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Intraannual variations in the pollinic spectrum of honey from the lower valley of the River Chubut (Patagonia, Argentina)

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Abstract

Thirty honey samples from the lower valley of the River Chubut, obtained by successive harvests in five apiaries over two consecutive apicultural periods (between 1997 and 2000) were analysed. Three hives per apiary were selected, from which three successive harvests were obtained at the end of the following periods: initial (September 1st-December 20th), middle (December 20th-January 30th) and final (January 30th-March 15th). Pollen types were identified by comparison with a reference collection. Both reference pollen and sample pollen were subjected to acetolysis**.** A total of fifty entomophilous pollen types were identified: thirty-six, thirty-five and thirty-seven in initial, middle and final periods, respectively. The most abundant pollen was similar over the whole season, due to the long flowering periods of the taxa producing dominant and secondary pollen. *Tamarix gallica* and *Medicago sativa* were main sources of nectar during the entire production period. Significant differences ($P<0.001$) in pollen content were detected when honey from the three production periods was compared. Most unifloral honeys were produced in Spring. In this season, the major contribution was recorded from the southern district of the Monte. Anemophilous pollen was present in all the samples. The number of pollen grains per gram of honey was less than 10,000 in the three periods considered.

Key words: bee-plants, pollen, melissopalynology.

Resumen

Variaciones intranuales en el espectro polínico de las mieles del valle inferior del río Chubut (Patagonia, Argentina)

Se analizó el contenido polínico de 30 muestras de miel obtenidas de forma secuencial en cinco colmenares del valle inferior del río Chubut durante dos temporadas apícolas consecutivas, entre los años 1997 y 2000. Se seleccionaron tres colmenas por apiario, en las que se efectuaron cosechas sucesivas correspondientes a tres períodos de la temporada apícola: inicial (1 de septiembre-20 de diciembre), medio (20 de diciembre-30 de enero) y final (30 de enero-15 de marzo). Los tipos polínicos fueron reconocidos por comparación con una colección de referencia de polen proveniente de plantas del lugar. En todos los casos el polen fue acetolizado. Se identificó un total de 50 tipos de polen entomófilo, 36 en el período inicial, 35 en el período medio y 37 en el período final. Al comparar los períodos de cosechas se detectaron diferencias significativas ($P<0,001$) en la composición polínica. En la primavera se obtuvo el mayor número y variedad de mieles monoflorales y se registró el mayor aporte del distrito austral del Monte. La composición del polen mayoritario fue similar en toda la estación, en concordancia con los extensos períodos de floración de los taxa representados en el polen dominante y secundario. *Tamarix gallica* y *Medicago sativa* fueron las principales fuentes de néctar durante todo el período de producción. La presencia de polen anemófilo fue constante en todas las muestras. El número de granos de polen por gramo de miel fue inferior a 10.000 en los tres períodos considerados.

Palabras clave: plantas melíferas, polen, melisopalinología

Introduction

Traditionally, pollen analysis in honey has been used to identify nectariferous plants used by *Apis mellifera* L. in a region and to classify honey according to its botanical and geographical origin (Louveaux, 1978).

Analysis of the evolution of the pollen spectrum of honey during its period of production is an useful tool to detect the contribution of the different sources of nectar over the apicultural period. This is of special interest for hive management and also permits probable

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periods of monofloral honey production to be identified, which have a greater commercial value.

Although few researches have focused on this issue, works at different latitudes have used melissopalynological analyses to study the nectariferous resources selected by *A. mellifera* during the apicultural period either by stepped harvests (Seijo *et al.*, 1992a and b; Basilio, 1998) or by examining at regular intervals the nectar deposited in the honeycombs (Adams *et al.*, 1979; Adams and Smith, 1981; Parent *et al.*, 1990).

In Argentina, although melissopalynological studies have been carried out in some provinces, most have aimed at classifying honey according to its botanical and geographical origin. There is only one study of the intraannual variations of the pollinic spectrum of honey, which was carried out in the Paraná Delta, a floristically highly heterogeneous area within the pampean phytogeographic province (Basilio, 1998).

Apiculture is largely undeveloped in the Chubut province. The most important apiculture area corresponds to the lower valley of the River Chubut, with around 2,500 hives in production. The apiaries, most of which are small (20-50 hives), are mainly located in the areas of Trelew, Gaiman and Dolavon (Fig. 1). The apicultural period has a shorter duration than in the north of the country owing to climatic conditions, since hive production begins in November and finishes at the end of March, with an average yield approximate of 35 kg/year/hive.

From palynological analyses of the final harvests, 55 nectariferous plants were found to be used by *A. mellifera* in the lower valley of the River Chubut (Forcone and Tellería, 1998). The aim of this work was to detect the resources most used in three stages of honey production by studying the samples from successive harvests and the differences in the pollinic composition of honey that could justify harvesting in stages.

Material and methods

Characteristics of the area

The lower valley of the River Chubut (43-44°S and 65-66°W) is situated in the province of the same name between the Departments of Rawson and Gaiman. The region has a temperate cold semidesertic climate. The mean annual temperature is 12.7°C and the average annual rainfall ranges from 175 to 180 mm. The frost-free period covers 117 days from mid-November to mid-March (Walter *et al.*, 1975; Arbuniez de Mc Karthy, 1994; Leon *et al.*, 1998). From a phytogeographical viewpoint, the lower valley of the River Chubut is situated in the southern district of the Monte (Cabrera, 1971; Leon *et al.*, 1998). Characteristic vegetation of this region is «jarillal» (*Larrea divaricata* Cav)., species accompanied among others by *Larrea nitida* Cav., *Prosopidastrum globosum* (Gillies ex Hook & Arn)

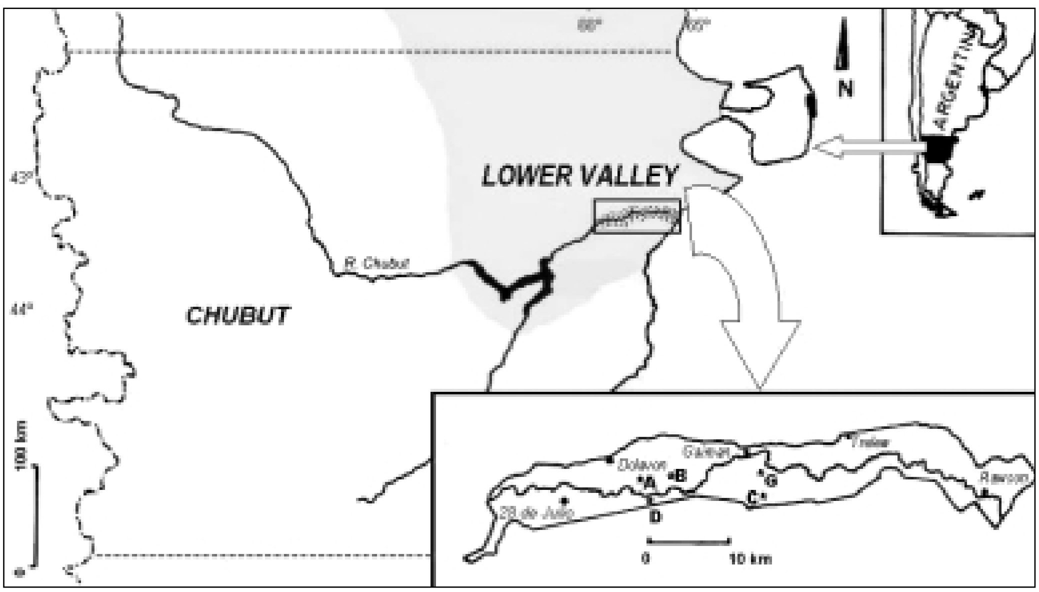

Figure 1. Location of the lower valley of the River Chubut. A, B, C, D, G: apiaries sampled. Grey area: phytogeographical province of the Monte.

Burkart, *Prosopis alpataco* Phil., *Schinus johnstonii* Barkley, *Chuquiraga erinacea* D. Don, *Ch. avellanedae* Lorentz, *Lycium chilense* Miers. ex Bertero, *L. ameghinoi* Speg., *Junellia ligustrina* (Lag.) Moldenke, *Atriplex lampa* (Moq.) D. Dietr., *Cyclolepis genistoides* D. Don and *Suaeda divaricata* Moquin. From this community only some species, mainly the halophiles, descend to the flood plain where saline soils with very low drainability are frequent (Soriano, 1950). Since over 100 years ago, seasonal irrigation from September-April has been carried out. The most important crops are foraging plants, mainly *Medicago sativa* L. To a lesser extent horticultural crops, fruit and cereals are grown. Among the forest crops, *Populus* spp. are predominant, especially *P. nigra* L., used to form wind breaks, and *Salix* spp. and *Tamarix gallica* L., the latter of which is naturalised and widespread in Patagonia (Rossow, 1988).

Sampling procedure

The pollinic contents of 30 honey samples obtained sequentially in five apiaries during two consecutive apicultural periods were studied. The apiaries were selected for having an area and honey production representative of the study area, with a mean size of 50 hives. In all cases hives were of the Dadant type. The locations of the different apiaries are indicated in Figure 1. In the G (Gaiman), D (Dolavon) and A (ABEPA) hives, harvests were carried out in the seasons 1997-1998 and 1998-1999, and in apiaries B (Siguero) and C (Neira) harvests were obtained in the seasons 1998-1999 and 1999-2000. Three hives per apiary were randomly selected and successive harvests were carried out in each one, corresponding to three apicultural periods: initial, from the start of the season (1 September) until the first operculation (20 December); middle, 20 December-30 January; and final, 30 January-15 March. Three empty labelled frames were placed in the hives at the start of each period, which were removed and replaced at the end of each of these steps as described in Seijo Coello *et al*. (1992a). The honey from the frames removed from all three hives was extracted together by centrifugation obtaining one sample per apiary for each period.

Palynological analysis

Qualitative analysis

In order to determine the percentage representation of each taxon in the pollen contents of the honey, the methodology proposed by Louveaux (1978) was followed. Pollen types were identified by comparing them with a reference collection obtained using plants from the area; this collection was deposited in the palynotheque of the Facultad de Ciencias Naturales of the Universidad Nacional de la Patagonia (Sede Trelew). Pollen from the samples and from the reference collection was acetolysed.

To determine frequency distribution classes, 500 pollen grains were counted per sample and anemophilous pollen was deducted. Pollen types, according to their percentages, were classified into: >45%, dominant pollen (D); 15-45%, secondary pollen (S); 3-15%, pollen of minor importance (M); <3%, pollen traces (T). The samples in which one pollen type represented ≥45% were classified as monofloral, and those in which no pollen type reached this percentage were classified as mixed (Louveaux, 1978). This criterion was not used in the classification of monofloral honeys of *Medicago sativa* and *Eucalyptus* spp., types under and over-represented, respectively, in the pollen contents of honey. In these plants, honeys were considered to be monofloral of *M. sativa* when 20% of the pollen was from this species and as monofloral honey of *Eucalyptus* spp., when 75% of the pollen was from this latter species according to Maurizio and Louveaux (1961) and Serra Bonvehi and Cañas Lloria (1988).

Quantitative analysis

To determine the absolute number of pollen grains per gram of honey the methodology proposed by Stockmarr was followed (1971). In all samples, the number of honeydew elements were counted and the HDE/P index (ratio of the number of honeydew elements/number of pollen grains) was calculated.

Palynological analysis was complemented with observations of *A. mellifera* activity on vegetation and records of the flowering phenology were carried out according to Anderson and Hubritch (1940).

Statistical analysis

The data of the qualitative palynological analysis of the harvests of each period were analysed by applying Chi-squared test with P<0.001. The 16 pollen types with the highest frequencies were considered. The samples from each harvesting period were compared

Table 1. Frequency distribution classes and frequency of occurrence of entomophilous pollen found in three periods of the apicultural season in the lower valley of the River Chubut

Family	Pollen type	Initial period					Middle period					Final period				
		D	S	M	T	FO	D	S	M	T	FO	D	S	M	T	FO
Anacardiaceae	*Schinus* spp.				60	60				30	30				20	20
Apiaceae	*Pastinaca sativa*				40	40					50					
	Ammi spp.									10	10					
	Daucus spp.									10	10				30	30
	Foeniculum vulgare									20	20				50	50
Asteraceae	*Anthemis cotula-Matricaria*				10	10				40	40				20	20
	*Astereae**			30	20	50		20	60	10	90			50	30	80
	Cirsium vulgare				10	10			10	70	80			30	60	90
	Carduus spp.		10	30	40	80		10	30	50	90			30	60	90
	Centaurea spp.				20	20		10	10	50	70			10	40	50
	*Cyclolepis genistoides**				10	10										
	Mutisieae				10	10			10	10	20				40	40
	Chuquiraga spp.*				10	10				30	30				20	20
	Onopordon acanthium			10	10	20				50	50			10	50	60
	Taraxacum officinale			20	60	80		10	20	40	70			20	70	90
	Helianthus annuus														10	10
	Xanthium spp.									10	10				10	10
	Flaveria bidentis														10	10
	Lactuca type									30	30				30	30
Brassicaceae	Brassicaceae			70	30	100			50	50	100		10	40	50	100
Convolvulaceae	*Convolvulus arvensis*			10	30	40				60	60				50	50
	Cressa type				20	20				50	50		10	10	30	50
Fabaceae	*Acacia* spp.				10	10									10	10
	*Glycyrrhiza astragalina**				20	20				20	20					
	Melilotus spp.			30	50	80		10	80	10	100		20	60	20	100
	Medicago sativa	10				100	20				100	20				100
	Prosopis spp.*		10		60	70										
	*Prosopidatrum globosum**	10		40	20	70			20	30	50			10	40	50
	Trifolium pratense			10	20	30				60	60			10	30	40
	Trifolium spp.			70	30	100			40	50	90			70	10	80
	Robinia pseudoacacia				30	30										
Lamiaceae	*Mentha* spp.									10	10					
Liliaceae	*Allium* spp.						10				10					
Malvaceae	*Malvella leprosa*		10	40	40	90		10	40	40	90			40	50	90
	Malva nicaensis														10	10
Myrtaceae	*Eucalyptus* spp.		10	10	60	80		10	20	60	90		10	20	30	60
Oleaceae	*Ligustrum* spp.								10	20	30					
Polygonaceae	*Polygonum aviculare*					20									20	20
Plumbaginaceae	*Limonium brasiliensis*					20									20	20
Rosaceae	*Rosaceae*	10			60	70				20	20				20	20
Salicaceae	*Salix* spp.				30	30										
Solanaceae	*Lycium* spp.*		10	30	50	90			30	40	70			20	60	80
Tamaricaceae	*Tamarix gallica*	40	40	20		100	10	70	20		100	20	40	40		100
Verbenaceae	*Acantholippia seriphioides**			10	10											
	Phylla canescens				20	20			10	10	20				20	20
	*Glandularia crithmifolia**				10	10				10	10					
	Junellia spp.*				10	10										
Zygophyllaceae	*Larrea* spp.*		10	10	50	70			10	40	50		10		50	60

Frequency classes: D (dominant), S (secondary), M (minor), T (traces). The values indicate the percentage of samples in which the pollen types appear in each class. Frequency of occurrence (FO): percentage of samples in which the pollen types appears. * Native plants.

and then the mean values for each period (Steel and Torrie, 1981).

Results

A total of 50 types of entomophilous pollen were identified, 36 in the initial period, 35 in the middle period and 37 in the final period. The pollen types were identified at different taxonomic levels: 24 to species, 20 to genus, 2 to tribe, 2 to family and 2 were assigned to the type category: *Cressa* type, that includes *Cressa truxillensis* Kunth and *Cuscuta indecora* Choisy*,* and *Lactuca* type, that also includes *Picris* spp., *Sonchus* spp. and *Hypochoeris condrilloides* (A. Gray) Cabrera. Table 1 lists the identified pollen types grouped per families and their frequencies of occurrence in the total of the samples analysed and in each of the frequency classes. Since *Medicago sativa* is an under-represented species in honey only the dominant category was recognised. Figure 2 shows the most abundant pollen types for each apiary and period.

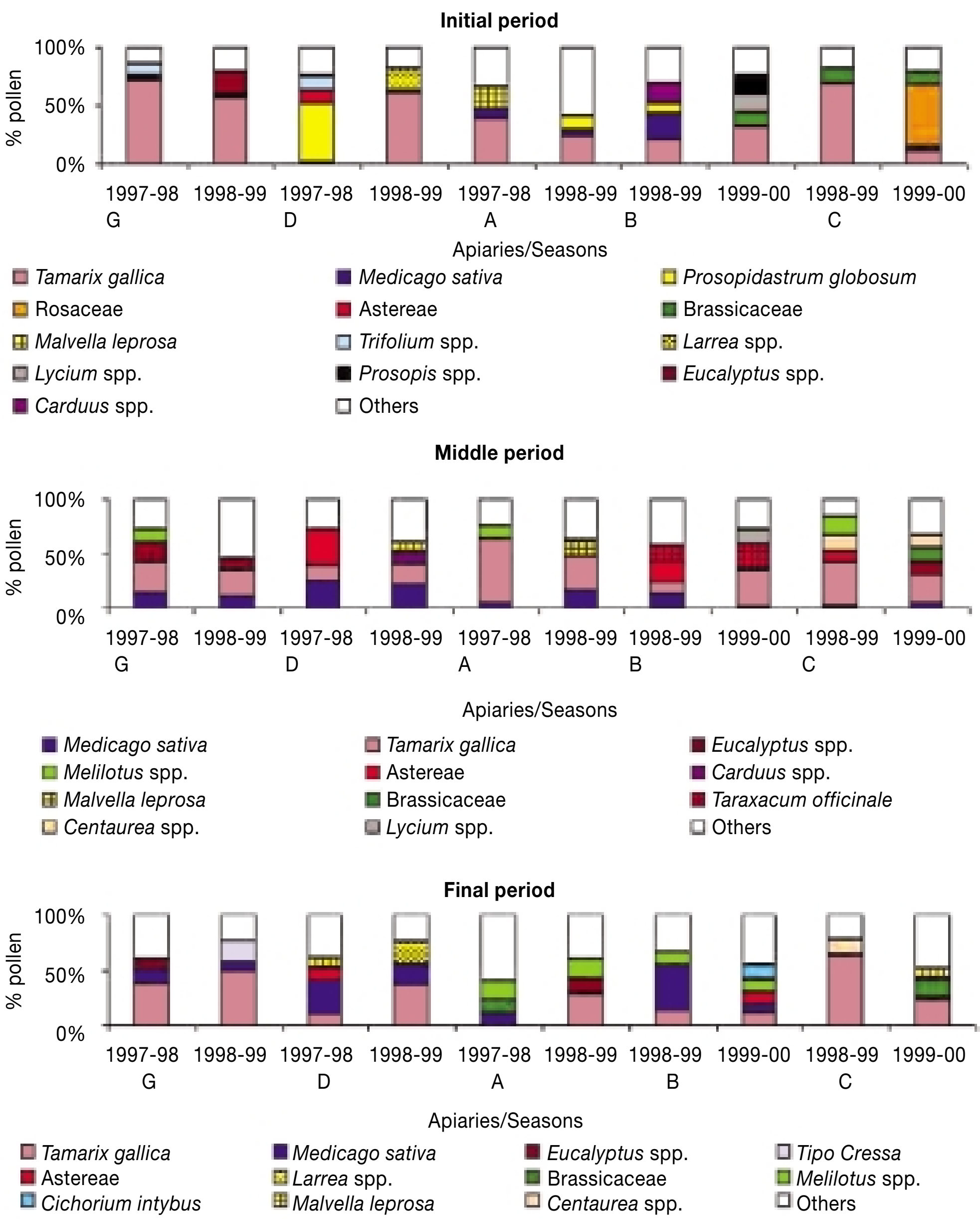

Figure 2. Entomophilous pollen with ≥10% representation in the samples.

Table 2. Comparison of pollen composition of honeys of the three harvesting periods using the χ^2 test

Periods	Pollen types																	χ^2
	Ast	Bra	*Car*	*Con*	*Euc*	*Lar*	*Lyc*	*Mal*	*Mel*	*Med*	*Pros*	*Pro*	Ros	*Tam*	*Tar*	*Tri*	Others	
Initial	88.8	4.5	4.2	1.4	9.9	24.1	13.8	0.3	150.8	99.2	289.0	77.6	321.6	55.8	13.9	15.0	190.4	1,360.3
Middle	135.7	7.3	2.6	0.0	9.3	68.7	5.2	0.0	31.8	9.9	75.3	12.0	91.0	15.6	67.9	7.1	14.1	553.7
Final	4.0	0.3	15.2	1.6	0.0	11.3	2.7	0.2	54.2	55.6	86.0	34.6	88.0	15.4	21.0	1.9	120.3	512.3
Total																		2,426.3*

* Highly significant value (P<0.001). Ast: Astereae; Bra: Brassicaceae; *Car: Carduus; Con: Convolvulus; Euc: Eucalyptus; Lar: Larrea; Lyc: Lycium; Mal: Malvella; Mel: Melilotus; Med: Medicago; Pros: Prosopidastrum; Pro: Prosopis;* Ros: Rosaceae; *Tam: Tamarix; Tar: Taraxacum; Tri: Trifolium.*

The dominant pollen in the three production periods corresponded to *Tamarix gallica* and *Medicago sativa*, types that presented a frequency of occurrence of 100%. In the initial period Rosaceae and *Prosopidastrum globosum* were also found in this category.

The most abundant accompanying pollen types (with a percentage>10%) found over the whole production period were: Astereae, Brassicaceae, *Eucalyptus* spp. and *Malvella leprosa*; the latter two with a frequency of occurrence of 90% in the three stages studied. In the initial period *Prosopis*, *Larrea*, *Lycium, Trifolium* and *Carduus* spp. were also found; in the middle period, *Centaurea*, *Melilotus, Taraxacum, Lycium* and *Carduus* spp.; and in the final period, *Centaurea, Melilotus*, *Cichorium*, *Larrea* and *Cressa* spp. (Fig. 2).

The pollen composition of honeys was heterogeneous in all (initial, middle and final) periods (P<0.001). On the other hand, when comparing pollen composition of honeys from the three periods, we found highly significant differences (χ^2= 2426.3; P< 0.001) (Table 2).

Anemophilous pollen was detected over the whole honey production period. In Spring, this mainly came from *Plantago* spp., and in Summer from *Plantago* spp. and Chenopodiaceae-Amaranthaceae. Other anemophilous types detected were *Typha* spp., Poaceae, Cyperaceae, *Artemisia absinthium* L., *Juglans regia* L., *Zea mays* L. and *Ambrosia tenuifolia* Spreng.

The number of pollen grains per gram of honey was low in all the samples (Fig. 3) and most were, therefore, placed in Group I described by Louveaux *et al.* (1978). The HDE/P index was lower than 1 in all cases.

Flowering periods of the taxa identified in the dominant and secondary pollen are shown in Figure 4.

Spring was the season with the greatest production of monofloral honey and most came from *Tamarix gallica*, coinciding with the optimum flowering of this species; the others were from fruit trees (Rosaceae), from the native shrubland (*Prosopidastrum globosum*) and from *Medicago sativa.* The latter started the phenophase of full flowering at the end of November, when there was a strong nectar flow to the hives. In this season too, the greatest contribution was made by plants from the Monte: *Larrea* spp., *Lycium* spp., *Prosopidastrum globosum* and *Prosopis* spp.

In Summer, the contribution of *Medicago sativa, Melilotus* spp., Astereae [mainly represented by *Grindelia tehuelches* (Speg.) Cabrera and *Baccharis* spp.] and some disturbance plants (*Centaurea* spp., *Cichorium intybus* L., *Taraxacum officinale* Weber ex F. H. Wigg.*,* and *Cressa* type) increased.

Discussion

The families most represented in pollen contents of the honey in the three periods considered were Fabaceae and Asteraceae. These families have been shown to be important sources of nectar at different latitudes (Crane, 1991) and in different honey-producing regions of Argentina (Tellería, 1988, 1992, 1996; Tellería and Forcone, 2000; Basilio and Romero, 1996; An-

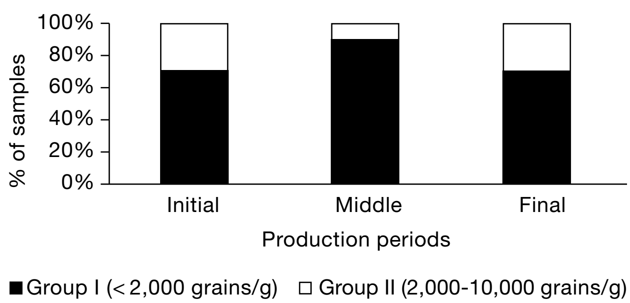

Figure 3. Classification of the samples according to the absolute number of pollen grains per gram of honey.

Figure 4. Flowering periods of the taxa found as dominant and secondary pollen.

drada and Tellería, 2002). Although both families predominated over the whole production period, the majority of species belonged to Fabaceae in Spring and to Asteraceae in Summer.

Although statistical differences were found when comparing pollen contents of the three production periods, the main components of pollen were the same over the whole season in accordance with the long flowering periods of the main sources of nectar. *Tamarix gallica* and *Medicago sativa* were the species that made the greatest contribution of nectar over the whole apicultural period and were the main components in monofloral honey.

The fact that the greatest number and diversity of monofloral honeys were obtained at the end of the Spring demonstrates the benefit of harvesting the honey produced at this time. Current practise in the study area is to harvest only once at the end of the season storing the honey on the hives and extracting a mixture of honeys from different flowerings.

The low pollen contents of the honey is related to the supply of nectar from *Medicago sativa,* present in all the samples. Similarly, some species frequently visited for nectar had very little or almost no presence in the honey pollen as occurred with *Salix* spp. and *Glycyrrhiza astragalina* Gillies ex Hook. & Arn. The low representation of these taxa is attributed to the predominance of the female foot in the most widespread *Salix* spp. (*S. fragilis* L.*, S. alba* L. and *S. babilonica* L.) and to the small pollen production of *G. astragalina* (personal observations).

The low presence of the willows and osiers, the abundance of tamarisk and the presence of pollen types characteristic of the south of the Monte (*Lycium* spp., *Prosopidastrum globosum*, *Larrea* spp.) distinguishes the Spring honey in the study area from that produced in the Paraná Delta, where *Salix* spp., Rosaceae, *Cytrus* spp., Myrtaceae and *Amorpha fruticosa* L. are the most abundant types at this time of year (Basilio, 1998). On the other hand, although summer honey from both areas is distinguished by its dominant types and by the presence of pollen associations specific to the different regions, a common characteristic is abundant pollen from Astereae, mainly represented by *Baccharis* spp. and *Solidago* spp. in the Paraná Delta and by *Grindelia tehuelches* and *Baccharis* spp. in the lower valley of the River Chubut.

The abundance of anemophilous pollen is a constant feature of extra-Andean Patagonian honeys studied to date (Forcone and Tellería, 1998, 2000; Tellería and Forcone, 2000). The presence of this pollen has been attributed to different factors, but mainly to contamination with corbicular loads (Louveaux, 1958; Fernández and Ortiz, 1994). However, this does not seem to be a very likely origin in honeys from the lower valley of the River Chubut since these usually present low values of pollen per gram. Probably, the anemophilous pollen results from contamination of the nectar with pollen particles in the air, especially owing to the incidence of wind in the region.

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