

Pollen collected by honey bees (*Apis mellifera* L.) from south of Caldén district (Argentina): botanical origin and protein content

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In the south of the Caldén district (Phytogeographical province of Espinal, Argentina) the availability of flowering along with the botanical origin and protein content of pollen harvested by honey bees, was studied. During two apiculture periods (from end August to early January) pollen loads were collected every fortnight using pollen traps. A total of 637.96 g of pollen loads was analysed. From 139 species recorded, only 29 were visited by honey bees. In terms of biomass the contribution of exotic plants was high at the end of the winter (Brassicaceae and *Erodium cicutarium*); during this period of pollen shortage, honey bees collected spores of *Puccinia interveniens*. During the spring, the native plants (*Condalia microphylla*, *Chuquiraga erinacea*, *Discaria americana*, *Grindelia tehuelches*, *Larrea divaricata*, *Prosopis* sp., *Prosopidastrum globosum* and *Vicia pampicola*) were utilised. Those pollen types of high protein level were collected most intensively.

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In Argentina, there are a number of studies on honey sources; however there are few studies on both the botanical origin and the nutritional value of pollen collected by honey bees (Andrada 2001, Forcone 2002). The present paper is part of a larger project whose objective was to determine and evaluate the apiculture resources of the Caldenal district. This region holds particular interest not only because of increasing beekeeper activity in the last years, but also the foraging behaviour of honey bees in the Caldenal region is poorly known. In a previous study, the nectariferous flora was investigated and the main sources of honey were detected (Andrada & Tellería 2002).

Pollen is a source of proteins, lipids and vitamins which are essential to growth and development of honey bees rather than energy production (Schmidt & Buchmann 1985). In particular, nitrogen is crucial for development of larvae and longevity of adults (Schmidt et al. 1987). It is important to detect which are the main pollen sources of a region and to determine their protein value as pollen is a major component of honey bees' diet.

The present paper provides additional data on plants visited by honey bees to obtain pollen and on the protein content of pollen collected. Thus, the aims of this paper are: to record the flowering availability during the apiculture period; to identify the pollen collected by honey bees; and to provide information about the protein content of the main pollen types foraged by honey bees.

MATERIAL AND METHODS

Sampling was performed in the south of the Caldén District, which lies within the Phytogeographical Province of Espinal (Fig. 1) where nectariferous resources were previously studied (Andrada & Tellería 2002). The Caldén District, usually called Caldenal extends over an area of 40,000 km² in the central semiarid region of Argentina (Fernández et al. 1989). In this area, vegetation consists basically of

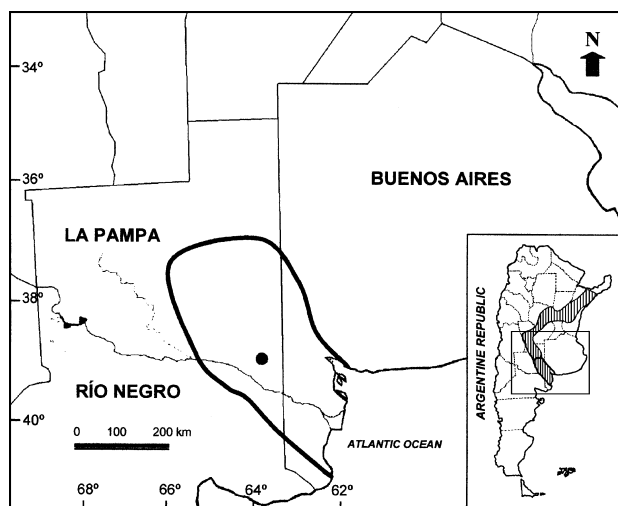


Fig. 1. Study area and location of the studied apiary (●).

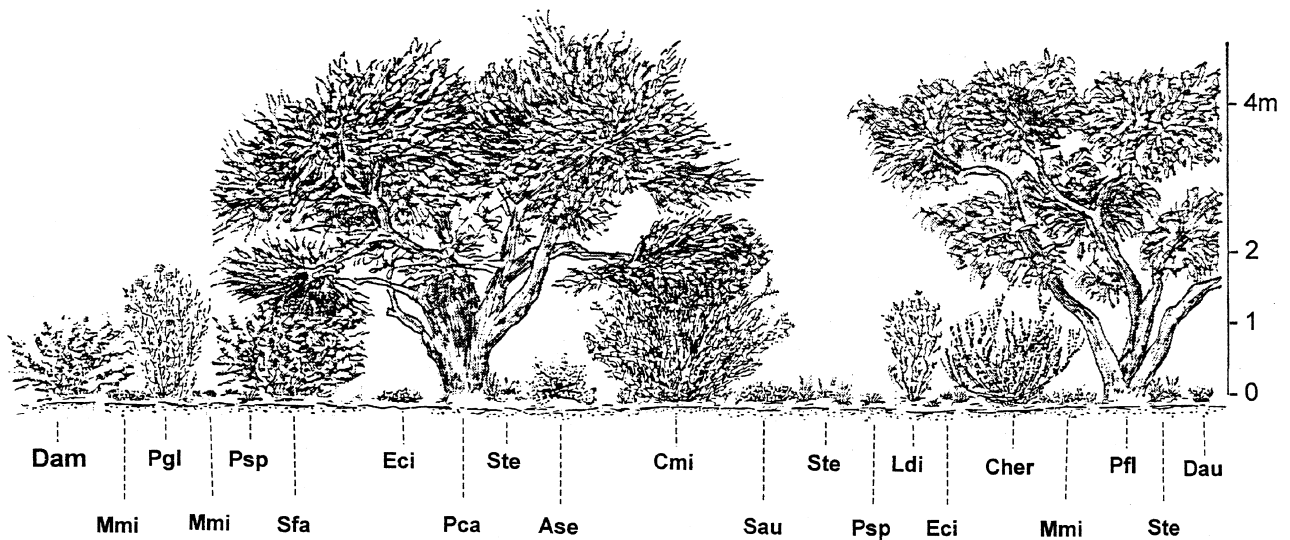


Fig. 2. Profile of typical vegetation of the Caldenal (modified from INTA, 1980).

Dam – *Discaria americana*, Mmi – *Medicago minima*, Pgl – *Prosopidastrum globosum*, Psp – *Plantago* sp., Sfa – *Schinus fasciculatus*, Eci – *Erodium cicutarium*, Pca – *Prosopis caldenia*, Ste – *Stipa tenuis*, Ase – *Acantholippia seriphoides*, Cmi – *Condalia microphylla*, Sau – *Sphaeralcea australis*, Ldi – *Larrea divaricata*, Cher – *Chuiraga erinacea*, Pfl – *Prosopis flexuosa*, Dau – *Draba australis*; m – meters.

open woods, a shrubby stratum and a herbaceous stratum rich in Poaceae. *Prosopis* sp., mainly *P. caldenia* Burkart, is the dominant arboreal species of the association (Cabrera 1971, Andrada & Telleria 2002) (Fig. 2).

Samples were collected every fortnight during two apiculture periods (August–January) of 1997–1998. The intensity of flowering – “coming into bloom”, “in full bloom” and “passing out of bloom” – was determined for each species (Anderson & Hubricht 1940) within an area of 1.5 km radius around the apiary. During study periods meteorological data, such as temperature, rainfall and wind, were recorded since these climatic parameters have a great influence on pollen collection (e.g., Percival 1947, Louveaux 1968). Meteorological data for the region were provided by the Río Colorado weather station.

Sixteen samples of corbicular pollen (637.96 g) were obtained from pollen traps (Louveaux 1968), placed in six beehives randomly chosen from an apiary. Pollen loads collected during each day of sampling were considered as one sample. Each sample was weighed, carefully mixed and stored frozen in a labelled vial until analysed.

To identify pollen types and to determine the relative importance of the different plants in the honey bees’ diet, pollen loads were classified by colour, shape and texture under camera lucida. Colour-classified pollen loads were weighed and the percentages calculated (Montenegro et al. 1992). We assumed that each pollen load contains pollen from a single plant species as was previously shown (Percival 1947). Pollen grains from pollen loads were mounted on slides in two ways: fresh and dehydrated loads were acetolysed with glacial acetic acid (Erdtman 1960); then they were identified by comparison with pollen reference collections of the Division of Sistemática Vegetal of the Departamento de Agronomía of the Universidad Nacional del Sur (Andrada & Telleria 2002).

For nitrogen content determination, 50 mg of pollen type (AOAC 1980) was analysed by the micro-Kjeldahl methods (Bremner & Mulvaney 1982), and crude protein was estimated using the factor: 6.25 (Roulston & Cane 2000). The pollen samples weighing less than 50 mg were excluded. The analysis of pollen nitrogen content was

made in LANAIS N15 (National Laboratory of Research and Services UNS-CONICET), Departamento de Agronomía, Universidad Nacional del Sur.

For scanning electron microscope (SEM) unacetolysed pollen grains were disaggregated in ethanol, pipetted onto an unexposed common film, air-dried, transferred onto specimen stubs and coated with a thin layer of palladium-gold. Observations were made with a Jeol-JSMU SEM.

RESULTS

Meteorological conditions

Mean monthly temperature values varied little during the two periods of sampling ranging between 2.4 and 33.2°C (Table I). Temperature, as well as wind, affected the foraging activity. On cold or windy days the pollen collected was scarce. As previously shown, honey bees are active when the velocity of wind is lower than 25 km/h and temperature ranges between 14°C and 37°C (e.g., Percival 1947, Crane 1991). Precipitation in the Caldenal is concentrated in winter months, followed by progressive drying during spring and summer. The amount of unusually high rainfall during the first year of sampling was presumably due to the impact of the weather phenomenon, “El Niño” (Magrin et al. 1998), and the result was a profusion of growth of annual herbs and the unusual weak blooming of *Prosopis* sp.

During the second year of study the amount of rainfall was the expected average for the region.

Flowering availability and pollen collected by the honey bees

Flowering of 139 species was recorded out of which 75% are natives and 25% exotic. The dominant plant families in the area were Asteraceae (22.5%) and Fabaceae (9%), followed

Table I. Meteorological data and weight of pollen loads collected by honey bees.

Date	Pollen loads (g)		T Average (°C)		Wind speed mean (km/h)	Rainfall Average (mm)	
	1997	1998	Min.	Max.		1997	1998
29-Aug	3.50		11.4	20.3	3.6		
19-Aug		0.31	6.6	13.4	29.5	0	20
10-Sep	1.89		2.4	12.2	10.0 (max. 20.0)		
23-Sep		15.86	8.2	21.8	4.0		
23-Sep	3.87		5.8	18.8	16.0 (max. 30.0)	40	19
10-Oct	90.60		13.3	24.5	8.0		
24-Oct	136.96		13.0	27.5	12.0		
24-Oct		24.77	10.5	28.0	19.0	146	0
07-Nov	59.56		15.4	28.6	37.0		
18-Nov		25.14	12.6	28.2	27.0 (max. 40.0)		
18-Nov	47.10		13.4	26.4	13.0 (max. 20.0)	11	43
03-Dec	3.52		10.0	21.5	54.0		
16-Dec		121.62	13.7	28.2	10.0		
16-Dec	47.71		14.7	26.4	30.0	24	7
03-Jan	26.37		19.2	31.0	15.0 (max. 30.0)		
03-Jan		29.18	13.0	33.2	27.0 (max. 38.0)	93	35

by Poaceae which are scarcely visited by honey bees (e. g., Louveaux 1968). Availability of pollen was low at the end of winter, and then increased sharply to the maximum in spring, and declined towards the early summer (Fig. 3A & B). Twenty-nine species were utilised by the honey bees to obtain pollen, and only 16 of these species displayed individual values greater than 1% of the total pollen loads (Table II A & B). Mixed pollen loads represent less than 0.01% of the total loads crop.

Towards the end of winter (August - September) flowering was scarce, the main pollen contribution was by exotic plants such as Brassicaceae, whose pollen cannot be distinguished from each other due to palynological similarities (*Brassica nigra* (L.) W. D. J. Koch, *Capsella bursa-pastoris* (L.) Medik., *Diploaxis tenuifolia* (L.) DC., *Eruca vesicaria* (L.) Cav., *Hirschfeldia incana* (L.) Lagr.- Fossat, *Sisymbrium irio* L., and the natives *Descurainia argentina* O. E. Schulz, *Draba australis* R. Br. and *Lepidium* sp.), *Erodium cicutarium* (L.) L' Hér.; and the native *Schinus fasciculatus* (Griseb.) Hieron. The collection of *Puccinia interveniens* (Peck.) Bethel spores which infected the leaves of *Sphaeralcea australis* Speng occurred in this period. In the following period (from October to mid November), pollen from *Discaria Americana* Gillies & Hook., *Medicago minima* (L.) Bartal. and *Vicia pampicola* (Jacq.) DC. was added. Towards the end of apiculture season (from mid November to early January) pollen of *Carduus* sp., *Centaurea solstitialis* L., *Condalia microphylla* Cav., *Prosopidastrum globosum* (Gillies) Burkart, *Prosopis* sp. and *Grindelia tehuelches* (Speg.) Cabrera was also collected (Table II A).

During the second year of sampling the flowering of *Prosopis* was exceptionally abundant, and an additional blooming was recorded during early summer. This unusual availability of pollen was intensely utilised by honey bees (Table II B). At the end of winter the anemophilous *Plantago* sp. was included in the pollen collection, and *Chuquiraga erinacea* D. Don, *Larrea divaricata* Cav., and

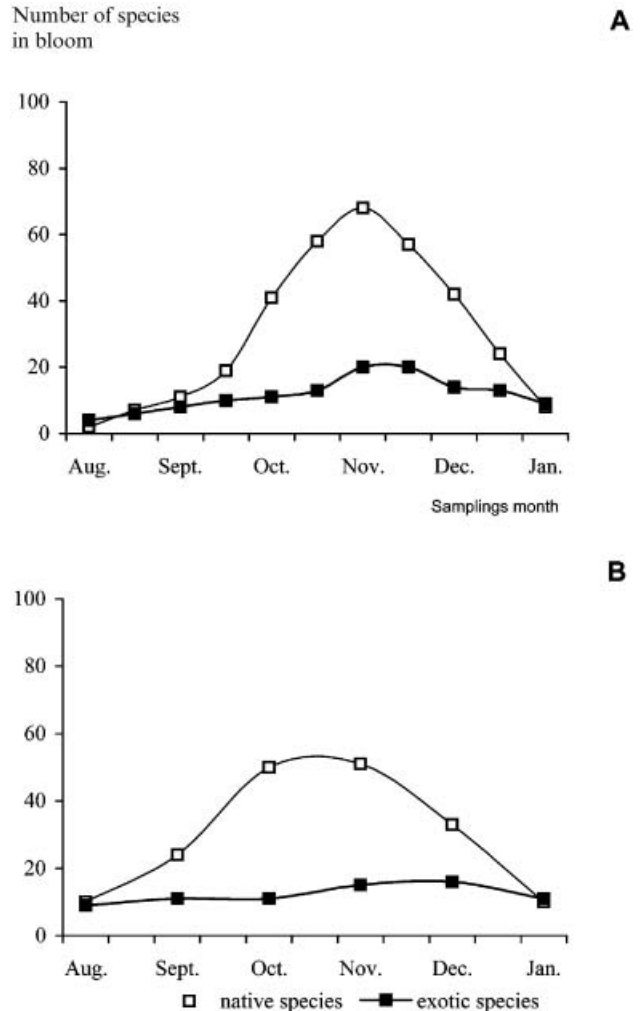


Fig. 3. Blooming of native and exotic plants in the Caldenal: A - in 1997; B - in 1998.

Table II. Data of blooming and percentages of pollen species, which were more than 1% of total loads collected during 1997 and 1998.

Heavy lines – “in full blooming”, thin lines – “coming into bloom” or “passing out of bloom”. * Native plants.

A. 1997. (1) Geraniaceae, (2) Anacardiaceae, (3) Rhamnaceae, (4) Fabaceae, and (5) Asteraceae.

B. 1998. (1) Geraniaceae, (2) Plantaginaceae, (3) Rhamnaceae, (4) Fabaceae, (5) Zygophyllaceae, and (6) Asteraceae.

A

Pollen types / 1997	AUG	SEP	OCT	NOV	DEC	JAN				
Brassicaceae	56	64	24	27	76	78	56	2	12	8
<i>Brassica nigra</i>			—————	—————	—————	—————				
<i>Capsella bursa-pastoris</i>	—————	—————	—————	—————	—————					
<i>Descurainia argentina</i> *			—————	—————	—————					
<i>Diplotaxis tenuifolia</i>				—————	—————	—————	—————	—————	—————	—————
<i>Draba australis</i> *	—————	—————								
<i>Eruca vesicaria</i>	—————	—————	—————	—————	—————					
<i>Hirschfeldia incana</i>					—————	—————				
<i>Lepidium</i> sp. *			—————	—————						
<i>Sisymbrium irio</i>			—————	—————	—————					
<i>Erodium cicutarium</i> (1)	30	31	71	19	9	<1				
<i>Schinus fasciculatus</i> * (2)	14	5	4	<1						
<i>Discaria americana</i> * (3)			<1	52						
<i>Medicago minima</i> (4)			<1	8	<1					
<i>Vicia pampicola</i> * (4)					11	<1				
<i>Carduus</i> sp. (5)					<1	7	13	3	<1	
<i>Centaurea solstitialis</i> (5)						<1	27	89		
<i>Condalia microphylla</i> * (3)					28	45	<1	<1		
<i>Prosopidastrum globosum</i> * (4)					4	22	12	2		
<i>Prosopis</i> sp. * (4)					<1	18	8			
<i>Grindelia tehuelches</i> * (5)						<1	36			
<i>Puccinia interveniens</i> (fungi spores)	<1	<1				<1				
other		1	<1	1	7	10	4	2	1	

B

Pollen types / 1998	AUG	SEP	OCT	NOV	DEC	JAN
Brassicaceae	20	45	94	12	2	8
<i>Brassica nigra</i>		—————	—————	—————		
<i>Capsella bursa-pastoris</i>	—————	—————	—————			
<i>Descurainia argentina</i> *			—————	—————		
<i>Diplotaxis tenuifolia</i>				—————	—————	—————
<i>Draba australis</i> *	—————	—————				
<i>Eruca vesicaria</i>	—————	—————	—————	—————		
<i>Hirschfeldia incana</i>				—————	—————	
<i>Lepidium</i> sp. *			—————	—————		
<i>Sisymbrium irio</i>			—————	—————		
<i>Erodium cicutarium</i> (1)	80	2	<1			
<i>Plantago</i> sp. * (2)		52				
<i>Condalia microphylla</i> * (3)				28	<1	
<i>Prosopidastrum globosum</i> * (4)				9	<1	1
<i>Prosopis</i> sp. * (4)				36	74	63
<i>Larrea divaricata</i> * (5)				12		
<i>Centaurea solstitialis</i> (6)				2	17	9
<i>Chuquiraga erinacea</i> * (6)					<1	18
<i>Onopordon acanthium</i> (6)					5	<1
other		1	5	1	1	1

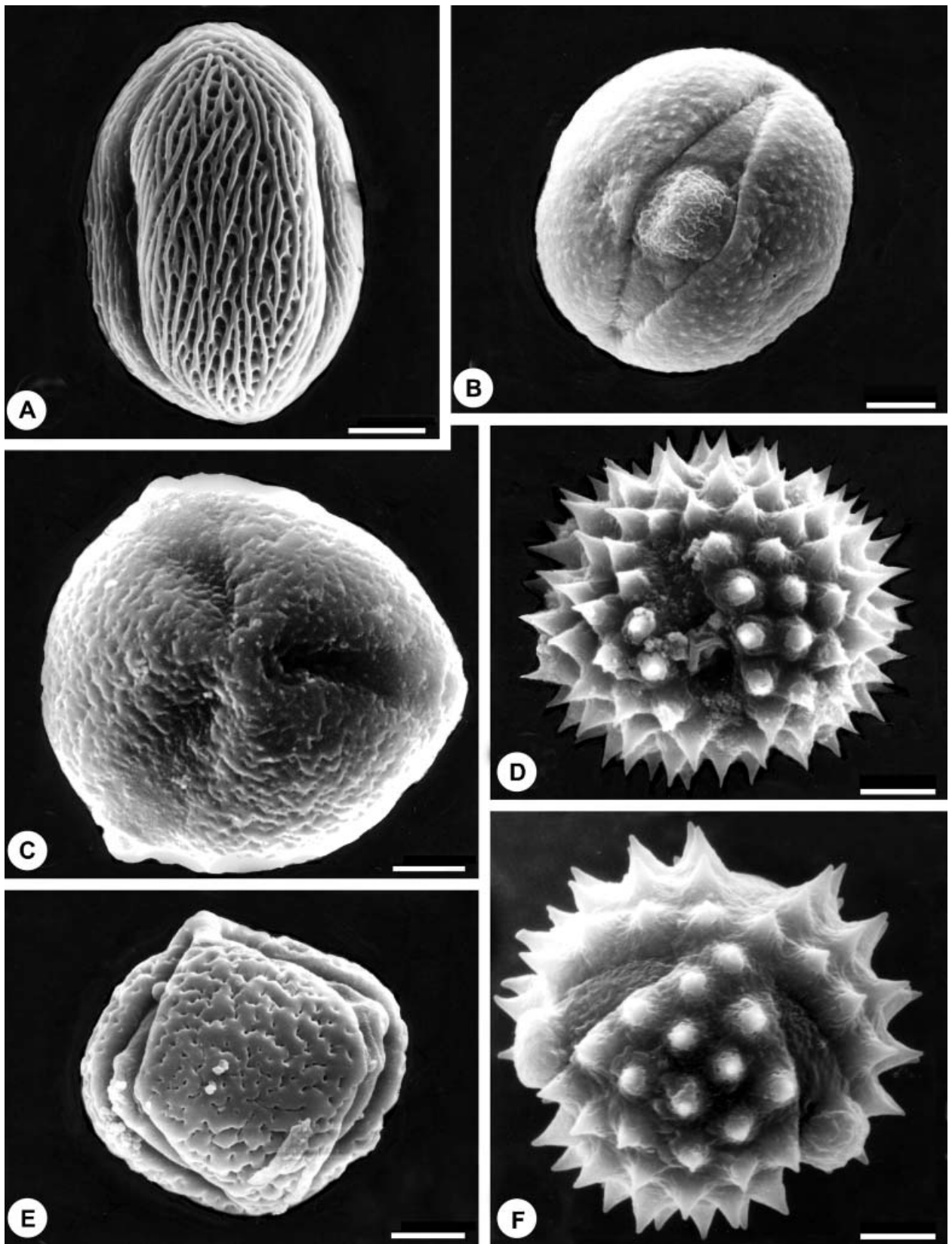


Fig. 4. SEM micrographs of main pollen types from native plants collected by the honey bees in the Caldenal: (A) *Schinus fasciculatus*; (B) *Chuquiraga* sp.; (C) *Prosopis alata*; (D) *Hysterionica jasionoides*; (E) *Prosopidastrum globosum*; (F) *Grindelia tehuelches*. Scale bar – 5 μ m (A, C, D & E); 10 μ m (B & F).

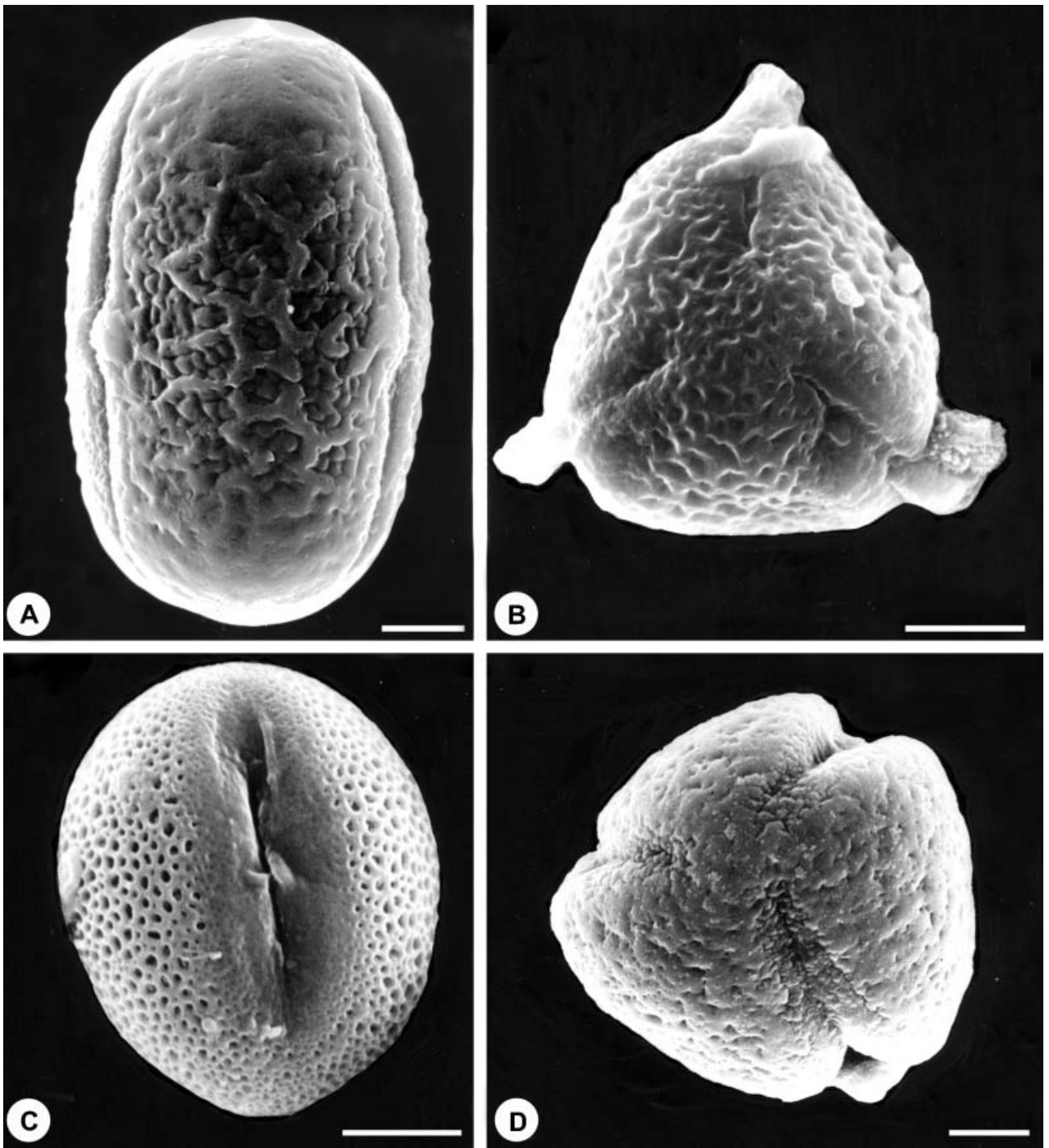


Fig. 5. SEM micrographs of main pollen types from native plants collected by the honey bees in the Caldenal: (A) *Vicia pampicola*; (B) *Condalía microphylla*; (C) *Larrea divaricata*; (D) *Discaria americana*. Scale bar – 5 μ m.

Onopordon acanthium L. during the end of spring and early summer (Table II B). Less than 1% of the pollen was represented *Lycium chilense* Miers, Poaceae, *Senecio* sp., *Sphaeralcea australis*, *Acantholippia seriphioides* (A. Gray) Moldenke, *Baccharis* sp., *Eucalyptus* sp., *Adesmia muricata* (Jacq.) DC. and *Hysterionica jasionoides* Wild. during the first year of sampling, and *Glandularia* sp., *Brachyclados* sp.,

Lactuca serriola L. and *Cereus aethiops* Haw. during the second year.

Throughout the study periods the pollen forage was constant on Brassicaceae, *Erodium cicutarium*, *Carduus* sp., *Condalía microphylla*, *Prosopidastrum globosum* and *Prosopis* sp.

Abundant pollenkitt was observed on pollen of

Table III. Nitrogen and protein content of pollen collected by honey bees in the Caldenal.

* Native plants.

Taxa	Nitrogen content	Crude protein (%)
<i>Acantholippia seriphoides</i> *	3.68	23.0
Brassicaceae	4.87	30.5
<i>Carduus</i> sp.	2.83	17.7
<i>Centaurea solstitialis</i>	3.63	22.7
<i>Cereus aethiops</i> *	3.41	21.3
<i>Chuquiraga erinacea</i> *	3.60	22.5
<i>Condalia macrophylla</i> *	5.04	31.5
<i>Discaria americana</i> *	5.11	31.9
<i>Erodium cicutarium</i>	2.27	14.2
<i>Grindelia tehuelches</i> *	2.67	16.7
<i>Hysterionica jasionoides</i> *	2.64	16.5
<i>Lycium chilense</i> *	4.06	25.4
<i>Medicago minima</i> *	3.53	22.1
<i>Onopordon acanthium</i>	3.08	19.3
<i>Plantago</i> sp.*	2.17	13.6
<i>Prosopidastrum globosum</i> *	3.57	22.3
<i>Prosopis</i> sp.*	4.35	27.2
<i>Puccinia interveniens</i> ("Rust")	2.54	15.9
<i>Schinus fasciculatus</i> *	3.86	24.1
<i>Senecio</i> sp.*	2.48	15.5
<i>Sphaeralcea australis</i> *	3.34	20.9

Brassicaceae, *Hysterionica jasionoides*, *Larrea divaricata*, *Chuquiraga erinacea*, and *Schinus fasciculatus*, and on the less collected pollen *Senecio* sp. and *Acantholippia seriphoides* (Figs 4 & 5).

Protein content of collected pollen

Protein content of most collected pollen was variable. Optimal levels (>20% of protein, according to Shaw 1999) were noted on pollen of: Brassicaceae, *Centaurea solstitialis*, *Chuquiraga erinacea*, *Condalia microphylla*, *Discaria americana*, and *Prosopis* sp. While low levels of protein were found in the pollen of *Erodium cicutarium*, *Plantago* sp., *Grindelia tehuelches*, and spores of *Puccinia interveniens* (Table III).

DISCUSSION

Flower availability in the Caldenal is brief as generally occurs in semi-arid ecosystems (Moldenke 1976). It begins towards the end of winter and continues through spring until early summer. The amount of rainfall for the first year sampling was unusually high and concurrent with the weak flowering of one of the most conspicuous elements of the Caldenal, *Prosopis* (*P. alpataco* Phil., *P. caldenia* and *P. flexuosa* DC.). This poor effect of rain on *Prosopis* flowering was also observed by other authors (Fisher et al. 1973, Distel & Peláez 1985, Lee & Felker 1992); but in other studies such effect is neglected (O'Neill & Waller 1984, Karling & Díaz 1988).

Within the great diversity of pollen sources, the plants intensely utilised by honey bees were few. This foraging

pattern was also observed in other studies, performed in different ecosystems (e. g., Percival 1965, Louveaux 1968, Telleria, 1993).

Fluctuations of flowering during the apiculture period are reflected in the composition of pollen collected by honey bees. Most native plants do not bloom until relatively late in the season in comparison with exotic species. Thus, at the end of winter and early spring, the exotic *Erodium cicutarium* and Brassicaceae, and the native *Schinus fasciculatus*, are the most important sources of pollen. The early flowering of exotic plants and their utilization by honey bees were also observed in the Paraná River delta (Basilio 1998) and in the lower valley of Chubut River (Forcone 2002). During the period of pollen shortage, honey bees collected spores of *Puccinia interveniens*, which infected the leaves of *Sphaeralcea australis* (Andrada & Telleria 2001). The collection of fungal spores by honey bees, instead of pollen, is related to a dearth of pollen. In spite of the lower nutritional value of fungal spores, they contribute to the pollen diet (Shaw 1999).

During spring the increase of flowering species was reflected in the pollen loads composition. Many native plants of the families Asteraceae (*Grindelia tehuelches*, *Hysterionica jasionoides* and *Senecio* sp.), Fabaceae (*Adesmia muricata*, *Prosopidastrum globosum*, *Prosopis* sp. and *Vicia pampicola*), Malvaceae (*Sphaeralcea australis*), Plantaginaceae (*Plantago* sp.), Rhamnaceae (*Condalia microphylla* and *Discaria americana*), Solanaceae (*Lycium chilense*), Verbenaceae (*Acantholippia seriphoides* and *Glandularia* sp.), Zygophyllaceae (*Larrea divaricata*), and Poaceae were collected together with the exotic Brassicaceae and *Carduus* sp. At the end of the season Asteraceae (*Centaurea solstitialis* and *Chuquiraga erinacea*) was also intensely collected. From the study of honey from the Caldenal, most of these taxa were also identified as important sources of nectar (Andrada & Telleria 2002). *Prosopis* was recognised as a relevant source of nectar for honey bees by Genise et al. (1990), but from our results it is evident that the pollen is also intensely utilised by honey bees.

Honey bees collected pollen widely different in protein content; but has, in general, high levels of protein (>20%), enough to satisfy the nutritional requirements of honey bees (Shaw 1999). Within this group, pollen from Brassicaceae and Rhamnaceae (*Condalia microphylla* and *Discaria americana*) have the major protein content, followed by Fabaceae (*Medicago minima*, *Prosopidastrum globosum* and *Prosopis* sp.), *Schinus fasciculatus*, *Acantholippia seriphoides*, and *Sphaeralcea australis*. Different protein content was found in Asteraceae. In this family, some species such as *Carduus* sp., *Grindelia tehuelches*, *Hysterionica jasionoides*, *Onopordon*, and *Senecio* have low protein content, while *Centaurea solstitialis* and *Chuquiraga erinacea* have high protein content. Forcone (2002) reported similar results. These results show that honey bees collected pollen with different protein content and not just those particularly rich in protein, as has been noted (Moezel van der et al. 1987, Roulston & Cane 2000).

In summary, pollen of native plants of the Caldenal is intensely utilised by honey bees and, in general, is a nutritional food for them because it combines high protein

content and lipids. Beekeepers should take these results into account in apiculture exploitation of the Caldenal. Studies carried out in different regions of our country showed that the introduction of honey bees reduces native bees' communities, and may be deleterious for many of the native plants whose pollination largely depends on these native insects (Aizen & Feinsinger 1994, Eynard & Galletto 2002). Consequently, it is necessary to increase the ecological studies in order to make decisions regarding sustainable resource management of this region.

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