Revista Chilena de Historia Natural
70: 91-99, 1997

Pollen loads: source of carotenoids originating from the mediterranean plant communities of the central zone of Chile

El polen corbicular: fuente de carotenoides proveniente de las comunidades de clima mediterráneo de la zona central de Chile

GLORIA MONTENEGRO, GUACOLDA AVILA, DENISE ROUGIER and BARBARA TIMMERMANN[1]

Departamento de Ecología, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile
[1]College of Pharmacy, University of Arizona, Tucson, USA

ABSTRACT

The purpose of this research was to asses the possibility of utilizing *Apis mellifera* as a natural harvester of carotenoids, contained in pollen loads from various plant species. A discussion of the utility of carotenoids in terms of their nutritional value to various life processes is followed by an analysis of carotene productivity of pollen loads from various beehives from El Litral, Paine locality, in the mediterranean zone of Central Chile. These data are then used to identify species that have been selected by honeybees as a pollen source and to determine the significance of various species of the native and introduced flora in terms of the carotene presence of their pollen loads. We conclude that the utilization of honeybees as vehicles for harvest period of carotenoids is feasible, since pollen loads from at least one species per month were shown to posses them in significant quantities.

Key words: Honeybee, carotenoid content, pollen loads, hive productivity, native plant species.

RESUMEN

El objetivo de esta investigación fue estudiar la posibilidad de utilizar a *Apis mellifera* L. como un recolector natural de carotenoides, los que están contenidos en las cargas polínicas de varias especies vegetales. Se discute la utilidad de los carotenoides en términos de su valor para varios procesos vitales y se hace un análisis de la productividad de cargas polínicas provenientes de varias colmenas de la localidad de El Litral, Paine, situada en la zona mediterránea de Chile central. Se identificaron las especies que son seleccionadas por las abejas como fuente de polen y se determinó la importancia de varias especies nativas e introducidas en términos de la presencia de carotenoides de sus cargas polínicas. Concluimos que es posible la utilización de las abejas melíferas como recolectores de carotenoides durante el período de cosecha dado que, al menos una especie en cada mes, muestra tener tales pigmentos.

Palabras clave: Abeja melífera, contenido de carotenoide, carga polínica, productividad de colmena, especie vegetal nativa.

INTRODUCTION

The microscopic analysis of pollen loads allows demonstration of which plant species are utilized as a source of pollen in a determined territory of the country (Synge 1947, Percival 1947, 1950, 1955, Loveaux 1958a, 1958b, Montenegro et al. 1989, Varela et al. 1991, Iturriaga et al. 1992). For this purpose the specific morphological characteristics of the pollen grain and pollen loads are a useful tool (Montenegro et al. 1991[1], Montenegro et al. 1992[2]). In Chile, this analysis has permitted the compilation of lists of plant species used by honeybees as a source of nectar and pollen

[1] MONTENEGRO G, M GOMEZ, G AVILA, AM MUJICA, L ITURRIAGA & R GINOCCHIO (1991). Pollen harvesting by honeybees in mediterranean shrublands of Chile. Procc. VI International conference on Mediterranean Climate Ecosystems. Maleme (Crete), Greece.

[2] MONTENEGRO G, G AVILA, L ALDA, E CARDALDA, R GINOCCHIO, M GOMEZ, L. ITURRIAGA, AM MUJICA, D RIOS, G RIZZARDINI, D

(Received 13 December 1995; accepted 30 October 1996; managed by Bernabé Santelices)

(Montenegro et al. 1989[3]), an important accomplishment since honeybees have been shown to be selective in their visits to flowers (Synge 1947, Free 1963, 1967, Rashad et al. 1979).

There has been a growing interest in the diversification of the use of products derived from beehive activity. It is known, for example, that pollen is the primary source of protein for bees (Haydak 1970, Van der Moezel et al. 1987). Several studies have confirmed the presence, in variable quantities, of proteins and/or free aminoacids in the pollen harvested by *Apis mellifera* (Todd & Bretherick 1942, Nielsen et al. 1955, Gilliam et al. 1980, Solberg & Remedios 1980, Loper & Cohen 1987, Rougier et al. 1994). The fact that *A. mellifera* appear to prefer protein-rich pollens (Rougier et al. 1994), raises the possibility of utilizing these pollens as nutritional compliments with the bee acting as a natural harvester of pollen, and therefore plant protein.

Another aspect of the pollen grain, the possession of pigments, has received little interest until recently. The color of pollen loads, and therefore of pollen grain, is determined by the presence of pigments such as flavonoids and/or carotenoids. Such flavonoids correspond to chemical compounds, some of which determine colors such as red, pink, purple and blue (Francis 1989). These compounds are water-soluble and therefore cannot be retained by animals that consume them. The carotenoids comprise a large family consisting of approximately 600 compounds of lipid nature derived from isoprenoids, which determine colors from pale yellow to dark red and pass through red-orange and yellow-orange (Tee 1992). It is known that the food color is an important factor in their selection by the consumer (Tee 1992), and consequently there exists international interest in the development of natural food colors. Many have been synthesized but in the face of strong opposition and generally at great cost (Francis 1989). The carotenoids are able to fuse with the plasmatic membrane through their lipid tails, thereby giving color to the animal tissue. In Chile, flowers rich in carotenoids, such as species of the genus *Tagetes*, have been utilized as a poultry nutriment, giving an attractive color to meat.

The importance of the carotenoids, however, is not limited to their ability to give color. The β carotene, for example, constitutes the principal source of vitamin A for animals, which is obtained through its cleavage (Bendich 1989, Tee 1992). Vitamin A is of vital importance for vision, bone growth and reproduction, and animals depend on food consumption to synthesize it (Tee 1992). Its deficiency constitutes a serious problem, particularly in children of underdeveloped countries (Tee 1992, Olson 1993). Furthermore, effects such as enhancing the immune response; inhibiting mutagenesis; diminishing nuclear damage; protecting tissue from light-induced damage; blocking reactions mediated by free radicals; and others, have been attributed to the carotenoids (Bendich 1989, Bendich & Olson 1989, Diplock 1991, Van Poppel 1993). Similarly, it has been observed in epidemiological studies that a diet rich in carotenoids diminishes the risk of some types of cancer (Ziegler 1989, Van Poppel 1993).

The hypothesis will be that by knowing the plant species selected by *Apis mellifera* as pollen source, it is possible to detect a corbicular pollen rich in carotenoids.

The objective of this investigation is to determine the productivity of species-specific pollen loads originating from beehives located in the Central Zone of Chile, and to determine the presence or absence of carotene pigments in these loads. This information can be used to ascertain the potential of the native flora for providing pollen rich in carotenes, with the honeybee serving simply as a vehicle for their harvest.

ROUGIER, C SILVA, S TEILLER, & D VARELA (1992). El grano de polen como herramienta para diagnosticar especies vegetales melíferas. Situación en Chile. Procc. III Encuentro Nacional de Ciencia y Tecnología Apícola. Universidad del Bío-Bío, Chillán, pp 85-115.

3 MONTENEGRO G, G AVILA, M GOMEZ, L ITURRIAGA, AM MUJICA, V POBLETE, M SCHUCK, J SEMPE, S TEILLER & D VARELA (1989) Implementación de una red nacional de especies vegetales utilizadas por abejas melíferas. Cuadragésimo Congreso de la Sociedad Agronómica de Chile. Simiente 59: 114.

MATERIALS AND METHODS

The sampling site, "El Litral", is situated in the Paine locality in central Chile (33° 49' S, 70° W) which corresponds to a semiarid Mediterranean zone. The existing vegetation consists of matorral formed by evergreen shrubs, summer deciduous shrubs, and a seasonal herbaceous stratum (Montenegro et al., 1978, Montenegro et al., 1979). Sampling to determine the presence of carotenoids was performed between January of 1987 and January of 1989 and involved the utilization of pollen collecting traps. These traps were placed at the entrance of the beehives and permitted the bees to enter but at the same time retained the pollen loads carried by them (Mohammed & Khattab, 1979).

Productivity of pollen loads

The traps were placed in three beehives for a period of eight hours. Pollen loads obtained were transferred to the laboratory where they were dried in an oven at 30° C. Later, they were separated by color and was determined, through microscopic analysis, the plant species corresponding to each pollen load (which were previously shown to be monospecific). Later, the dry weight of each group of pollen loads was determined and the relative percentages in which species had been harvested by the bees in each month of the year were obtained. With this data it was possible to calculate average monthly amounts of pollen load productivity for each of the beehives analyzed.

Determination of carotenoid presence in pollen loads

The presence of carotenoid pigments was evaluated using high-pressure chromatography liquid (HPLC) in pollen loads arriving at the hives in different amount. The process involved the extraction of carotenoids which was achieved by macerating the pollen samples with acetone for twenty minutes. This process was repeated four times at room temperature to eliminate wax and other compounds that make the extraction of carotenoids difficult. After vacuum filtering, the acetonic extract was eliminated and the pollen was extracted again with hexane-acetone (4:6) for twenty minutes at room temperature. The hexane-acetone extract was evaporated under reduced pressure. This extract was preserved at 5° C in nitrogen and in the dark to avoid carotenoid decomposition.

The hexane-acetone extracts of each species were analyzed separately with high pressure liquid chromatography (HPLC) using an Altech 5u-Spherisorb OD-2 (4.6*250mm) column. The extracts were dissolved in dicloromethane. The injection volume was 10 µl. A solvent system in gradient was used beginning with H20-methanol-acetonotrile (8:1:1). The percentage of acetonotrile was incremented until reaching 100 % in 20 minutes. After five additional minutes of isocratic elution with acetonotrile to 100 %, a gradient system of acetonotrile-dicloromethane was used in which the dicloromethane was increased from 0 % to 100 % during the next 35 minutes. The eluiting compounds were detected using an ultraviolet/visible diode array detector scanning between 19-600 nm. All data was acquired using a 486-33 computer and was transferred to a 4 mm tape for storage. For primary identification, based on ultraviolet spectra and retention time, standard α carotene, β carotene, licopene and xanthophyll were used.

RESULTS

Data obtained previously in the same location permit a comparison of the productivity of pollen loads in two periods (September 1987-January 1988 and October 1988-January 1989), as shown in Figs. 1 and 2. The tendency of the three beehives shown is the same, although the productivity of hive A is significantly greater ($p<0.05$) than that of hives B and C (T student test), with a greater differentiation in periods of full bloom of the species. This data indicates a strong relationship between productivity and vital state of the beehive. Furthermore, bee activity is shown to be strongly dependent on environmental temperature and precipitation. It can be observed in Fig. 2 that on December 6, 1988, a day with a

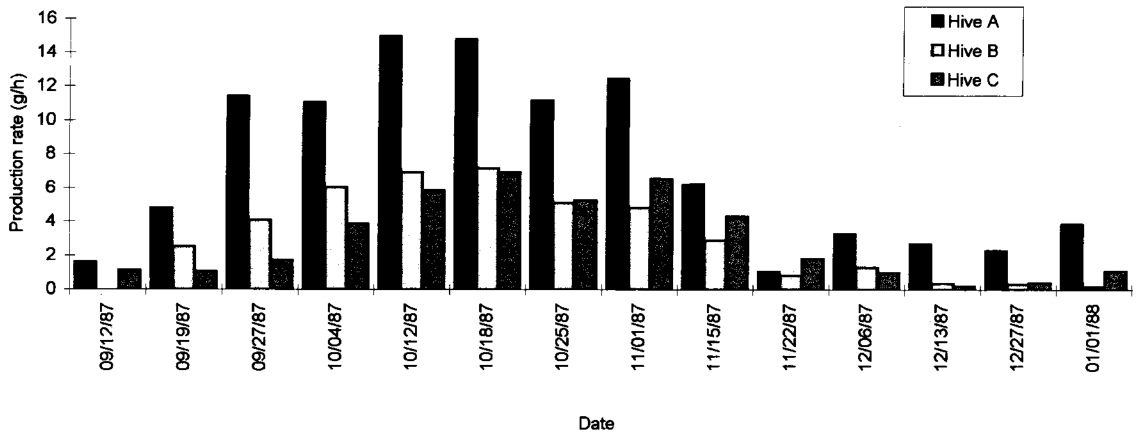

Fig. 1: Pollen load collection, expressed as rate as production rate (g/h), for the three hives located in El Litral, Paine, from September 1987 to January 1988.

Acopio de polen corbicular, expresado como tasa de producción (g/h), para tres colmenas ubicadas en el fundo El Litral, Paine, en el período Septiembre 1987-Enero 1988.

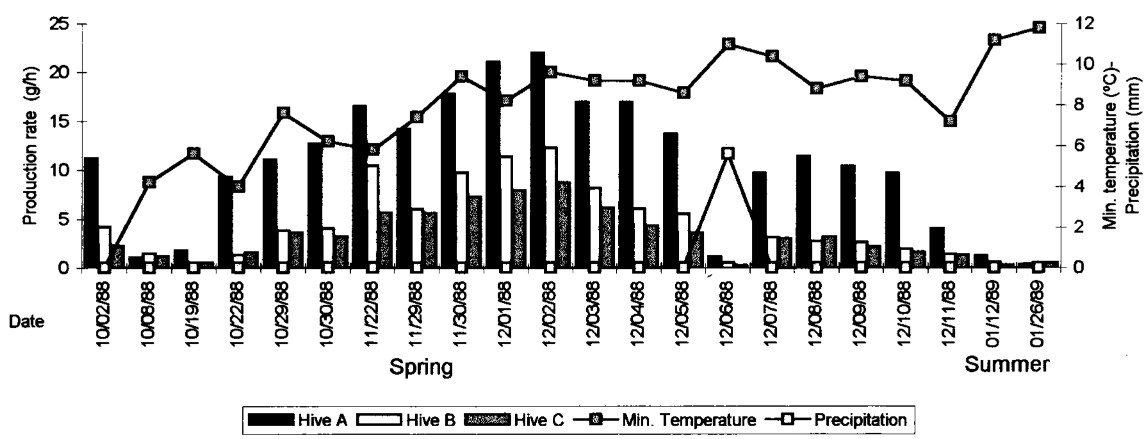

Fig. 2: Pollen load collection, expressed as production rate (g/h) for the three hives located in El Litral, Paine, from October 1987 to January 1988. The variations in minimum temperature and precipitation are shown.

Acopio de polen corbicular, expresado como tasa de producción (g/h), para tres colmenas ubicadas en el fundo El Litral, Paine, en el período octubre 1988-enero 1989. Se muestra la variación de la temperatura mínima y del nivel de precipitaciones en el mismo período.

summer shower, the harvesting of pollen fell drastically. It is also evident that there is an increase in harvesting with temperature increments, but maximum harvesting occurs at the end of spring at 10° C.

Monthly average rates of productivity for each beehive in two successive years show significant differences within the same beehive occurring in this period (data not showed) as it reflect in the total productivity of pollen loads for two seasons (Figs. 3, 4 y 5). Tendency within each beehive, in different periods, is the same, with maximum values corresponding to the period of greatest bloom (Montenegro et al. 1989, Montenegro et al. 1989[3])

Table 1 shows the plant species utilized by the bees and the relative percentages in which they are used during the 92-93 season (Montenegro et al. 1994[4]). It can be observed that the relative percentage contributed

4 MONTENEGRO G, G AVILA, R GINOCCHIO, M GOMEZ, L GONZALEZ AM MUJICA, E RAVANAL, G RIZZARDINI, D ROUGIER & I TORREALBA (1994). Uso sustentable de la flora nativa por la actividad apícola. Procc. IV Congreso Nacional de Ciencia y Tecnología Apícola. Universidad Católica de Valparaíso, 28 y 29 de julio, Olmué.

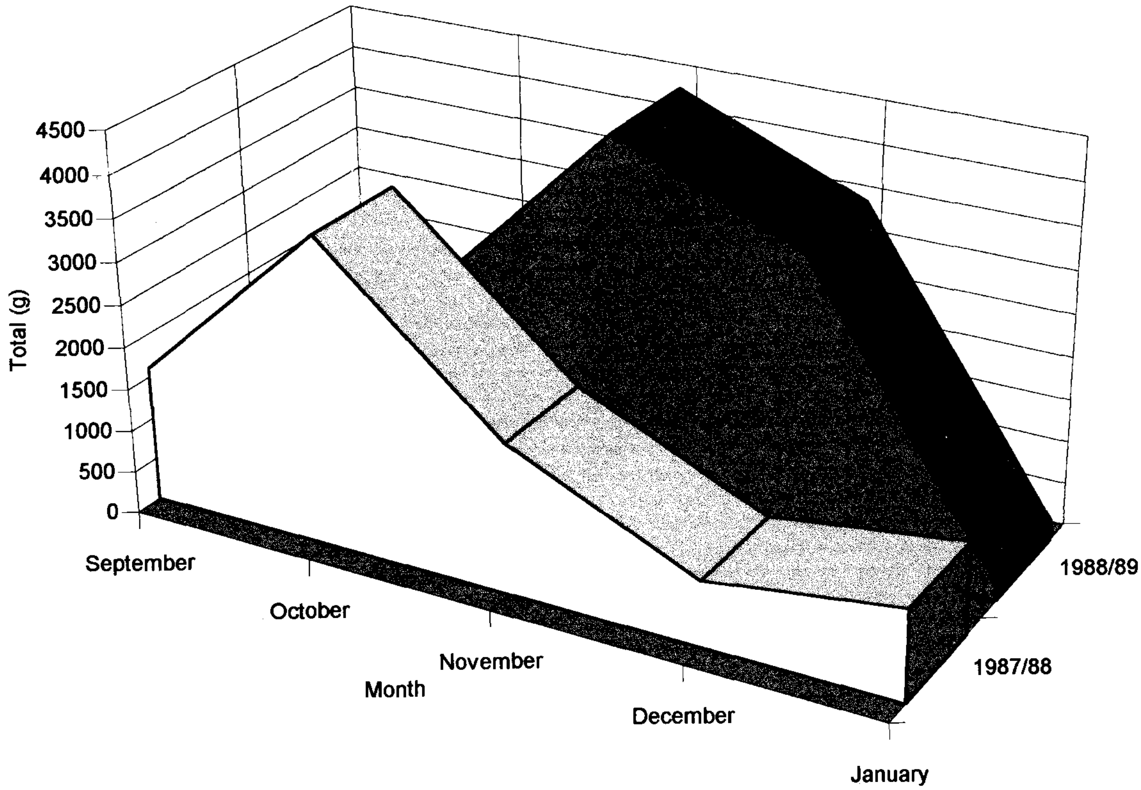

Fig. 3: Pollen load productivity (g) of hive A of El Litral, Paine, from September 1987 to January 1988, and from September 1988 to January 1989.

Productividad total de polen corbicular en gramos, de la colmena A del fundo El Litral, Paine, en el período entre septiembre de 1987 a enero 1988, y entre septiembre de 1988 y enero de 1989.

by a specific plant species varies considerably. For example, 2.3% was contributed by *Trevoa trinervis* in September while slightly over 95% was contributed by *Lithrea caustica* in November.

Pollen load productivity appears to be similar in native and introduced in terms of quantity. The harvesting productivity of native species reaches a maximum in November and December. Table 1 shows that *L. caustica* again reached over 95% of the contribution in November and over 58% in December. The introduced species demonstrate greater productivity at the beginning of the season; in September (*Hypochaeris radicata,* 44.1 %) and October (*H. radicata,* 53.2 %). They did not, however, reach the levels of relative productivity observed for native species. In four of the five months tested it was found that one native species, L. caustica, contributed more than 40 % of the total harvest, which would confirm the previously described selectivity of the bees and their preference for this particular species.

The analysis of the carotenoids shows that highly productive species in the pollen loads present carotenoids (Table 2). Is important to note that, during certain months of the year, species such as *L. caustica, H. radicata* and *P. chilensis* contribute significant percentages of pollen loads to the hive (Table 1). The possibility of the commercial exploitation of this product is viable because the contribution period of species-specific pollen loads to the hive is known.

DISCUSSION

The quantification of pollen loads that reach the beehives of a specific region permit the determination of the relative importance of plant species in proximity to the beehive. This is fundamental if a prediction of the potential use of each species as a carotenoid

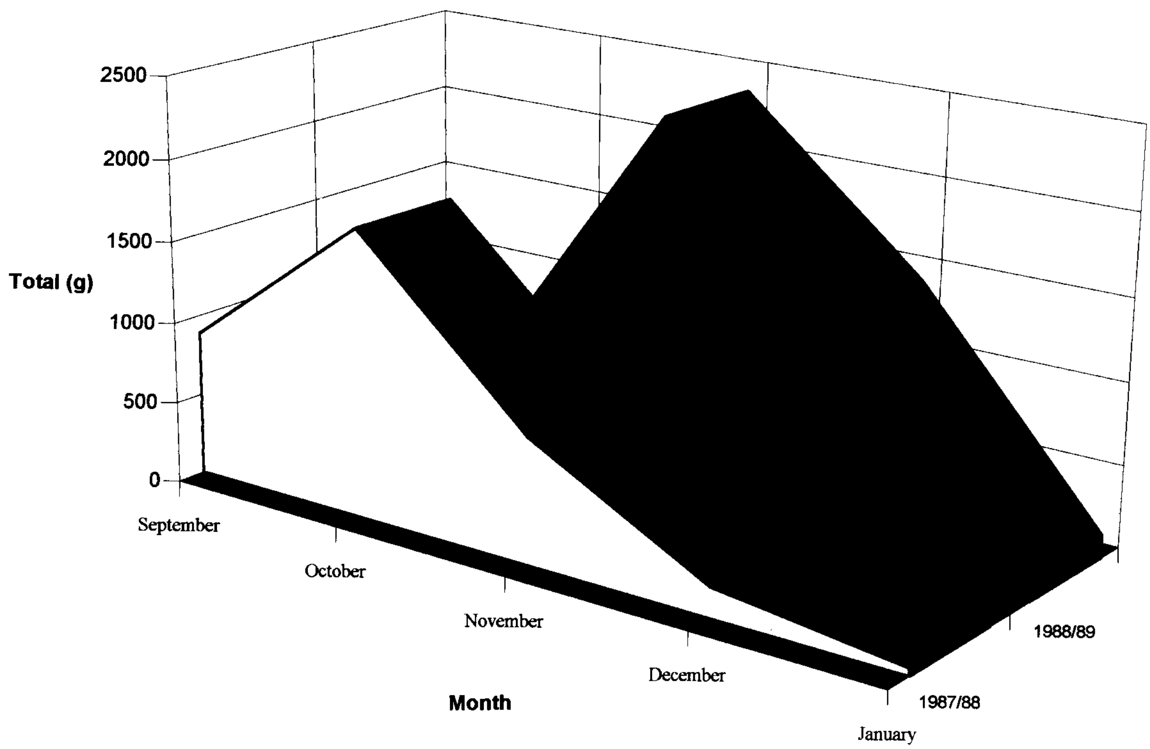

Fig. 4: Pollen load productivity (g) of hive B of El Litral, Paine, from September 1987 to January 1988, and from September 1988 to January 1989.

Productividad total de polen corbicular en gramos, de la colmena B del fundo El Litral, Paine, en el período entre septiembre de 1987 a enero 1988, y entre septiembre de 1988 y enero de 1989.

TABLE 1

Principal plant sources in pollen loads collected from September 1992-January 1993 at "El Litral", Paine (% of total)

Fuentes vegetales principales de polen corbicular colectado desde septiembre de 1992 a enero de 1993 en "El Litral", Paine (% del total)

Month	Species	Family	Growth Form	% of total
September	*Hypochaeris radicata* L.	Asteraceae	introduced perennial herb	44.1
	Colliguaja odorifera Mol.	Euphorbiaceae	native semideciduous shrub	17.8
	Peumus boldus Mol.	Monimiaceae	native evergreen shrub	8.4
	Trevoa trinervis Miers	Rhamnaceae	native deciduous shrub	2.3
October	*Hypochaeris radicata* L.	Asteraceae	introduced perennial herb	53.2
	Lithrea caustica (Mol.) H. et A.	Anacardiaceae	native evergreen shrub	11.8
	Podanthus mitique Lindl.	Asteraceae	native evergreen shrub	5.8
	Trevoa trinervis Miers	Rhamnaceae	native deciduous shrub	5.2
November	*Lithrea caustica* L.	Anacardiaceae	native evergreen shrub	95.2
	Rubus ulmifolius Schott	Rosaceae	introduced evergreen shrub	2.5
December	*Lithrea caustica* (Mol.) H. et A.	Anacardiaceae	native evergreen shrub	58.4
	Rubus ulmifolius Schott	Rosaceae	introduced evergreen shrub	21.6
	Echinopsis chilensis (Colla) Fried.et Rowl.	Cactaceae	succulent shrub	8.6
January	*Rubus ulmifolius* Schott	Rosaceae	introduced evergreen shrub	23.7
	Echinopsis chilensis (Colla) Fried.et Rowl.	Cactaceae	succulent shrub	14.2
	Puya chilensis Mol.	Bromeliaceae	native rossette-like evergreen shrub	10.2

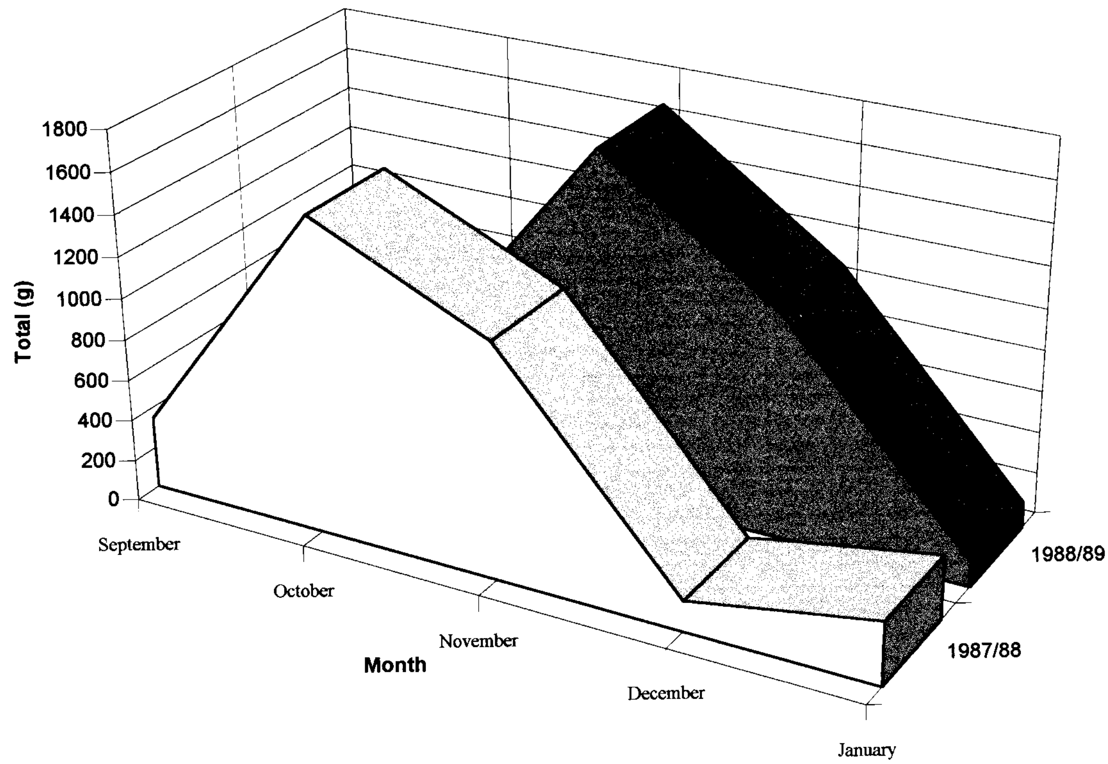

Fig. 5: Pollen load productivity (g) of hive C of El Litral, Paine, from September 1987 to January 1988, and from September 1988 to January 1989.

Productividad total de polen corbicular en gramos, de la colmena C del fundo El Litral, Paine, en el período entre septiembre de 1987 a enero 1988, y entre septiembre de 1988 y enero de 1989.

TABLE 2

Species with carotenoid pigments in the pollen loads

Especies con pigmentos carotenoides en las cargas polínicas

Species	Family	Growth Form
Acacia caven (Mol.) Mol.	Mimosaceae	native evergreen shrub
Acacia melanoxylon R. Br.	Mimosaceae	introduced evergreen shrub
Adesmia arborea Bert. ex Savi	Papilionaceae	native evergreen shrub
Baccharis elaeoides Remy	Asteraceae	native evergreen shrub
Baccharis linearis (R.et P.) Pers.	Asteraceae	native evergreen shrub
Baccharis pingraea DC.	Asteraceae	native evergreen shrub
Brassica rapa L.	Brassicaceae	introduced perennial herb
Cichorium intybus L.	Asteraceae	introduced perennial herb
Eschscholzia californica Cham.	Papaveraceae	introduced perennial herb
Flourensia thurifera (Mol.) DC.	Asteraceae	native deciduous shrub
Gochnatia foliolosa (D.Don) D. Don ex H. et A.	Asteraceae	native evergreen shrub
Haplopappus foliosus DC.	Asteraceae	native evergreen half-shrub
Haplopappus glutinosus Cass.	Asteraceae	native evergreen half-shrub
Hypochaeris radicata L.	Asteraceae	introduced perennial herb
Hypochaeris scorzonerae (DC.) F. Muell.	Asteraceae	native perennial herb
Lithrea caustica (Mol.) H. et A.	Anacardiaceae	native evergreen shrub
Maytenus boaria Mol.	Celastraceae	native evergreen shrub
Ochagavia carnea (Beer) L. B. Sm. et Looser	Bromeliaceae	native succulent
Puya berteroniana Mez.	Bromeliaceae	native rossette-like evergreen shrub
Puya chilensis Mol.	Bromeliaceae	native rossette-like evergreen shrub
Schinus latifolius (Gillex Lindl) Engler	Anacardiaceae	native evergreen shrub
Senecio cerberoanus Remy	Asteraceae	native evergreen half-shrub
Silybum marianum L. Gaertn.	Asteraceae	introduced annual herb
Sonchus oleraceus L.	Asteraceae	introduced annual herb
Tagetes sp.	Asteraceae	introduced annual herb
Verbascum virgatum Stockes	Scrophulariaceae	introduced biannual herb

source is[5] desired. Furthermore, knowing the productivity values of a determined region permits an estimation of the potential of that area in terms of carotenoid production (Poblete et al. 1989[6]). Moreover, the protein content could be used as a possible indicator of nutrient value (Rougier et al. 1994).

L. caustica stands out as an important species in terms of carotenoid productivity given that it was collected in 3 of the 5 months tested and attained 95.2 % of the sample in one month. *P. chilensis* appears significant, but to a lesser extent (10.2 % of the sample). Also of significance, parallel to the utilization of the native species previously mentioned, are the introduced species. For example, *H. radicata* was the species most harvested in two of the five months tested (44.1 % and 53.2 %, respectively).

During the entire period tested, at least one species per month was seen to possess carotenoid pigments in the pollen loads which would permit the year-round harvest of pollen rich in this type of pigment. However, other species such as *Colliguaja odorifera,* with a relative importance of 17.8 %, do not present carotenoids.

The cross pollinization by honeybees on species of native could have also an impact on the breeding system of plant communities in Chilean Mediterranean Ecosystems, but little informations currently avaible on this matter (Fuentes et al. 1994). However, the possibility using the communities in a sustainable manner to harvest carotenoid pigments is certain.

ACKNOWLEDGMENTS

The authors thanks Dr. Thomas McClure for helping in the carotenoid analysis and Robin Hemmings for English revision, and Liliana Iturriaga for helpful suggestions. This research was financed by the FONDECYT project 1940655 to Professor Gloria Montenegro and NIH 1U01TW/CA 00316-01 awarded to Professor Barbara N. Timmermann.

5 POBLETE V, D VARELA, L MOYA, AM MUJICA, M SCHUCK & G MONTENEGRO (1989) La flora nativa de la región mediterránea semiárida de Chile como recurso para mantención de apiarios. Simiente Chile 59: 113.

LITERATURE CITED

BENDICH A & JA OLSON (1989) Biological actions of carotenoids. The FASEB Journal of 3: 1927-32.

BENDICH A (1989) Symposium conclusion: biological actions of carotenoids. Journal of Nutrition 119: 135-6.

DIPLOCK AT (1991) Antioxidant nutrients and disease prevention: an overview. American Journal of Clinical Nutrition 53(Suppl): 189S-193S.

DOSKEY PV & BJ UGOAGWU (1992) Macronutrient chemistry of pollen. Communical Soil Science Plant Annals 23(1&2): 15-23

FRANCIS FJ (1989) Food colorants: anthocyanins. Critical Reviews in Food Science and Nutrition 28: 273-314.

FREE JB (1963) The flower constancy of honey bees. Journal of Animal Ecology 32: 119-131.

FREE JB (1967) Factors determining the collection of pollen by honey bees foragers. Animal Behaviour 15: 134-144.

FUENTES ER, G MONTENEGRO, PW RUNDEL, MTK ARROYO, R GINOCCHIO & F M YAKSIK (1994) Functional approaches to biodiversity in the mediterranean-type ecosystems of central Chile. In: Davis GW & DM Richardson (eds) Mediterranean-type ecosystems. The function of biodiversity: 185-232. Springer-Verlag, Berlin.

GILLIAM M, WF McCAUGHEY & B WINTERMUTE (1980) Amino acids in pollens and nectars of *Citrus* cultivars and in stored pollen and honey from honeybee colonies in *Citrus* Groves. Journal of Apiculture Research 19: 64-72.

HAYDAK M (1970) Honey bee nutrition. Annual Review of Entomology 15: 143-156.

ITURRIAGA, L, G ÁVILA, M GÓMEZ, S TEILLER & G MONTENEGRO (1992) Especies vegetales utilizadas como fuente de polen por *Apis mellifera* en la región mediterránea subhúmeda de Chile. Simiente 62: 19-23.

LOPER GM & AC COHEN (1987) Amino acid content of *Dandelion* pollen, a honey bee (Hymenoptera: Apidae) Nutritional Evaluation. Journal Economical Entomology 80: 14-17.

LOVEAUX J (1958a) Recherches sur la recolte du pollen par les abeilles *Apis mellifera*. Annals Abeille 1(B): 113-188.

LOVEAUX J (1958b) Recherches sur l' origine dans la miel du pollen de plantes entomophiles dépourvues de nectaires. Annals Abeille 1: 89-92.

MOHAMED MI & MM KHATTAB (1979) Pollen plants and pollen gathering activity of honey bees *Apis mellifera* L. in mashtohor region. Research Bulletin Animal Shams University 1135: 1-6.

MONTENEGRO G, O RIVERA & F BAS (1978) Herbaceous vegetation in the Chilean matorral. Dynamics of growth and evaluation of allelopathic effects of some dominants shrubs. Oecologia 36: 237-244.

MONTENEGRO, G, ME ALJARO & J KUMEROW (1979) Growth dynamics of Chilean matorral shrubs. Botanical Gazette 140: 114-119.

MONTENEGRO G, M SCHUCK, AM MUJICA & S TEILLER (1989) Flora utilizada por abejas melíferas *(Apis mellifera)* como fuente de polen en Paine, Región Metropolitana, Chile. Ciencia e Investigación Agraria (Chile) 16: 47-53.

NIELSEN N, GROMMER J & R LUNDEN (1955). Investigation on the chemical composition of pollen from some plants. Acta.Chem.Scand 9: 1100-1106.

OLSON JA (1993) 1992 Atwater Lecture. The irresistible fascination of carotenoids and vitamin A. American of Journal Clinical Nutrition 57: 833-9.

PERCIVAL MS (1947) Pollen collection by *Apis mellifera* L. New Phytologist 46: 142-173.
PERCIVAL MS (1950) Pollen presentation and pollen collection. New Phytologist 49: 40-63.
PERCIVAL MS (1955) The presentation of pollen in certain Angiosperms and its collection by *Apis mellifera*. New Phytologist 54: 345-368.
RASHAD SE, IM MOHAMED & SMA EL-SHAKAA (1979) Behaviour of honey bee workers on major pollen sources in Giza region. Ann.Agric.Sc.Moshtohor 12: 380-383.
ROUGIER D, B TIMMERMANN, E FUENTES, L YATES, F BAS & G MONTENEGRO (1994) Relación entre la selectividad de la abeja melífera (*Apis mellifera*) y el contenido de proteína cruda del grano de polen. Diagnóstico en la flora de Chile. Ciencia e Investigación Agraria (Chile) 21: 47-52.
SOLBERG Y & G REMEDIOS (1980) Chemical composition of pure and bee-collected pollen. Scientific Reports of the Agricultural University of Norway 59: 1-12.
SYNGE AD (1947) Pollen collection by honeybees Apis mellifera. Journal of Animal Ecology 16: 122-38.
TEE ES (1992) Carotenoids and retinoids in human nutrition. Critical Reviews in Food Science and Nutrition 31: 103-63.
TODD FE & O BRETHERICK (1942) The composition of pollen. Journal of Economical Entomology 35: 312-317.
VAN DER MOEZEL PG, JC DELFS, JS PATE, WA LORENAGAN & DT BELL (1987) Pollen selection by Honeybees in shrublands of the northern sandplains of western Australia. Journal of Apicultural Research 26: 224-232.
VAN POPPEL G (1993) Carotenoids and cancer: an update with emphasis on human intervention studies. European Journal of Cancer 29A: 1335-44.
VARELA D, M SCHUCK & G MONTENEGRO (1991) Selectividad de *Apis mellifera* en su recolección de polen en la vegetación de Chile central (Región Metropolitana). Ciencia e Investigación Agraria (Chile) 18: 73-78.
ZIEGLER RG 1989) A review of epidemiologic evidence that carotenoids reduce the risk of cancer. Journal of Nutrition 119: 116-122.