, A. mellifera had a highly polyphagous and generalist behaviour showing an opportunistic foraging strategy.
- (7) Multivariate analysis did not allow differentiation of samples according to origin and/or date of harvest. The current extensive agricultural area of the department minimises the expected differentiation of honeys attributable to different sub-environments within the department.
- (8) Honeys from the Diamante Department are characterised by the association of pollen from native and exotic species: *Ammi* spp.,

Asteraceae Asteroideae (mainly natives such as type Baccharis, Solidago chilensis, type Bidens and/or Grindelia pulchella), Glycine max, Lotus corniculatus, Melilotus albus, and Salix humboldtiana. They also present abundant aquatic and marsh taxa such as Eichhornia spp., type Ludwigia peploides, Nymphoides indica, type Polygonum hydropiperoides, Pontederia sp., Sagittaria montevidensis, Sapium haematospermum, type Serjania and Tessaria integrifolia, found on the coast and islands of the Paraná River and a large hydrographic network inland.

Acknowledgements

This study was supported by the National Council of Scientific and Technical Research (CONICET). Special thanks to the beekeepers who provided samples for this study, to Mg. Sc. Diego Blettler for statistical advice and critical reading of the manuscript, to Dr. Pablo Aceñolaza for his assistance in the statistical analysis, and to the referees for their valuable suggestions.

Disclosure statement

No potential conflict of interest was reported by the author.

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