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Characterization of honeys from west and south Buenos Aires Province, Argentina

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Abstract

Pollen analyses were carried out on 33 honey samples from Espinal, Monte de Llanuras y Mesetas and Pampeana phytogeographical Provinces, collected during the 2000-2001 period. Sample processing as well as qualitative and quantitative analyses were performed according to standard techniques. Sixty-seven morphological pollen types were identified. The association of *Eucalyptus* sp. (*E. camaldulensis* Dehnh., *E. viminalis* Labill.), *Centaurea* sp. (*C. solstitialis* L., *C. calcitrapa* L.) and *Diplotaxis tenuifolia* DC. characterized these honeys. Twelve samples were unifloral: six from *Eucalyptus* sp., five from *Helianthus annuus* L., and one from Brassicaceae. Asteraceae and Fabaceae were the most representative botanical families.

Key words: botanical origin, geographical origin, pollen analysis, Melissopalynology.

Resumen

Origen botánico de las mieles del oeste y sur de la provincia de Buenos Aires, Argentina

Se analizaron 33 muestras de miel recolectadas entre los años 2000 y 2001, provenientes de las provincias fitogeográficas Espinal, Monte de Llanuras y Mesetas y Pampeana. Las muestras fueron procesadas y analizadas cualitativa y cuantitativamente según técnicas convencionales. Se identificaron 67 tipos polínicos. La combinación *Eucalyptus* sp. (*E. camaldulensis* Dehnh., *E. viminalis* Labill.), *Centaurea* sp. (*C. solstitialis* L., *C. calcitrapa* L.) y *Diplotaxis tenuifolia* DC. caracterizó a dichas mieles. Los resultados indican que 12 mieles fueron monoflorales: seis de *Eucalyptus* sp., cinco de *Helianthus annuus* L. y una de Brassicaceae. Las familias más representadas fueron Asteraceae y Fabaceae.

Palabras clave: origen botánico, origen geográfico, análisis polínico, Melisopalinología.

Introduction

Argentina is one of the main world's suppliers of honey, exporting 70,314 tons from January to December 2003 (SAGPyA, 2004). The important growth of local apiculture in recent years has led to the development of melissopalynological research, in order to classify honeys from Argentina (Tellería, 1988, 1992, 1996; Costa *et al*., 1995; Basilio and Romero, 1996; Forcone and Tellería, 1998; Salgado and Pire, 1999; Naab *et al*., 2001; Andrada and Tellería, 2002).

Pollen analyses of honeys from south-west Buenos Aires Province have been published (Valle *et al*., 1995, 2000, 2001; Andrada *et al*., 1998a, 1998b), although these do not include Guaminí, Adolfo Alsina, Puán, Villarino and Patagones districts (Fig.1).

The aim of this work was to study the botanical and geographical origins of honeys from these districts, which are located in the Caldén District of Espinal Province, a small area of Monte de Llanuras y Mesetas Province, and the Austral District of Pampeana Province (Burkart *et al*., 1999).

Material and Methods

Characteristics of the area

The region has a temperate cold dry climate. Mean annual temperature is 14ºC, and mean annual rainfall varies from 750 to 350 mm, with precipitation decreasing from NE to SW. Predominant vegetation consists of herbaceous stratum; the most representative family is Poaceae with several species: *Stipa papposa* Nees, *S. ambigua* Speg., *S. caudata* Trin., *S. ichu* Kunth, *Nassella*

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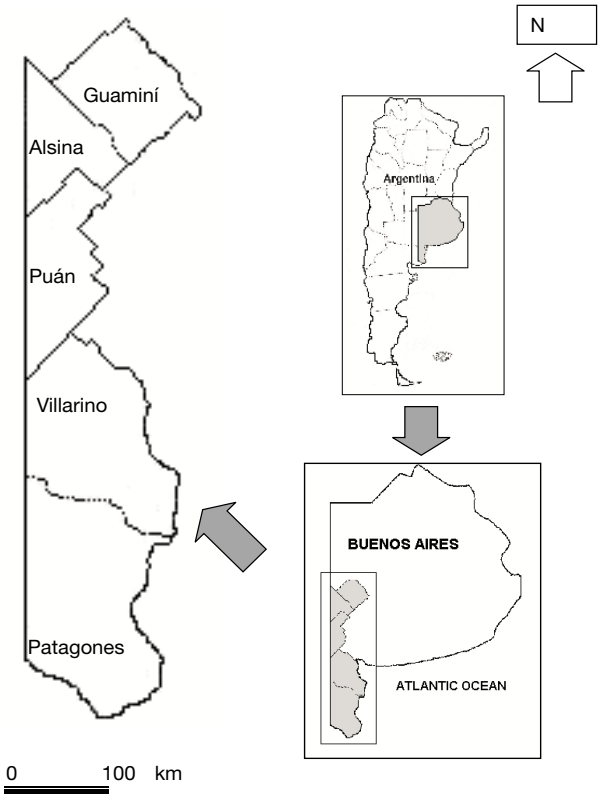

Figure 1. Geographical location of the studied districts.

tenuis (Phil.) Barkworth, *N. neesiana* Barkworth, *N. clarazii* Barkworth, *N. trichotoma* Arechav. and *N. tenuissima* Barkworth. These are accompanied by shrubs such as *Condalia microphylla* Cav., *Discaria americana* Hook., *Prosopidastrum globosum* Burk., *Schinus fasciculatus* Barkl., *Lycium chilense* Miers, *Geoffroea decorticans* Burk., *Larrea divaricata* Cav. and *Prosopis alpataco* Philippi. There are also arboreal species such as *Prosopis caldenia* Burk. and *Prosopis flexuosa* DC. (Cabrera, 1976).

Natural vegetation 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, particularly along the Lower Valley of Colorado river in Villarino and Patagones districts. *Eucalyptus* sp. L'Herit, *Schinus areira* L. and *Styphnolobium japonicum* Schott are the predominant woody species.

Preparation of samples and analyses

The pollen content of 33 honey samples obtained by centrifuging was studied: seven samples from Patagones District, seven from Villarino District, six from Puán District, nine from Adolfo Alsina District and four from Guaminí District.

Material preparing and analyses were carried out according to the methodology proposed by the International Bee Research Association (Louveaux *et al*., 1978).

Quantitative analyses consisted of counting the number of pollen grains contained in 10 g of honey (pollen abundance class –10) by means of an haemocytometer Neubauer Improved Double (Feller-Demalsy *et al.*, 1987). Results are expressed according to Louveaux *et al.* (1978): group I (< 20,000 grains), II (20,000-100,000), III (100,000-500,000), IV (500,000-1,000,000) and V (> 1,000,000).

Qualitative analyses consisted of counting up to 1,000 pollen grains per sample. Pollen types were identified by comparing them with the acetolyzed reference pollen collection belonging to the regional palynotheca of *Departamento de Agronomía, Universidad Nacional del Sur*. Specific literature was also used for identification (Erdtman, 1966; Markgraf and D'Anthoni, 1978; Tellería, 1995, 2000; Tellería and Forcone, 2002). Publications were consulted for scientific nomenclature (Zuloaga and Morrone, 1999).

Pollen types were identified up to species, genus, tribe, subfamily or family. A small list of potential species to which pollen could belong was added to some genera.

Frequency of occurrence of pollen grains, expressed as percentage, was determined by counting the number of honey samples in which pollen types appeared. Frequency classes of pollen grains were as follows: dominant pollen (D > 45%), secondary pollen (S = 16-45%), important minor pollen (M = 3-15%) and traces (T < 3%). Nectarless plant pollen was excluded when calculating percentages of frequency classes (Louveaux *et al.*, 1978).

Generally, samples are classified as monofloral when a nectariferous pollen type, excluding honeydew elements, is ≥ 45% (Louveaux *et al.*, 1978). However, this rule was not used for *Eucalyptus* sp. and *Helianthus annuus* honeys. Honeys were considered to be monofloral of *Eucalyptus* sp. when its pollen reached 70%, according to Resolution 274/95 (SAGPyA, 1998). Since the Argentinean legislation does not contemplate the conditions that honeys should fulfil to be considered monofloral of *H. annuus*, the Italian criterion was adopted (Accorti *et al.,* 1986). Investigations carried out by Bedascarrasbure and Bailez (1987) and Andrada

et al. (2004) showed that *H. annuus* pollen could be underrepresented (group I). For this reason, honeys were considered to be of *H. annuus* when its pollen reached 15%.

The number of honeydew elements was scarce in all samples; therefore the HDE/P index (ratio of the number of honeydew elements/number of pollen grains) was not calculated.

Pollen (P) and pollen-nectar (PN) producing plants were indicated.

Results

Quantitative analyses (PAC-10) are presented in Figure 2.

Qualitative analyses, frequency of occurrence, frequency classes, pollen-plants, and pollen-nectar plants are given in Table 1. Pollen types were identified at different taxonomic levels: 28 to species, 26 to genus, one to tribe, one to subfamily and 11 to family. All of them belong to 28 families. Asteraceae and Fabaceae gave the greatest number of types. *Eucalyptus* sp. (*E. camaldulensis* Dehnh., *E. viminali*s Labill.), *Centaurea* sp. (*C. solstitialis* L., *C. calcitrapa* L.) and *Diplotaxis tenuifolia* DC. were found in all honey samples.

Dominant pollens were from *Eucalyptus* sp. (48% of total samples) and Brassicaceae (3%). Secondary types were from *Eucalyptus* sp. (42% of total samples), *Centaurea* sp. (39%), *Condalia microphylla* (18%), *Helianthus annuus* (15%), *Melilotus* sp. (*M. indicus* Allioni, *M. albus* Medik.) (15%), *Diplotaxis tenuifolia* (9%), Brassicaceae (9%), *Larrea divaricata* (3%), *Prosopis* sp. (*P. alpataco*, *P. caldenia, P. flexuosa*) (3%), *Tamarix* sp. (*T. juniperina* Bunge, *T. gallica* L.)

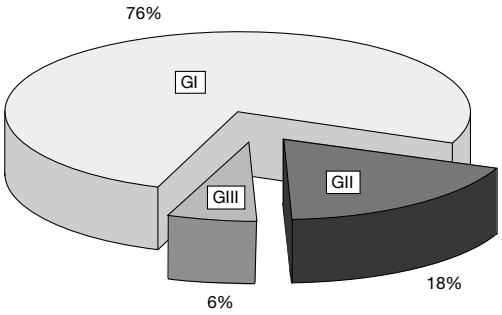

Figure 2. PAC-10: number of pollen grains in 10 g of honey. GI: < 20,000 grains. GII: 20,000-100,000 grains. GIII: 100,000-500,000 grains.

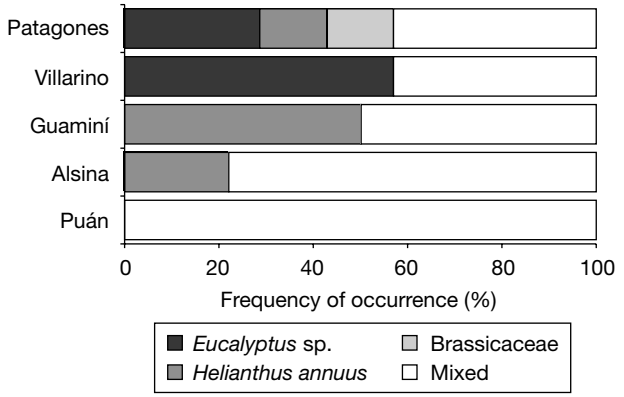

Figure 3. Honey distribution according to botanical origin.

(3%). Important minor pollens came from the following families: Anacardiaceae, Asteraceae, Brassicaceae, Fabaceae, Myrtaceae, Rhamnaceae, Verbenaceae and Zygophyllaceae. It must be outlined that 51 pollen types appeared only in traces.

From the total samples, 36% were monofloral and 64% were mixed. Among monofloral samples, six were from *Eucalyptus* sp. (four group II and two group I), five from *Helianthus annuus* (group I) and one from Brassicaceae (group I).

Figure 3 shows the distribution of honeys in the studied districts according to their botanical origin.

Accompanying pollens of eucalyptus and sunflower honeys (frequency of occurrence > 50%) are presented in Figures 4 and 5.

Most of the morphological types identified (86%) in honey samples belonged to pollen-nectar plants (Table 1).

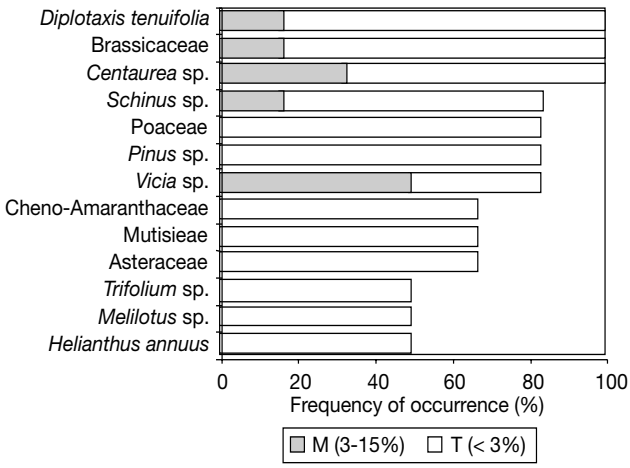

Figure 4. Accompanying pollen grains of Eucalyptus honeys. Frequency of occurrence: important minor pollen (M) , traces (T).

Table 1. Frequency classes and frequency of occurrence of pollen types

Family	Pollen type	Frequency classes				FO%
		D	S	M	T	
Anacardiaceae	*Schinus* sp. *(S. areira, S. fasciculatus)* (PN)			2	17	58
Apiaceae	Apiaceae (PN)				6	18
	Conium sp.- *Ammi* sp. (PN)				15	45
Asteraceae	Asteraceae (PN)				22	67
	Baccharis sp. *(B. ulicina, B. trimera)* (PN)				8	21
	Brachychlados lycioides (PN)				3	9
	Carduus sp.- *Cirsium* sp. (PN)				26	79
	Carthamus lanatus (PN)				2	3
	Centaurea sp. *(C. solstitialis, C. calcitrapa)* (PN)		13	13	7	100
	Chuquiraga erinacea (PN)				2	6
	Cichorium intybus (PN)				10	27
	Cynara cardunculus (PN)				11	33
	Gaillardia megapotamica (PN)			1	1	6
	Grindelia tehuelches (PN)				1	3
	Helianthus annuus (PN)		5	4	18	82
	Hyalis argentea (PN)				5	15
	Matricaria recutita (PN)				7	21
	Mutisieae (PN)				18	55
	Onopordon acanthium (PN)				1	3
	Senecio sp. *(S. montevidensis, S. pampeanus)* (PN)				5	15
	Sonchus sp. *(S. oleraceus, S. asper)* (PN)				3	9
	Taraxacum officinale (PN)				5	15
	Verbesina encelioides (PN)				1	3
Boraginaceae	*Heliotropium* sp. *(H. curassavicum, H. amplexicaule)* (PN)				3	9
Brassicaceae	Brassicaceae (PN)	1	3	18	10	97
	Diplotaxis tenuifolia (PN)		3	15	15	100
Cactaceae	Cactaceae (PN)				1	3
Caryophyllaceae	Caryophyllaceae (PN)				3	9
Cheno-Amaranthaceae	Chenopodiaceae-Amaranthaceae (P)				24	73
Convolvulaceae	*Convolvulus* sp. *(C. arvensis, C. bonariensis)* (PN)				3	9
Cupressaceae	*Cupressus* sp. *(C. sempervirens, C. arizonica)* (P)				9	27
Cyperaceae	Cyperaceae (P)				3	9
Ephedraceae	*Ephedra triandra* (P)				4	12
Fabaceae	*Acacia* sp. *(A. retinodes, A. dealbata)* (PN)				2	6
	Adesmia sp. *(A. incana, A. muricata)* (PN)				5	15
	Caesalpinioideae (PN)				3	9
	Geoffroea decorticans (PN)				2	6
	Lotus glaber (PN)				26	76
	Medicago sp.*(M. sativa, M. minima)* (PN)			1	22	70
	Melilotus sp. *(M. indicus, M. albus, M. officinalis)* (PN)		5	8	16	88
	Prosopidastrum globosum (PN)				13	39
	Prosopis sp. *(P. alpataco, P. caldenia, P. flexuosa)* (PN)		1	5	12	55
	Styphnolobium japonicum (PN)				1	3
	Trifolium sp. (PN)			1	19	61
	Vicia sp. *(V. sativa, V. villosa)* (PN)			6	22	85

Table 1 (continued). Frequency classes and frequency of occurrence of pollen types

Family	Pollen type	Frequency classes				FO%
		D	S	M	T	
Malvaceae	Malvaceae (PN)				5	15
	Sphaeralcea sp. *(S. australis, S. mendocina)* (PN)				5	15
Myrtaceae	*Eucalyptus* sp. *(E. camaldulensis, E. viminalis)* (PN)	16	14	2	1	100
Onagraceae	*Onagraceae* (PN)				4	12
Oxalidaceae	*Oxalis* sp. *(O. conorrhiza, O. lasiopetala)* (PN)				2	6
Pinaceae	*Pinus* sp. *(P. halepensis, P. radiata)* (P)				12	48
Plantaginaceae	*Plantago* sp. *(P. patagonica, P. myosuros, P. lanceolata)* (P)				9	24
Poaceae	Poaceae (P)				29	88
	Zea mays (P)				1	3
Polygalaceae	Polygalaceae (PN)				1	3
Portulacaceae	*Portulaca* sp. *(P. oleracea, P. grandiflora)* (PN)				2	6
Rhamnaceae	*Condalia microphylla* (PN)		6	8	6	61
	Discaria americana (PN)				9	27
Solanaceae	*Lycium* sp. *(L. chilense, L. tenuispinosum)* (PN)				11	33
Tamaricaceae	*Tamarix* sp. *(T. juniperina, T. gallica)* (PN)		1		13	42
Ulmaceae	*Ulmus pumila* (P)				1	3
Verbenaceae	*Acantholippia seriphioides* (PN)				1	3
	Glandularia sp. *(G. peruviana G. pulchella, G. platensis)* (PN)				1	3
	Phyla canescens (PN)			2	7	27
	Verbena sp. *(V. bonariensis, V. intermedia)* (PN)				4	12
Zygophyllaceae	*Larrea divaricata* (PN)		1	3	14	55
	Tribulus terrestris (PN)				6	18

Frequency classes: values indicate the number of samples in which the different pollen types appeared, with the following percentages: D, predominant pollen, > 45%; S, secondary pollen, 16-45%;M, minor important pollen, 3-15%;T, minor pollen, < 3%; R, present pollen, < 1%. FO: frequency of occurrence. PN: pollen-nectar producing plants. P: pollen producing plants.

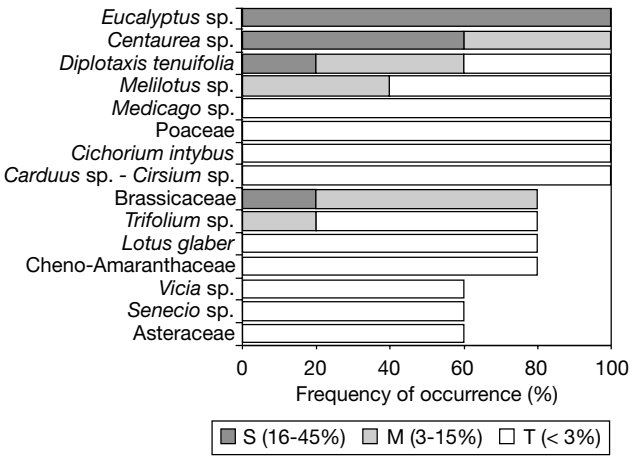

Figure 5. Accompanying pollen grains of sunflower honeys. Frequency of occurrence: secondary pollen (S), important minor pollen (M), traces (T) .

Discussion

Quantitative pollen analyses (PAC-10), showed that most samples (76%) belong to group I (Fig. 2), according with results obtained in honeys from the mountain region and the surrounding plains of Sistema Ventania (Andrada *et al.*, 1998b; Valle *et al.*, 2001).

Pollen spectrum analyses revealed the presence of three morphological types in all samples: *Eucalyptus* sp., *Centaurea* sp. and *Diplotaxis tenuifolia*. These are characteristic types of honeys coming from other places of the pampeana region (Valle *et al.*, 1995, 2000, 2001; Tellería, 1996; Andrada *et al.*, 1998a, 1998b) and correspond to introduced species spread in this area. Moreover, Brassicaceae, Poaceae, *Melilotus* sp., *Vicia* sp., *Helianthus annuus*, *Carduus*

sp. - *Cirsium* sp. and *Lotus glaber*, present in more than 75% of samples, contributed to identify these honeys (Table 1).

The importance of Fabaceae and Asteraceae in samples coincides with honeys coming from other apicultural areas of the world (Crane, 1991) and from different regions of Argentina (Tellería, 1988, 1992, 1996; Forcone and Tellería, 1998; Valle *et al*., 2000, Naab *et al*., 2001).

Monofloral samples had a poor pollen content; 92% of them were eucalyptus and sunflower honeys. Considering *Eucalyptus* sp. as an over-represented pollen type, it should be classified in group III (Louveaux *et al.*, 1978). However, results obtained in this work agree with values expressed by Aira *et al*. (1998) in Portugal. Results for *Helianthus annuus* honeys also agree with those obtained in Italy by Accorti *et al*. (1986).

Thirty four pollen types were identified in eucalyptus honeys; 79% of them belonged to pollen-nectar plants. Five taxa with important minor pollen frequency class were observed. *Eucalyptus* sp. honeys differed from those harvested in surrounding plains of Sistema Ventania (Valle *et al.*, 2001) because they had other accompanying types: *Schinus* sp., *Vicia* sp., Mutisieae and *Trifolium* sp. (Fig. 4).

Thirty nine pollen types were identified in sunflower honeys; 82% of them belonged to pollen-nectar plants. Four taxa showed values ranging from 16 to 45% (S). This could be explained by the low pollen percentage established for sunflower honeys, according to Accorti *et al*. (1986). Sunflower honeys differed from those harvested in the southern Austral District of Pampeana Province (Valle *et al.*, 2000) because of the presence of Asteraceae, *Senecio* sp., *Vicia* sp., *Medicago* sp. and *Cichorium intybus* (Fig. 5).

Sunflower honeys come from Guaminí (50%), Adolfo Alsina (22%) and Patagones (14%) districts. Most of samples from Villarino and Patagones districts were monofloral (57%); the highest diversity was found in the last district (Fig. 3).

Accompanying pollen types of monofloral honeys come mainly from introduced species.

Honeys from Puán district were mixed and presented elements from Caldén District (*Condalia microphylla*, *Prosopis* sp. and *Larrea divaricata*) with frequency class values of secondary and important minor pollen (Andrada and Tellería, 2002). Similar characteristics were reported for La Pampa honeys (Tellería, 1996; Naab *et al.*, 2001).

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