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A melissopalynological map of the south and southwest of the Buenos Aires Province, Argentina

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Abstract

The aim of this work was to produce a melissopalynological map of the south and southwest of the Buenos Aires Province, Argentina, using pollen analysis data pertaining to 127 honey samples from the Pampa, Espinal (the Calden District), and Monte de Llanuras y Mesetas ecoregions, collected over the period 1992-2002. Using principal components and hierarchical cluster analysis, the different districts were grouped into five regions: I (Tres Arroyos, San Cayetano, Coronel Pringles and Coronel Dorrego), II (Guaminí, Saavedra, Coronel Suárez and Adolfo Alsina), III (Coronel Rosales, Monte Hermoso, Bahía Blanca and Villarino), IV (Patagones and Tornquist), and V (Puán). In Regions I, III and IV, 80% of honey samples were monofloral: Region I was characterized by the presence of 50% *Helianthus annuus* honeys and 10% clover honeys, Region III by 65% *Eucalyptus* sp. honeys, and Region IV by 30% *Diplotaxis tenuifolia* honeys. In Regions II and V, 50% of honeys were monofloral. Region II was distinguished by the presence of 50% *H. annuus* honeys, and Region V by 15% *Larrea divaricata* and 15% *Vicia* sp. honeys. The multifloral honeys of Region V included samples containing *Condalia microphylla* pollen. The families Fabaceae and Asteraceae provided the greatest diversity of pollen types. The association of *Eucalyptus* sp., *Centaurea* sp., and *Diplotaxis tenuifolia* characterised the honeys from all five regions. The natural variability of honey samples renders it very difficult to define the boundaries between the different regions.

Additional key words: botanical origin, geographical origin, honey, melissopalynological, pollen analysis.

Resumen

Mapa melitopalinológico del sur y sudoeste de la provincia de Buenos Aires, Argentina

El objetivo de este trabajo fue elaborar un mapa melitopalinológico del sur y sudoeste de la provincia de Buenos Aires, Argentina, con datos de análisis polínicos de 127 muestras de miel provenientes de las eco-regiones Pampa, Distrito del Caldén en el Espinal, y Monte de Llanuras y Mesetas, recolectadas en el período 1992-2002. Utilizando análisis de componentes principales y de cluster, los partidos se agruparon en cinco regiones: I (Tres Arroyos, San Cayetano, Coronel Pringles y Coronel Dorrego), II (Guaminí, Saavedra, Coronel Suárez y Adolfo Alsina), III (Coronel Rosales, Monte Hermoso, Bahía Blanca y Villarino), IV (Patagones y Tornquist) y V (Puán). En las Regiones I, III y IV el 80% de las muestras fueron monoflorales. La Región I se caracterizó por la presencia de un 50% de mieles de *Helianthus annuus* y de un 10% de mieles de trébol; la Región III por 60% de mieles de *Eucalyptus* sp.; y la Región IV por 30% de mieles de *Diplotaxis tenuifolia*. En las Regiones II y V el 50% de las mieles fueron monoflorales: la Región II se distinguió por la presencia de 50% de mieles de *H. annuus* y la Región V por 15% de mieles de *Larrea divaricata* y 15% de mieles de *Vicia* sp. Las mieles multiflorales de la Región V se destacaron por la presencia de pólen de *Condalia microphylla*. La mayor diversidad de tipos polínicos correspondió a las familias Fabaceae y Asteraceae. La asociación de *Eucalyptus* sp., *Centaurea* sp. y *Diplotaxis tenuifolia* caracterizó a las mieles de las cinco regiones. La variabilidad natural de las muestras de miel hace muy difícil definir límites precisos entre las diferentes regiones.

Palabras clave adicionales: análisis polínico, melisopalinología, miel, origen botánico, origen geográfico.

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Introduction

Argentina is one of the world's main suppliers of honey, and in recent years local apicultural activity has grown appreciably. In 2005 the country exported 100,000 tons of this product (SAGPyA, 2006). Honey production in the Buenos Aires Province represents more than 50% of the national output (AACREA, 2003). Only about 8% of Argentinean honey is consumed domestically; most is exported in bulk.

The growing demand for «guarantee of origin» honey on the international market, and the interest in improving the profitability of beekeeping, have led to increased melissopalynological research in the Buenos Aires region (Valle *et al*., 1995, 2000, 2001, 2004; Andrada *et al*., 1998a,b, 1999; Gallez *et al*., 2005). However, to date, no Argentinean melissopalynological maps have been available; only a few studies on apibotanical resources in the Pampa (Tellería, 1993), Delta e Islas del Paraná (Gurini and Basilio, 1995), Espinal (Andrada, 2001) and Monte de Llanuras y Mesetas ecoregions (Forcone, 2003a, b) have been performed.

The aim of this work was to construct a melissopalynological map of the south and southwest of the Buenos Aires Province, and to establish the geographical distribution of honeys according to their botanical origin.

Material and Methods

Characteristics of the study area

The study area, the south and southwest of the Buenos Aires Province, includes the fifteen districts shown in Figure 1. The area is located in the south of the Pampa and Espinal ecoregions and includes a small area of the Monte de Llanuras y Mesetas ecoregion (Burkart *et al*., 1999). This area has a temperate cold, dry climate; snow occasionally falls in the hills. The mean annual temperature is 14ºC. The mean annual rainfall varies from 300 to 750 mm, with precipitations decreasing from the northeast towards the southwest (Cabrera, 1976).

The flat landscape of the Pampa ecoregion is only broken by the Ventania System which runs through the Puán, Tornquist, Saavedra, Coronel Pringles and Coronel Suárez districts. The predominant vegetation consists of a herbaceous stratum; the most representative family is Poaceae with several species of *Stipa* L. and *Nassella* E. Desv. (Barkworth). Shrub communities of *Discaria americana* Gillies and Hook., *Prosopidastrum globosum* (Gillies ex Hook. & Arn.) Burkart, *Geoffroea decorticans* (Gill.) Burkart, *Condalia microphylla* Cav., *Schinus fasciculata* Cav., and *Lycium chilense* Miers ex Bertero are common. Halophyllous and psammophyllous communities are also present (Cabrera, 1976; Verettoni and Aramayo, 1976; Cabrera and Zardini, 1978; Lamberto *et al*., 1997). The south of the Pampa ecoregion also includes the San Cayetano, Monte Hermoso and Tres Arroyos districts, plus the north of the Puán, Tornquist, Bahía Blanca and Coronel Rosales districts. The Espinal ecoregion in Buenos Aires Province, includes the Patagones and Villarino districts, plus the south of the Puán, Tornquist, Bahía Blanca and Coronel Rosales districts. This region contains isolated specimens of *Prosopis caldenia* Burkart and *Prosopis flexuosa* AD. (left over from woodlands destroyed by anthropic action), and a herbaceous stratum rich in Poaceae. There are also dunes with psammophyllous vegetation and saline soils with halophyllous heathland and steppes.

The Monte de Llanuras y Mesetas ecoregion in the Buenos Aires Province includes a small part of the south of the Patagones district. The natural vegetation here is shrub steppe; the predominant species are *Larrea* sp., *Disacaria americana* and *Chuquiraga* sp.

The natural vegetation of the study area has been disturbed by agricultural activities. The most important crops are wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.) and sorghum (*Sorghum bicolor* (L.) Moench. There are also horticultural crops along the lower valley of the Colorado River in the Villarino and Patagones districts. The most widespread melliferous weeds are *Carduus* sp. (*C. thoermeri* Weinm, *C. tenuiflorus* Curtis), *Carthamus lanatus* L., *Cirsium vulgare* (Savi) Ten., *Cynara cardunculus* L., *Onopordon acanthium* L., *Centaurea calcitrapa* L., *C. diffusa* Lam., *C. iberica* Trev., *C. solstitialis* L. and *Diplotaxis tenuifolia* DC. Arboreal melliferous species such as *Eucalyptus* sp., *Robinia pseudo-acacia* L., *Schinus areira* L. and *Styphnolobium japonicum* (L.) Schott are also present.

Preparation of samples and analysis

Melissopalynological data were obtained from 127 honey samples collected at apiaries in 15 districts of the south and southwest of the Buenos Aires Province over the period 1992-2002 (Valle *et al*., 1995,

2000, 2001, 2004; Andrada *et al.*, 1998a,b, 2000a,b; Andrada and Tellería, 2002). A melissopalynological map was constructed using the pollen identified after its separation from the honey by centrifugation. Figure 1 shows the districts of origin and the number of samples obtained from each. The material was prepared and analysed according to the methods recommended by the International Bee Research Association (Louveaux *et al.*, 1978).

Qualitative analyses were undertaken by counting and attempting to identify up to 1,000 pollen grains per honey sample. Pollen grains were identified by comparing them with those of a pollen reference collection produced by the authors for the plants of the study area. Plant samples were deposited at the Regional Herbarium of the Agronomy Department of the Universidad Nacional del Sur, Bahía Blanca, and the pollen preparations deposited at the palynotheca of the same institution. Pollen from the collected plants and honey samples were acetolyzed (Erdtman, 1960), mounted in glycerine gelatine and sealed with paraffin. Pollen types were identified at the level of species, genus, tribe or family. Specialized literature was also used in this task (Erdtman, 1966; Kremp, 1968; Heusser, 1971; Markgraf and D'Anthoni, 1978; Tellería, 1995, 2000; Tellería and Forcone, 2002). Publications regarding the flora of the Buenos Aires Province were consulted regarding scientific nomenclature (Cabrera, 1968; Lamberto *et al.*, 1997; Zuloaga and Morrone, 1999).

The frequencies of occurrence of the different types of pollen grain (recorded as a percentage) were deter-

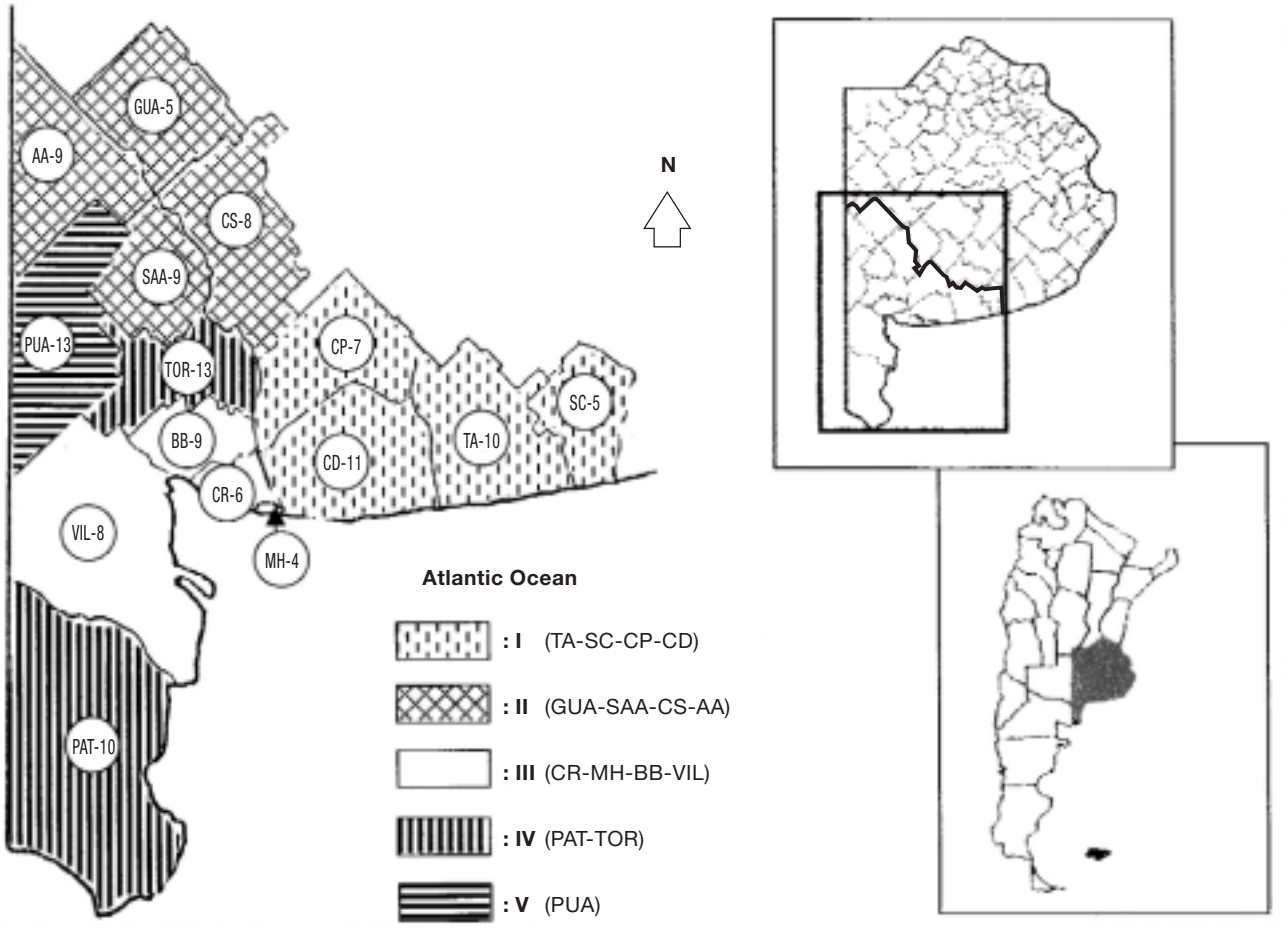

Figure 1. Geographical location of the study area in the south and southwest of the Buenos Aires Province. Region I: TA: Tres Arroyos; SC: San Cayetano; CP: Coronel Pringles; CD: Coronel Dorrego. Region II: GUA: Guaminí; SAA: Saavedra; CS: Coronel Suárez; AA: Adolfo Alsina. Region III: CR: Coronel Rosales; MH: Monte Hermoso; BB: Bahía Blanca; VIL: Villarino. Region IV: PAT: Patagones; TOR: Tornquist. Region V: PUA: Puán. The number of honey samples from each district is shown at the right side.

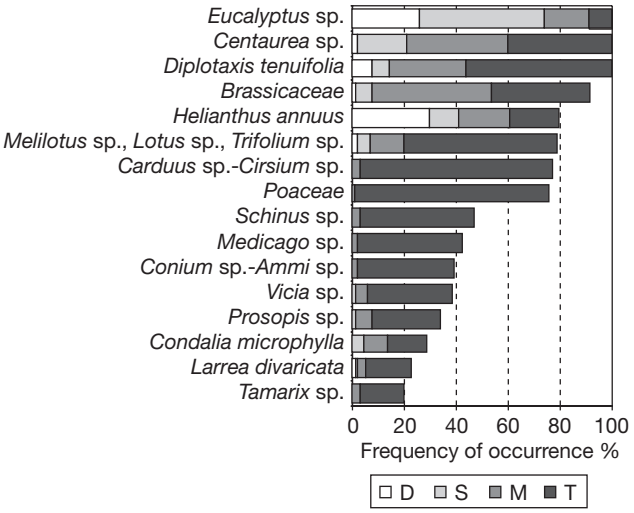

Figure 2. Frequency classes of pollen types present in the 127 honey samples: predominant pollen (D) > 45%; secondary pollen (S) 16-45%; important minor pollen (M) 3-15%; minor pollen (T) < 3%. Although 98 pollen types were identified, only the 16 types with the highest frequency of occurrence (≥ 20%) were used in analyses.

mined by counting the number of honey samples in which they appeared. The pollen grain frequency classes were designated as dominant (D), secondary (S), important minor (M), and trace (T < 3%) (Fig. 2) (Louveaux *et al.*, 1978). Nectarless plant pollen was excluded when calculating percentages (Louveaux *et al.*, 1978).

The honey samples were classified as monofloral or multifloral. The relative dominance percentages required for such classification differed according to pollen type following the criteria of other authors: *Eucalyptus* sp. > 70% (SAGPyA, 1998), *Helianthus annuus* > 15% (Accorti *et al*., 1986), and other species > 45% (Louveaux *et al*., 1978).

Ninety eight pollen types were identified, but only the 16 types with the highest frequency of occurrence (≥ 20%) were selected for further study. Pollen types that were non- representative of the studied region, with a frequency of occurrence < 20%, and in the T frequency class, were not taken into account in statistical analyses.

Statistical analysis

In order to group the districts in terms of species of similar importance, the mean value of the ordinal scale of the species for each district was first calculated. The resulting matrix (matrix H; 15 districts × 16 species) was subjected to multivariate analysis. The covariance matrix (using matrix H as the data source) was subjected to principal components analysis (PCA), and the districts represented graphically on the planes of the first two axes. Each axis was interpreted according to its correlation with each species. Hierarchical cluster analysis was used to group the districts as a measure of their Euclidean distances and complete linkage. The resulting groups (regions) were obtained by considering the division points to lie at the mid points of the maximum distances obtained.

Results

The main characteristic of the honey samples from the study area was the association of *Eucalyptus* sp., *Centaurea* sp. and *Diplotaxis tenuifolia* pollen types in all samples (Fig. 2). Other pollens included those from Brassicaceae, *Helianthus annuus,* clovers (*Melilotus* sp., *Lotus* sp. and *Trifolium* sp.), *Carduus* sp., *Cirsium* sp., and Poaceae.

Table 1 shows the frequency of occurrence and frequency classes of the 98 pollen types identified in the 127 samples. The families Fabaceae and Asteraceae provided the greatest number of pollen types.

Figure 3 shows the PCA results. The distributions of the districts are shown on the plane of the first two PC axes.

Figure 4 shows the five regions obtained by cluster analysis. These regions are also shown in Figure 1. Region I includes the Tres Arroyos, San Cayetano, Coronel Pringles and Coronel Dorrego districts; Region II the Guaminí, Saavedra, Coronel Suárez and Adolfo Alsina districts; Region III the Coronel Rosales, Monte Hermoso, Bahía Blanca and Villarino districts; Region IV the Patagones and Tornquist districts; and Region V the Puán district (Fig. 4).

Regions I, III and IV have about 80% monofloral honeys, while in regions II and V this figure only reaches 50% (Fig. 5). The presence of taxa with S frequency class values, such as *Eucalyptus* sp., *Helianthus annuus* and clovers in Region I, *Centaurea* sp., *Eucalyptus* sp. and *Condalia microphylla* in Region II, *Eucalyptus* sp., *Diplotaxis tenuifolia* in Region III, *Centaurea* sp. and *Eucalyptus* sp. in Region IV, and *Diplotaxis tenuifolia, Centaurea* sp., *Larrea* sp., *Condalia microphylla* and *Prosopis* sp. in region V, is important in multifloral honeys (Fig. 2, Table 1).

Table 1. Frequency classes and frequency of occurrence of the 98 pollen types identified in the different samples

Family	Pollen type	D	S	M	T	FO
Anacardiaceae	Schinus sp. (*S. areira*)			4	53	45
Apiaceae	Apiaceae			1	20	17
	Conium sp.-*Ammi* sp.			3	49	41
	Eryngium sp.				4	3
Asteraceae	*Ambrosia tenuifolia*				4	3
	Artemisia sp.				1	1
	Asteraceae (Astereae)			1	58	46
	Baccharis sp.				25	20
	Bidens sp.				2	2
	Brachychlados sp.				3	2
	Carduus sp.-*Cirsium* sp.			4	93	76
	Carthamus lanatus				4	3
	Centaurea sp. (*C. solstitialis, C. calcitrapa, C. diffusa*)	3	28	48	48	100
	Chuquiraga erinacea				4	3
	Cichorium intybus				24	19
	Cyclolepis genistoides				1	1
	Cynara cardunculus				25	20
	Gaillardia megapotamica			1	1	2
	Grindelia sp.				1	1
	*Helianthus annuu*s	5	33	20	43	80
	Hyalis argentea				8	6
	Hysterionica jasionoides				1	1
	*Matricaria chamomill*a				19	15
	Mutisieae				32	25
	Onopordum acanthium				1	1
	Senecio sp.				11	9
	Sonchus sp. (*S. oleraceus, S. asper*)			1	23	19
	Taraxacum officinale				12	9
	Verbesina sp.				3	2
Berberidaceae	*Berberis* sp.				1	1
Betulaceae	*Alnus* sp.				1	1
	Betula sp.				1	1
Boraginaceae	*Echium plantagineum*			1	5	5
	Heliotropium sp.				4	3
Brassicaceae	Brassicaceae	2	8	59	50	94
	Diplotaxis tenuifolia	10	9	38	70	100
Cactaceae	Cactaceae				2	2
Casuarinaceae	*Casuarina* sp.				3	2
Caryophyllaceae	Caryophyllaceae				12	9
Chenopodiaceae-Amaranthaceae	Chenopodiaceae-Amaranthaceae				73	57
Convolvulaceae	*Convolvulus* sp.				6	5
Cucurbitaceae	Cucurbitaceae				2	2
Cupressaceae	*Cupressus* sp.				26	20
Cyperaceae	Cyperaceae				8	6
Ephedraceae	*Ephedra* sp.				5	4
Euphorbiaceae	*Manihot* sp.				2	2
Fabaceae	*Acacia* sp.				7	6
	Adesmia sp.				9	7
	Caesalpinioideae				6	5
	Geoffroea decorticans			1	2	2
	Lotus sp.		4	6	56	52
	M*edicago* sp. (*M. sativa, M. minima*)			3	52	43

Table 1 (cont.). Frequency classes and frequency of occurrence of the 98 pollen types identified in the different samples

Family	Pollen type	D	S	M	T	FO
Fabaceae (cont.)	*Melilotus* sp. (*M. indicus, M. albus, M. officinalis*)	3	5	17	73	77
	Parkinsonia aculeata				6	5
	Prosopidastrum globosum				27	21
	Prosopis sp.		2	8	35	35
	Styphnolobium japonicum			2	5	6
	Trifolium sp.			3	47	39
	Vicia sp. (*V. sativa, V. villosa*)	2		6	43	40
Fagaceae	*Nothofagus* sp.				2	2
Geraniaceae	Geraniaceae				1	1
Juglandaceae	*Juglans regia*				1	1
Lamiaceae	*Salvia* sp.				4	3
Malvaceae	Malvaceae				16	13
	Sida sp.				1	1
	Sphaeralcea sp.				12	9
Myrtaceae	*Eucalyptus* sp. (*E. camaldulensis, E. viminalis*)	32	72	15	8	100
Onagraceae	Onagraceae				9	7
	Ludwigia peploides				1	1
Oxalidaceae	*Oxalis* sp.				16	13
Pinaceae	Pinus				40	31
Plantaginaceae	*Plantago* sp. (*P. patagonica, P. myosuros*)				28	22
Poaceae	Poaceae			1	95	76
	Zea mays				4	3
Polygalaceae	Polygalaceae				3	2
Polygonaceae	*Polygonum* sp.				4	3
	Fagopyrum esculentum				1	1
Portulacaceae	*Portulaca* sp.				5	4
Rhamnaceae	*Condalia microphylla*		6	12	20	30
	Discaria longispina			1	15	13
Rosaceae	*Acaena* sp.				2	2
	Prunus sp.				1	1
	Rosaceae				3	2
Rutaceae	*Citrus* sp.				1	1
Salicaceae	*Salix* sp.			1	1	2
Scrophulariceae	*Gerardia genistifolia*				1	1
Solanaceae	*Lycium* sp.				17	13
	Solanum sp.			1	11	9
Tamaricaceae	*Tamarix* sp. (*T. juniperina, T. gallica*)			2	22	20
Ulmaceae	*Celtis* sp.				2	2
	Ulmus sp.				1	1
Verbenaceae	*Acantholippia seriphioides*				3	2
	Glandularia sp.				2	2
	Phyla canescens			2	15	13
	Verbena sp.				6	5
Vitaceae	*Parthenocissus* sp.				1	1
Zygophyllaceae	*Larrea divaricata*	2	1	4	23	24
	Tribulus terrestris				14	11

Frequency classes: values indicate the number of samples in which the different pollen types appeared at the following percentages: > 45%: predominant pollen (D); 16-45%: secondary pollen (S); 3-15%: important minor pollen (M); < 3%: minor pollen (T). FO: frequency of occurrence (in percentages).

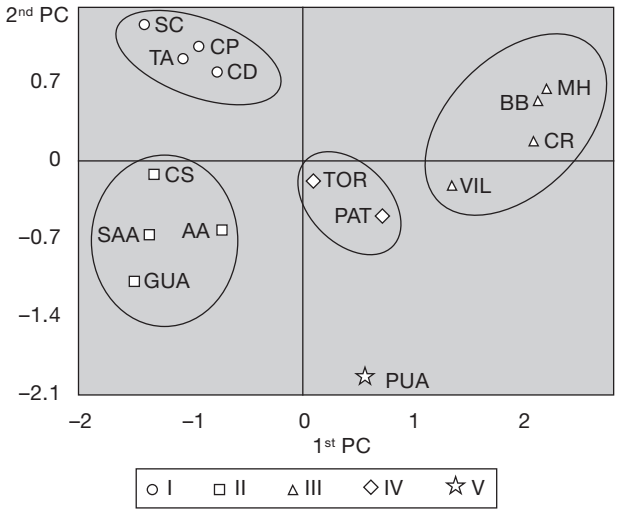

Figure 3. Coordinates of the districts in the plane of the first two principal components. I: San Cayetano (SC), Tres Arroyos (TA), Coronel Pringles (CP) and Coronel Dorrego (CD); II: Adolfo Alsina (AA), Coronel Suárez (CS), Guaminí (GUA) and Saavedra (SAA); III: Bahía Blanca (BB), Coronel Rosales (CR), Monte Hermoso (MH) and Villarino (VIL); IV: Patagones (PAT) and Tornquist (TOR); V: Puán (PUA).

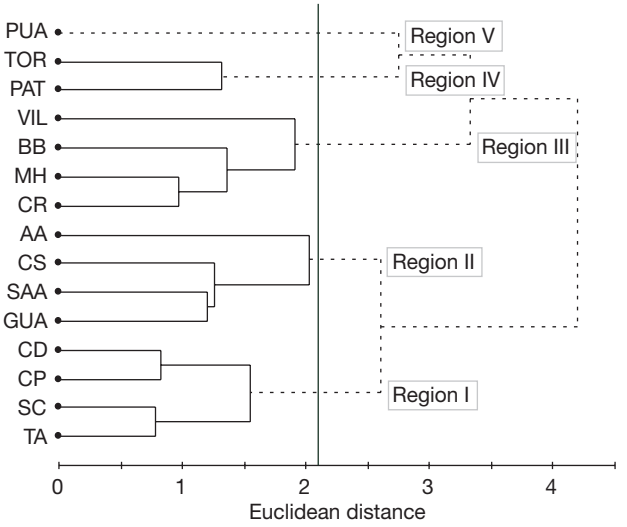

Figure 4. Hierarchical tree for the 15 districts studied. Complete linkage. Euclidean distances. AA: Adolfo Alsina. BB: Bahía Blanca. CD: Coronel Dorrego. CP: Coronel Pringles. CR: Coronel Rosales. CS: Coronel Suárez. GUA: Guaminí. MH: Monte Hermoso. PAT: Patagones. PUA: Puán. SAA: Saavedra. SC: San Cayetano. TA: Tres Arroyos. TOR: Tornquist, and VIL: Villarino.

Discussion

In this work, the families Fabaceae and Asteraceae were those that showed the greatest diversity of pollen types. The importance of these families agrees with data from other apicultural areas of the world (Louveaux, 1985; Crane, 1991), and from the Pampa (Tellería, 1988, 1992, 1996a,b; Valle *et al*., 2000, 2004), Monte de Llanuras y Mesetas (Forcone and Tellería, 1998, Forcone *et al*., 2005), and Espinal (Andrada, 2001; Naab *et al*., 2001) ecoregions of Argentina.

The pollen of *Eucalyptus* sp. showed a 60% frequency of occurrence in the honeys of the central region (Region III), a value not reached in other honeys from the Buenos Aires or La Pampa Provinces (Tellería, 1992, 1996a,b; Naab *et al.*, 2001) (Fig. 5). This can be explained by the widespread distribution of *Eucalyptus* sp. in Region III, where it is cultivated as a forest and ornamental species.

Although the eastern region (Region I) showed 50% sunflower honeys, this area is also characterized by 10% clover honeys; clover is characteristic of the humid prairies of the northeastern districts of the Buenos Aires Province (Tellería, 1992, 1996a,b).

The northwestern region (Region II) had 50% sunflower honeys and 50% multifloral honeys, with secondary pollen provided by *Centaurea* sp., *Eucalyptus* sp. and *Condalia microphylla*. The honey types of this region differ from those produced in the adjoining Utracán district of La Pampa Province (Naab *et al*., 2001) due to the absence of certain types of pollen typical of the Calden District flora, and to the presence of the sunflower honeys (attributed to agricultural activity).

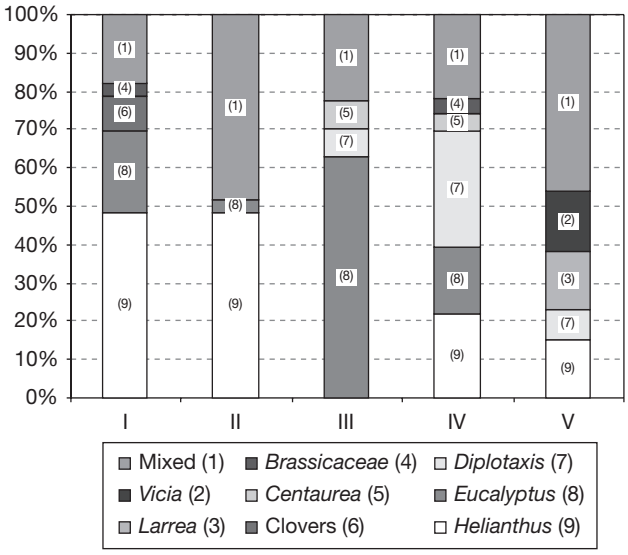

Figure 5. Percentage of monofloral and mixed honey samples collected in each region.

Region IV showed 30% *Diplotaxis tenuifolia* honeys, while region V produced honeys from native species such as *Larrea* sp. and *Vicia* sp. These species were present in mixed honeys too, along with other representative species of the south of the Calden District such as *Condalia microphylla* and *Prosopis* sp. (Andrada, 2001).

The pollen spectrum of the honeys from the study area is characterised by the presence of pollen belonging to *Diplotaxis tenuifolia,* a perennial invading weed introduced into Argentina as a melliferous plant. It is now widespread in the southwest of the Buenos Aires and La Pampa Provinces (Rodríguez, 1974). This species is absent in honeys from the east and northeast of the Buenos Aires Province (Tellería, 1992, 1996a).

The honeys from the study area resemble those from La Pampa Province because of the presence of *Diplotaxis tenuifolia*, but differ from them in the low frequency of native pollen types (Tellería, 1996b; Naab *et al*., 2001).

In general, the differences among the regions were not well defined, although the following conclusions can be drawn: i) the coastal districts, and their adjoining districts such as Tornquist and Coronel Pringles, are characterized by the presence of monofloral honeys; ii) in the northwestern districts, 50% of the honey samples are mixed; accompanying pollens came from *Eucalyptus* sp*, Centaurea* sp., and, in some samples, *Condalia microphylla*; with respect to the monofloral honeys, most were of *Helianthus annuus* although a few were of *Larrea* sp., and *Vicia* sp. (from the Espinal ecoregion); and iii) the natural variability of honey samples makes it very difficult to define borders between the different honey regions.

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