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Pollen and Nectar Sources used by Honeybee Colonies pollinating Sunflower (*Helianthus annuus*) in the Colorado River Valley, Argentina

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Summary: Pollen traps and beeswax foundations were set in order to study pollen and nectar sources used by honeybee colonies pollinating sunflower (*Helianthus annuus* L.) for the production of hybrid seed in the lower valley of the Colorado river in southern Argentina. Thirty-seven plant species in bloom were registered in the area surrounding the sunflower field. Most of them were scarcely represented. The honeybee maximum density recorded on the sunflower male-fertile line was 10.00 bees per 100 heads, while on the male-sterile line was 25.33 bees per 100 heads. Seven plant taxa were identified in the pollen loads and 26 in the honey samples. About 84% of the collected pollen came from three taxa: *Centaurea solstitialis* L., *Eucalyptus camaldulensis* Dehnh. and *Cirsium vulgare* (Savi) Airy - Shaw, and only 11% came from *H. annuus*. The taxa most gathered had high protein values, above 20%. The dominant and secondary pollen types in honey samples were *Tamarix gallica* L., *E. camaldulensis*, Brassicaceae and *C. solstitialis*. The results indicate that honeybees (*Apis mellifera* L.) foraged pollen and nectar mainly from the flora surrounding the sunflower field.

Key words: pollen loads, nectar, *Apis mellifera*, melliferous flora, sunflower.

Resumen: Fuentes de polen y néctar utilizadas por colonias de *Apis mellifera* que polinizan girasol (*Helianthus annuus*) en el valle inferior del río Colorado, Argentina. Se colocaron trampas caza-polen y cuadros con cera estampada para estudiar las fuentes de polen y néctar utilizadas por colmenas que polinizan girasol para la producción de semilla híbrida en el valle inferior del río Colorado, Argentina. Treinta y siete especies en floración fueron registradas en cercanías al cultivo de girasol. La mayoría de ellas estuvieron escasamente representadas. La máxima densidad de abejas en la línea androfértil de girasol fue de 10,00 abejas por cada 100 inflorescencias, mientras que en la línea androestéril fue de 25,33 abejas por cada 100 inflorescencias. En las cargas polínicas se identificaron siete taxa, y 26 en las muestras de miel. El 84% en peso del polen recolectado correspondió a tres taxa: *Centaurea solstitialis* L., *Eucalyptus camaldulensis* Dehnh. y *Cirsium vulgare* (Savi) Airy - Shaw y sólo el 11% provino de *H. annuus*. Los taxa más cosechados tuvieron valores de proteína altos, superiores a 20%. Los tipos polínicos dominantes y secundarios en las muestras de miel fueron *Tamarix gallica* L. y *E. camaldulensis*, Brassicaceae y *C. solstitialis*. Los resultados indican que las abejas (*Apis mellifera* L.) recolectaron polen y néctar principalmente de la flora circundante al cultivo de girasol.

Palabras clave: cargas polínicas, néctar, *Apis mellifera*, flora melífera, girasol.

Introduction

Honeybees (*Apis mellifera* L.) show different levels of preference for the flora surrounding the hive. Both pollen and nectar foragers participate in the pollination of crops, being the nectar foragers usually in a larger number (Free, 1964; Robinson, 1978; Ortiz & Fernández, 1992). Honeybee pollination activity is very important in seed production of hybrid sunflower (*Helianthus annuus* L.), although other insects can cooperate (Smith, 1978; DeGrandi-Hoffman & Watkins, 2000).

Even when the crop is in full flower, a good nectar or pollen flow from other plants can attract the bees away from it (Crane, 1990). Pollen sources nearby sunflower fields attract pollinating honeybees as demonstrated by Bedascarrasbure *et al,* (1985) in the Buenos Aires province, Argentina.

The aim of the present work was to study nectar and pollen sources used by honeybee colonies pollinating a sunflower crop to produce hybrid seed in the lower valley of the Colorado river, Argentina.

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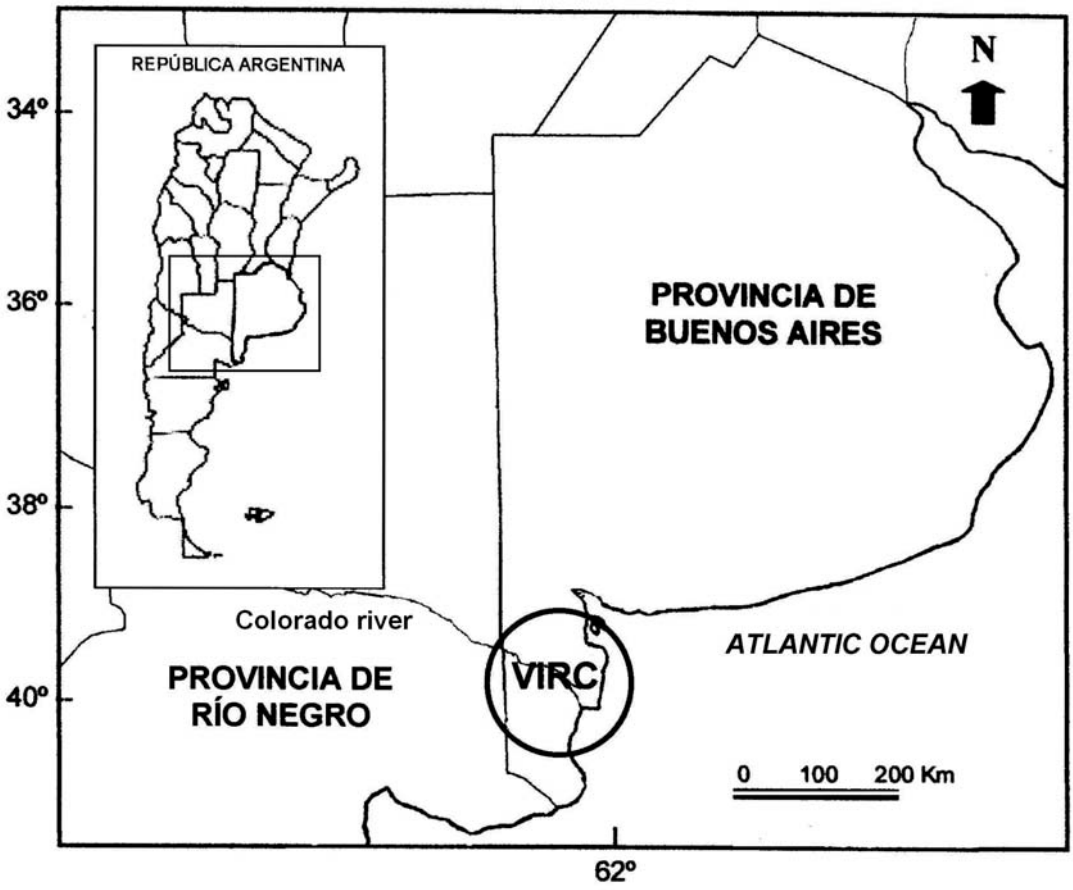

Figure 1. Geographical location of the lower valley of the Colorado river (VIRC), south of Buenos Aires province, Argentina.

Material and Methods

The study was conducted in the area surrounding a 30 ha field for hybrid sunflower seed production in the irrigation district of the lower valley of the Colorado river, south of Buenos Aires province, Argentina (Fig. 1).

Considering that 95% of bee foraging activity takes place within the surrounding 3000 m (Visscher & Seeley, 1982), the identification of plant species in blossom all around the sunflower field was made within a distance of 2500 m approximately. Regional flora identification was performed according to Lamberto *et al.,* (1997). Abundance-coverage was assessed using the Braun-Blanquet (1950) scale for phyto-sociological census.

The blooming season of the different species was registered according to Anderson & Hubritch (1940), which consider three phenological stages: "coming into flower", "in full bloom", "out of bloom".

The field was rectangular in shape, north-south orientated. The two sunflower genotypes were seeded 0.70 m apart on October 8th 1999, alternating ten rows of the male-sterile line and two of the male-fertile line, and adding six days later a third male-fertile line between the other two ones.

At the beginning of the flowering period (December 30th, 1999), when male-sterile line (S) was at R 5.1 development stage according to the Schneiter & Miller (1981) scale, 60 Langstroth hives were set with a brood chamber and one super in the study area. Hives were placed on both longer sides of the plot, named East and West. The seeding rows were perpendicular to those sides. Within each group there were three sub-groups of ten hives, 5 m apart from the field and 250 m apart one from another approximately. The density was of two hives ha^{-1}, commonly used in hybrid sunflower seed production in Argentina (Zorzín &

Woodward, 1998) and the minimum recommended by Goebel (1984). Two beehives were randomly selected at each side, and a marked beeswax foundation was placed in the super. These marked honey-combs were taken out 20 days later for pollen analysis.

In order to evaluate if the foraging activity was highly enough, the number of honeybees per 100 sunflower heads was recorded every two hours, from 09:00 to 17:00 hours (solar time) when line S was at R5.5 and the male-fertil line (M) at R5.9 (January 5th, 2000). Three replications were done on both parental lines. A double way analysis of variance was performed.

Pollen traps were placed at the hive entrances in the selected hives on the same date. Pollen loads were sampled between 08:00 and 18:00 hours (solar time).

The pollen loads were firstly sorted by color shape and texture, assuming that each pellet was an homogeneous mass of pollen from a single plant (O'Neal & Waller, 1984). This classification was checked under a stereoscopic microscope and then pollen loads were weighed and the percentages calculated (Montenegro *et al.,* 1992). Pollen grains from pellets were mounted on slides using the technique proposed by Wodehouse (1935) and the microacetolysis technique (Pla Dalmau, 1961). Pollen types were identified using the pollen reference collection from the botanical laboratory of the Departamento de Agronomía, Universidad Nacional del Sur.

Dry weight (55°C during 48 hours) per load of each pollen type was calculated on five replicates of 30 loads each. Nitrogen content determination was performed on 50 mg samples of each pollen type (AOAC, 1980) by the micro-Kjeldahl method (Bremner & Mulvaney, 1982), and crude protein was estimated using the factor 6.25 (Roulston & Cane, 2000). 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.

Pollen analysis of honeys followed the International Bee Research Association rules (Louveaux *et al.,* 1978). Morphological types identification was made using a reference palynotheca and phototheca, as well as specialized literature (Erdtman, 1966; Markgraf & D'Antoni, 1978).

The pollen types were identified at species level when possible, or to genus, tribe or family level. Pollen grains of *Cirsium vulgare* and *Carduus* L. sp., morphologically very similar, were included in the same pollen type of *Carduus* sp.- *Cirsium vulgare* (Tormo Molina & Ubera Jiménez, 1995), being recognized in the pollen loads by their colour.

Results

Thirty-seven plant species in bloom were registered in the area surrounding the sunflower field. Most of them were scarcely represented, 86% belonging to the «rare specimens» score, individuals infrequently seen, in the Braun-Blanquet frequency/abundance scale (Table 1).

Bee foraging activity showed differences between both lines of sunflower, and a highly significant effect ($P \leq 0,01$) of the "sunflower line-foraging time" interaction. Line S showed a remarkable increase during the morning, reaching its maximum at 13:00 h. The number of bees per head in line M was lower than in line S from 11:00 h onward ($P \leq 0,05$), and the variations in the studied time interval were small (Table 2). The maximum activity registered, 25 bees for every 100 heads on line S.

Wild pollinators were collected and identified, most of them belonging to the native species *Melissoptila* (M.) *tandilensis* Holmberg (Hymenoptera: Apidae: Eucerini). Individuals belonging to *Pseudagapostemon* sp. (Hymenoptera: Halictidae: Agapostemonini) and *Dialictus* sp. (Hymenoptera: Halictidae: Halictini) were also present.

Although *Centaurea* and *Eucalyptus* are stenopalynous genera (Erdtman, 1966; Tormo Molina & Ubera Jimenez, 1988), the pollen types identified appointed to *Centaurea solstitialis* and *Eucalyptus camaldulensis* respectively, species that were present in the surrounding area of the hives.

The analysis of the pollen loads revealed that 95% of the collected pollen came from four taxa: *C. solstitialis, E. camaldulensis, C. vulgare* and *H. annuus*. The most important pollen source was *C. solstitialis*. The relative contribution of *H. annuus* was small in all samples, even when sunflowers were in full bloom (Fig. 2).

The most abundant pollen load types had protein values over 20% (Table 3). Sunflower, showing 14.2% protein, contributed with 11% of the collected pollen only.

A total of 26 pollen types were identified in honey analysis, 81% belonging to nectar or pollen-nectar plants. The dominant pollen category comprised *Tamarix gallica* and *Eucalyptus camaldulensis*. The secondary pollen types were Brassicaceae, Casuarinaceae, Chenopodiaceae–Amaranthaceae, Elaeagnaceae, Fabaceae, Myrtaceae, Pinaceae, Plantaginaceae, Poaceae, Polygonaceae, Solanaceae, Tamaricaceae, Verbenaceae and Zygophyllaceae (Table 4).

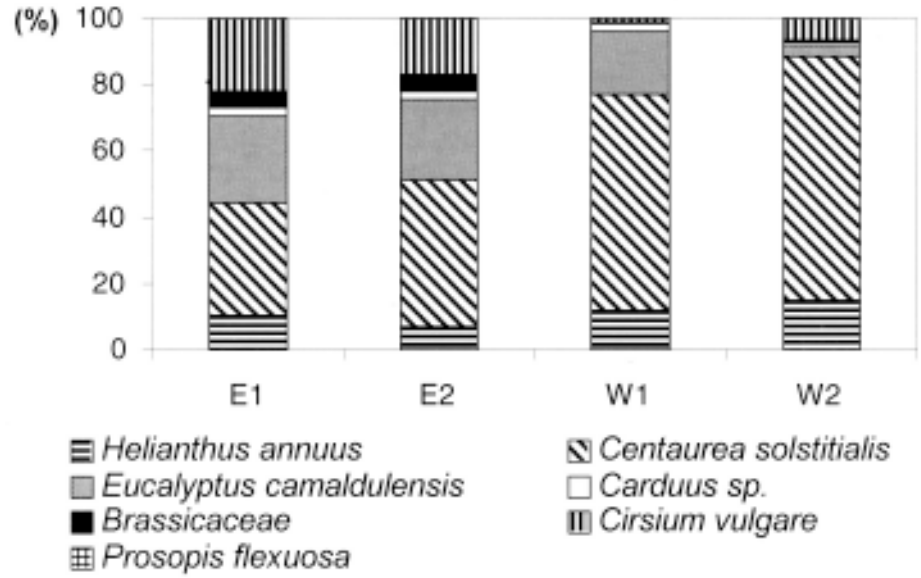

Figure 2. Pollen types present in the pollen loads, as dry weight percentage of the total collected pollen in each site. E1 and E2: East samples. W1 and W2: West samples.

Discussion

A large number of plant species in bloom were present in the area surrounding the hives, but most of them had very low levels of abundance-coverage. Only few species were used by honeybees as pollen and nectar sources in comparison with the large number of species recorded, which is in accordance with other authors (Louveaux, 1968; Parent *et al.,* 1990; Andrada, 2001).

The sunflower field in full bloom offered nectar and pollen. The maximum honeybee density on line S was similar to that considered enough for pollinating a commercial field (INTA, 1983) while on line M the bee density was much lower. At harvest, the percentage of hollow achenes was low (13,07± 15,22%; Paoloni, personal communication).

Bees that only get pollen from sunflower may suffer from moderate physiological and nutritional stress, as revealed by their reduced survival (Schmidt *et al.,* 1995), while collection of pollen from different species ensures a varied diet, satisfactory for their development (Louveaux, 1968).

Pollen is the main source of protein in the diet of honeybees, and it is fundamental for their development and growth (Stanley & Linskens, 1974; Pernal & Currie, 2000). Protein content is considered by many authors as a factor influencing the preference of pollen by bees (Louveaux, 1958; Schmidt & Johnson, 1984), however other works do not found evidences of this relationship (van der Moezel *et al.*, 1987).

Protein content in sunflower pollen is under 15%, what would contribute to explain its low proportion in pollen loads (Table 3). The low preference of bees to this pollen has been already reported by several authors (Louveaux, 1959; Fonta *et al.,* 1985).

Pollen loads of *Prosopis flexuosa* DC., *E. camaldulensis* and *C. solstitialis* had more than 20% protein. Eucalypt and yellow star thistle (*C. solstitialis*) were in full blossom and represented together 70% of the collected pollen. Eucalypt contribution was high even when the abundance-coverage estimation was rare in the area, confirming that its presence near a sunflower field is highly attractive to bees (Bedascarrasbure *et al.,* 1985). Yellow star thistle is a melliferous weed well known by local beekeepers. *P. flexuosa* was scarcely represented because its abundance-coverage score was very low and its flowering period was ending (Tables 1 and 3).

The former results agree with observations made by Shaw (1999), who found that levels of crude protein less than 20% did not satisfy the colony requirements, being the ideal levels those that overpass the 23%. Other factors like weight and humidity of loads did not exhibit any relationship with the collected pollen quantity (Table 3). The pollen loads of bull thistle (*Cirsium vulgare*) had a nitrogen content somewhat lower than those of yellow star thistle and eucalypt, but it was gathered in a significant proportion. These three species totalize about 84% of the collected pollen.

Table 1. Abundance-coverage estimation and blooming stages of plant species registered in the area surrounding the sunflower field (east and west zones). Taxa are alphabetically sorted by family. B-B: Braun-Blanquet score. +: rare, with small cover; 1: few, 5-10 % cover; 2: numerous, 10-25 % cover; 3: very numerous, 25-50 % cover. A&H: Anderson & Hubritch stages. C: coming into flower; F: in full bloom; O: out of bloom; X: present

B-B		A & H			TAXA	
East	West	C	F	O	Family	Cientific name
+			X		Apiaceae	*Ammi majus* L.
	+		X		Asteraceae	*Baccharis* sp.
+	1			X		*Carduus thoermeri* Weinm.
+				X		*Carduus pycnocephalus* L.
+	+		X			*Centaurea solstitialis* L.
	+	X				*Cichorium intybus* L.
+	+	X	X			*Cirsium vulgare* (Savi) Ten
+			X			*Cynara cardunculus* L.
	+	X				*Gnaphalium* sp.
3	3		X			*Helianthus annuus* L.
+	+		X			*Sonchus oleraceus* L.
	+			X		*Taraxacum officinale* Webwe
1			X			*Tessaria absinthioides* (Hook. et Arn.) DC
+			X		Boraginaceae	*Heliotropium curassavicum* Vahl
+			X		Brassicaceae	*Diplotaxis tenuifolia* (L.) DC.
2	+		X			*Hirschfeldia incana* (L.) Lagr.-Fossat
+	+			X		*Sisymbrium irio* L.
+			X			*Sisymbrium orientale* L.
+		X			Chenopodiaceae	*Atriplex semibaccata* R. Brown
+	+	X				*Atriplex* sp.
+			X		Convolvulaceae	*Cuscuta indecora* Choisy
	+	X				*Convolvulus arvensis* L.
+	+		X		Cucurbitaceae	*Cucumis anguria* L.
+			X		Fabaceae	*Hoffmannseggia trifoliata* Cav.
2	3		X			*Medicago sativa* L.
+			X			*Melilotus albus* Desr.
	+			X		*Prosopis flexuosa* DC.
+			X		Malvaceae	*Sida leprosa* (Ort.) K. Schum.
+			X			*Sphaeralcea australis* Speg.
+	+	X	X		Myrtaceae	*Eucalyptus camaldulensis* Dehnh.
+			X		Poaceae	*Thinopyrum ponticum* (Podp.) Barkw. & Dewey
+			X		Polygonaceae	*Polygonum aviculare* L.
+		X	X	X	Solanaceae	*Lycium chilense* Miers ex Bertero
+			X			*Solanum elaeagnifolium* Cav.
+				X		*Solanum pyrethrifolium* Griseb.
+			X		Tamaricaceae	*Tamarix gallica* L.
+			X		Zygophyllaceae	*Tribulus terrestris* L.

Table 2. Abundance of bees (*Apis mellifera* L.) observed on sunflower heads of both parental lines. Values in table show the average number of bees every 100 heads ± standard error.

Time	9:00 h	11:00 h	13:00 h	15:00 h	17:00 h
Line S	7.33 ± 0.33	20.33 ± 0.88	25.33 ± 0.88	20.00 ± 0.57	19.67 ± 0.33
Line M	10.00 ± 0.57	7.33 ± 0.33	9.67 ± 0.88	8.33 ± 0.33	10.00 ± 0.57

Table 3. Percentage of the collected pollen belonging to each taxa, and characteristics of the pollen loads: dry weight, humidity and protein content (N x 6.25). * Only one pollen load was collected.

Pollen type	Crude protein [%] (N x 6.25)	Collected pollen [% by weight]	Pollen loads dry weight [mg]	Humidit [% dry weight basis]
		--- Average ± standard error ---		
Helianthus annuus	14.2	10.95 ±1.68	8.33 ± 0.26	8.00 ± 0.21
Centaurea solstitialis	20.7	54.46 ±9.22	10.75 ± 0.21	7.25 ± 0.23
Eucalyptus camaldulensis	23.0	18.20 ±5.22	9.65 ± 0.21	7.12 ± 0.16
Carduus sp.	15.7	1.97 ±0.44	7.61 ± 0.37	7.44 ± 0.11
Brassicaceae	18.8	2.89 ±1.37	13.31 ± 0.33	6.92 ± 0.27
Cirsium vulgare	18.2	11.52 ±4.76	10.94 ± 0.60	7.33 ± 0.16
Prosopis sp.	27.2	0.01 ±0.01*		

Table 4. Identified pollen types in honey samples and corresponding proportions. E1 and E2: East samples. W1 and W2: West samples. * anemophilous pollen

Plant taxa	E 1	E 2	W 1	W 2
	Pollen content in honey (%)			
Apiaceae				<1
Astereae	<1	<1		<1
Brassicaceae	10.8	27	17.9	26.6
Carduus sp.-*Cirsium vulgare*	2.2	<1	2.7	<1
Centaurea solstitialis	1.8	26.4	7	11.1
Cichorium intybus			<1	
Elaeagnus sp.	<1	<1	<1	<1
Eucalyptus camaldulensis	8.5	41.3	47.6	50.5
Helianthus annuus	2.2	1.3	14.5	6
Lotus sp.		<1		
Lycium chilense		<1		<1
Matricaria recutita	<1			
Medicago sativa	2.7	<1	2.4	3.1
Melilotus albus	5.2	<1		
Phyla canescens	<1			
Polygonum aviculare	<1			
Solanum sp.	<1	<1		
Sonchus oleraceus		<1		
Tamarix gallica	64	1.5	7	1
Tribulus terrestris	<1			
Vicia sp.			<1	
Casuarina sp. *	<1		<1	<1
Chenopodiaceae-Amaranthaceae *	<1	<1		<1
Pinus sp. *		<1	<1	<1
Plantago sp. *	<1		<1	
Poaceae *		<1	<1	

The honey analysis showed the presence of several taxa, even though only eight overpassed 1% of the pollen grains content in one or more samples. The low sunflower pollen content in all samples could be explained considering the high proportion of sunflower plants offering only nectar (*i.e.* 77% male-sterile plants) and the fact that sunflower pollen is underrepresented in honeys (Accorti *et al*., 1986).

Colonies located in the East side used the white sweet-clover (*Melilotus albus* Desr.) as a nectar source, a species found only in that sector. Tamarisk plants (*Tamarix gallica*) located near the sunflower field in the East side made an important nectar contribution to the nearest sampled hive (Tables 1 and 4). The absence of tamarisk pollen in the pellet samples could be attributed to its low protein content (17.8 %) according to Forcone (2002).

Lucerne (*Medicago sativa* L.) offers nectar and pollen to honeybees, but the pollination-tripping mechanism in the flower hits the bee. Honeybees often learn to avoid the blow to the head, while still robing the nectar and leaving the tripping mechanism unsprung (Morse & Hooper, 1992). This behaviour explains that, having lucerne high values of abundance-coverage, the pollen of this species was found in honeys in percentages lower to 4% and absent in pollen loads (Tables 1 and 4).

The Brassicaceae family, of great nectar importance (Crane *et al.*, 1984), was well represented in honeys and poorly in pollen loads. Eucalypt pollen grains were found in all honey samples despite there were very few specimens within the foraging area. The great attractiveness of this genus, present in almost all honeys of the south-east Pampas (Valle *et al.,* 1995; Andrada *et al.,* 1998), and the fact that its pollen is over-represented in honey (Ortiz & Fernández, 1992), would explain this findings. Other outstanding taxa were *C. solstitialis* and *Carduu*s sp.-*Cirsium vulgare*, melliferous weeds frequently found in Buenos Aires province honeys (Tellería, 1996; Andrada *et al.,* 1999) (Tables 3 and 4).

Considering the association between the morphologic types determined in pollen loads and honeys, and the abundance-coverage of the flowering species in the East or West sides (Table 1), it can be assumed that only in some cases the pollen loads or honey samples reflect the flora surrounding the hives.

Some authors recommend weed control in the areas around the sunflower field to prevent the presence of competitive flora (Zorzín & Woodward, 1998). In contrast, it was suggested that farmers should have small areas with other cultures in the neighborhood of the sunflower fields, or allow the presence of weeds in areas along the edges of the field to provide supplementary pollen sources for bees in order to reduce the problem of a potential stress derived from the ingest of sunflower pollen as the only source (Schmidt *et al.,* 1995).

Honeybee colonies located in the lower valley of the Colorado river foraged intensely the flora surrounding a sunflower field, in order to obtain pollen and nectar, being the main sources *C. solstitialis*, *E. camaldulensis, C. vulgare-Carduus sp., T. gallica* and some species belonging to the Brassicaceae family.

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