

## Poster session e

## ENTOMOPALYNOLOGY AND MELISSOPALYNOLOGY

Diet and pollen transport of *Cheilosia flavipes* (Panzer, 1798) (Diptera: Syrphidae)Radisić, P.<sup>1</sup>; Papadopoulos, G.<sup>1</sup>; Vujic, A.<sup>1</sup> & Simic, S.<sup>1</sup><sup>1</sup>Department of Biology and Ecology, Trg Dositeja Obradovica 2, 21000 - Novi Sad, Serbia and Montenegro.

*Cheilosia flavipes* (Panzer, 1798) belongs to "flavipes" group of species of genus *Cheilosia* (VUJIĆ, 1996). This species is distributed in Siberia, North and Central Europe (SPEIGHT, 1998). It has been recorded on the whole territory of the Balkans.

Influence of flowering plants on the behaviour of adult hoverflies is scarcely known, although plant species from which flowers adults were collected, are often noted. Aim of this study was to improve knowledge of the biology and ecology of *C. flavipes* by analysing pollen diet and pollen transport.

30 specimens collected from National park Kopaonik mountain (Serbia) and Jahorina mountain (Bosnia and Herzegovina) were examined. Pollen from the integument was collected using vacuum method of RADISIĆ et al. (1992). In analysis of gut content specimens were first softened in excicator and then dissected. Gut content was isolated and the permanent preparations were made with glycerine-gelatine and fuchsin.

The qualitative and quantitative analysis of registered pollen spectrum on the integument showed that the number of visiting flower species is much higher than the number of pollen species at feeding. The analysis has shown that the Fenestrate and Aster type were the most important pollen types for the specimens of *C. flavipes*. Fenestrate type of pollen is present at pollen diet and pollen transport in every examined specimens *C. flavipes*.

RADISIĆ, P. VUJIĆ, A. SIMIĆ, S. & RADENKOVIĆ, S. 1992. Pollen transport of species *Cheilosia grossa* Fallen, 1817 (Diptera: Syrphidae) *Ekologija* 2, vol. 27:41-46.

SPEIGHT, J. H. 1998. Species accounts of European Syrphidae (Diptera): The Atlantic zone species (revised). *Syrph the Net publications*, Vol. 7, 25 pp, Dublin.

VUJIĆ, A. 1996. Genus *Cheilosia* Meigen and related genera (Diptera: Syrphidae) on the Balkan Peninsula. Monographs, Matica srpska, Novi Sad.

Identification of food sources of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) in BrazilLorioux, A. M.<sup>1</sup>; Castelo Branco, M.<sup>2</sup> & Jones, G. D.<sup>3</sup><sup>1</sup> ESA.<sup>2</sup> Embrapa Hortaliças, Caixa Postal 218, 70.359-970, Brasília-DF, Brazil. E-mail: marina@cnpq.embrapa.br.<sup>3</sup> USDA-ARS, APMRU, 2771 F & B Rd. College Station, TX 77845, USA. E-mail: g-jones@tamu.edu.

*Diabrotica speciosa* adults feed on leaves and flowers of several horticultural crops. To control their damage growers spray their crops with several insecticides, including the toxicol metamidophos. Management of the pest is being studied in Brazil. In this work plants that can be used as food source by the insect were identified. *Diabrotica* adults were collected in three different fields in Brasília, between February and August 2002. Insects were washed with alcohol and them processed by the acetolysis method. Pollen grains were extracted and them identified. More than 440 pollen grains representing 7 families were extracted from insects. In most of case, 1 to 5 pollen grains per insect was found. Asteraceae pollen dominated both the percentage of total pollen and the frequency of occurrence as well; 284 grains of this family plant were obtained from the insects.

Asteraceae plants such as *Galinsoga parviflora*, *Bidens pilosa*, etc. were commonly observed in the fields where *D. speciosa* adults were collected and this suggested that the adults could have feed on those plants.

Pollen grain belonging to the Chenopodiaceae-Amaranthaceae family was the second common one. 116 grains from this family were found. Other grains found belonged to the families Fabaceae, Myrtaceae, Pinaceae, Poaceae, Ulmaceae. So, it was found that *D. speciosa* feed on very different plant families, including weeds. This fact can make its management very difficult.

## Pollen foraging by solitary bees (Hymenoptera: Apidae) from Jalisco, México

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The plant species visited by Centridini bees for food resources were analyzed by means of palynological analysis of female scopal pollen loads. Some ecological parameters were used in order to compare foraging among species of bees. We analyzed the scopal contents of bees collected between August 1995 and September 1997 in the Estacion de Biología Chamela, Jalisco (19° 30' N, 105° 03' W) and other samples were taken from some nests. The pollen samples were prepared by standard acetolysis methods.

This study demonstrates that these solitary bees visit a relatively wide spectrum of plants. A total of 116 plant species were recorded, of the total taxa observed, only 24 were of importance, with frequency value of  $\geq 10\%$ . The pollen analysis showed genera with two or more species with different degrees of importance. These represent simultaneous flowering of several congeneric species. Fluctuations in flowering were reflected in the pollen content. The plant families better represented for number of species were: Leguminosae, Malpighiaceae, Compositae, Euphorbiaceae, Solanaceae, Cucurbitaceae and Flacourtiaceae. The Leguminosae was the best represented in the samples, this corresponding with the vegetation in the area where there are 116 species of this family. The principal life form used by Centridini bees is trees, in second place shrubs and epiphytes.

Palynological analysis of samples was used to show total number of resources that visit these bees, to calculated size of trophic niche and the uniformity of samples. The foraging of resources was homogeneous and only some times was heterogeneous.

According with resources utilized many species were polylectic (10 species), polylectic with strong preference for a single plant family (5 species) and oligolectic (2 species). Comparing the results obtained in this study with those from other studies for solitary bees in the same area, it is evident that a resource partition exists among the solitary bees in this zone.

The morphologic characteristics of plants visited were small flowers (0.3-1.8 cm), arrangement in raceme or panicle, corolla white, yellow, pink and violet, with production of nectar, pollen or oils.

Pollen morphology of the important taxa was monad, some times polyad, medium size, ornamentation reticulate, striate or echinate; the aperture better represented was tricolporate.

Females bees of the subgenera *Centris*, *Heterocentris* and *Trachina* show specialized organs implicated in the collection of floral oils and found on the fore- and midlegs.

The relationships between plants with epithelial elaiophores (Malpighiaceae) and their oil-collecting pollinators present a more case of coevolution.

## The break of sporoderm integrity by bees

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The process of pollen collection by bees and peculiarities of formation of pollen loads and beebread are well studied. However mechanical, osmotic and fermentative influences of bees on pollen grains are still unknown. Only fungicide impact of substances adding into pollen grains in the process of pollen load and beebread formation was the subject of special interest. There are some opinion, that pollen sporoderm (both exine and intine) and palasmalemma stay undisturbed (Klungness L.M. and Ying-Shin P., 1983). We believe that in the process of gathering bees mechanically break sporoderm. In the pollen loads, beebread and inside bee stomach pollen grains are exposed to enzymatic influence, that disturbs not only protoplasts but also pollen sporoderm.

We studied the number and peculiarities of damaged pollen collected from different pollen loads (*Trifolium*, *Taraxacum*, *Ribes*, *Galium*, *Rhinanthus*, *Melampyrum*), beebread of different maturity (fresh and last year's) and contents of different parts of bee stomach.

pollen load with palynotype	Color	% of damaged pollen grains
Trifolium	brown	3%
Trifolium	light brown	6%
Trifolium	yellow	1%
Taraxacum	orange	2%
Ribes	reddish	2%
Galium	reddish	1%
Melampyrum	grey	14%
Melampyrum	grey	1%
Melampyrum	grey	4%
Melampyrum	yellow	22%

	% of damaged pollen grains
Fresh beebread	7%
Last year's beebread	44%
Fore-gut	22%
Hindgut	25%

The number of damaged pollen grains in pollen loads collected from different plant species or from different individuals of the same species varies considerably. Usually the number of broken pollen grains in pollen loads is about a few percents and can be compared with the same in fresh beebread. Pollen grains of some species (for example, *Scrophulariaceae*) can be strongly damaged by bees in the process of gathering. Usually these pollen grains are big, colpate or colpate, damages are associated with apertures. Gaps in interaperturate regions are rare, they observed in porate and colpate pollen grains with thick sporoderm. The number of damaged pollen grains in fore-gut is several times bigger than average number for pollen loads and fresh beebread. The number of damaged pollen grains and morphological peculiarities of pollen passing through mouth parts and gut are similar. It means that maximum damage takes place in the mouth. Future fermentative treatment is not so important, because pollen grains stay in the gut for a very short period of time. The number of damaged pollen grains is maximal in last-year's beebread, it can run up to 50%. We suppose these high values are connected with enzyme activity of 1) substances, eructated by bees during the process of beebread formation and 2) poured out content of pollen grains. There are twice more broken pollen grains in last-year's beebread, than in hind-gut. It can be connected with the duration of enzyme treatment.

One would think, that maximal damage should be observed in pollen grains from stomach because of mechanical breaks by limbs and mouth parts. But evidently the main way to receive nutrients is fermentative one, and pollen grains loose their contents in the stomach through apertures without exine damage. The same process is possible connected with biological features of beebread.

Klungness L.M. and Ying-Shin Peng. 1983. A scanning electron microscopic study of pollen loads collected and stored by honeybees. //Journal of apicultural research. Vol. 22. N.4. P.264-271.

### The importance of Plantain (*Plantago* spp.) as a pollen source in the honeybee diet

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The pollen of Plantain, with great starch content, is known to have a low nutritional value for insects. For this reason, the honeybees only collect this pollen when none better pollen source is available.

The pollen collected by the honeybees was sampled, in two northern Portuguese regions during 2002 and 2003, using hives equipped with upper pollen traps.

In the two years studied, differences of one week in the flowering dates, were observed.

Throughout the year the Plantain pollen collection was present at very low level in the honeybees diet. However an important increase in yield was registered in three different times (June, end of July and in the beginning of autumn).

This behaviour is exactly related with the beginning and ending dates of the main nutritional pollen sources, without the influence of any other factor. This pollen collection was observed between the "gaps" in the flowering periods of *Eucalyptus*, chestnut (*Castanea sativa*), wild blackberry (*Rubus* spp.) and *Ericaceae*.

Our results showed that pollen of Plantain is, to the honeybees, a substitute of other more nutritive pollen types, being only collected as a supplementary source and without fidelity but that can be of great value for the colony development.

Keywords: Honeybees, *Plantago* sp., pollen, Portugal.

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### Analysis of protein content in pollen loads

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An analysis was made of the proteic content of the pollen loads produced by the bees in a hive situated in Viana do Bolo (Ourense, NW Spain), to establish whether or not the relative quantity of protein in the pollen of each vegetal species influences the selection made by the bee of the flowers that supply pollen to the hive. This analysis was performed on all the types of pollen that formed more than 5% of the pollinic spectrum (Díaz et al. 1998; De Sá et al. 2001). The pollen load samples were collected directly from the hive at the rate of one per week, from April to September. The pollen loads were separated by colour and their specific homogeneity was confirmed microscopically.

The Bradford method has been used for protein extraction and Spectrophotometry for the determination of protein content.

The results show that the different pollen loads have high protein content. The pollen of the pollinic types that reached relatively higher percentages in the pollinic spectrum (Díaz et al. 1998; De Sá et al. 2001) are also those that have the highest proteic content. These were *T. Cytisus scoparius*, *Poaceae sylvestris*, *Quercus robur*, *Sanguisorba minor*, *Salix fragilis* and *T. Spergularia rubra*.

The pollen of the systematic units, which had pollen loads that could be identified at the level of species, have maintained a constant value of proteic content independently of the date the samples were obtained. The pollen of the systematic units, which had pollen loads that could be identified at the level of pollinic type, have varied in proteic content in the analyses performed on samples obtained on different dates. This result is due to the fact that the different species that integrate the pollinic type, flower on different dates and thus have a pollenkit with different characteristics.

### Pollen types of honey and pollen loads from Bahia (Northeastern Brazil)

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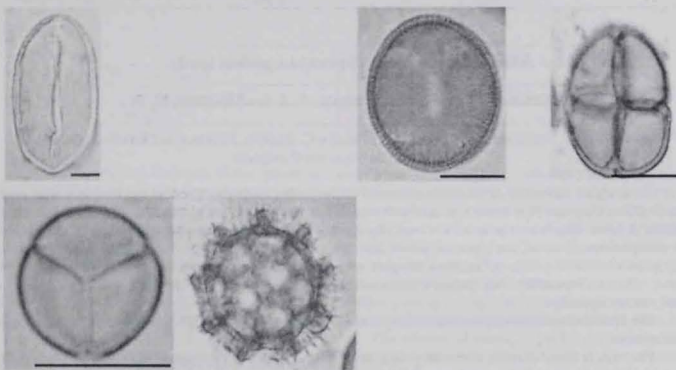
<sup>1</sup> PIBIC/CNPq Fellowship;

<sup>2</sup> FAPESB Fellowship;

<sup>3</sup> CNPq Research Grant Laboratório de Micromorfologia Vegetal, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, 44.031-460, Feira de Santana, Bahia, Brazil.

Bahia is a state located in Northeastern Brazil, which flora is the most biodiverse. The coastal Atlantic forest and the xerocytic "caatinga" in inwards areas characterize Bahian vegetation, mainly. The production of

honey is high in the kinds of vegetations; this state is the biggest producer of pollen loads from Brasil. The palynological analysis of honey and pollen loads was carried out according to acetolysis method, and by the count of 1.500 pollen grains from each sample. The results presented here is from a study of honey produced especially in Paraguassu region and pollen loads collected in municipality of Canavieiras (Southern coastal zone). The analysis revealed a great number of pollen types in the samples, the most from plants of the families Leguminosae (Mimosoideae mainly), Myrtaceae, Asteraceae, Araceae, Apiaceae. The honey samples studied (18) exhibit multifloral pollen spectra, characterized by the presence of *Syagrus coronata* (Fig. 1), *Herissantia* (Fig. 2) and *Evolvulus* (Fig. 3) types. In only one sample, a monofloral spectrum was identified, in what the *Piptadenia moniliformis* type (Fig. 4) presented a frequency of 98%. Seventeen pollen loads samples from Canavieiras presented a biodiverse pollen spectra. In these samples the types most frequent were *Mimosa scabrella* (Fig. 5), *Elephantopus* (Fig. 6), *Vernonia* (Fig. 7), *Cecropia*, *Cocos* and *Spondias* (Fig. 8). All pollen loads presented great number of pollen types, from X to X. Pollen spectra of honey and pollen loads revealed their vegetational origin, where elements from Atlantic forest and Caatinga (e.g. *Syagrus coronata*) were identified. (Supported by CNPq, FAPESP)



### Pollen characterization of some honeys from Northwest of Argentine

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The pollen content of nineteen honey samples from different locations in the Salta, Jujuy, Santiago del Estero and Tucumán provinces of Argentine were subjected to qualitative and quantitative melissopalynological analysis.

For the quantitative analysis natural honey (Vorwohl 1967) was used, following the methodology proposed by Méndez et al. 1994, modified by La Serna et al. 1999 for a suspension of 50 ml of acid water with 30 g of honey. And also for computing the honeydew index -- ratio of honeydew elements (fungal spores and hyphae, algae and wax particles) and pollen grains of nectariferous plants --, following the methodology proposed by Louveaux et al. (1978).

The qualitative analysis used acetolysed honey (Gadbin 1979) and based on the acetolysis method of Erdtman (1969) slightly modified by Hideux (1972).

Nevertheless, permanent slides of natural honey were prepared (Louveaux et al. 1978) in order to confirm the presence or absence of honeydew elements and fragile grains that cannot withstand acetolysis.

The quantitative analysis showed that 21% of the honey belonged to Maurizio's (1939) Class I, 26.3% to Class II, 47.4% to Class III and 5.3% to Class V. The pollinic density range from 1,042 grains/g of honey to 214,575 grains/g with average of 24,848 grains/g.

According to the qualitative analysis, eight honeys were typified as unifloral (42%) and eleven as multifloral (58%). The unifloral honeys samples were broken down as follows: two of "algarrobo" (*Prosopis*), two of *Rumex*, one of "cebil" (*Anadenanthera columbiana*), one of "sauce" (*Salix humboldtiana*), one of *Leonurus sibiricus*, and other of *Citrus*.

Honeydew elements were practically absent. 105 pollen types were identified belonging 50 different families. This number ranges between 10 and 29 (mean of 22.2). Most represented families in this honeys are: Asteraceae (100% of samples), Mimosaceae (94.7%), Nictaginaceae (84.2%), Anacardiaceae (73.7%), Lamiaceae (73.7%), Salicaceae (68.4%), Myrtaceae (68.4%), Euphorbiaceae (63.2%) and Brassicaceae (63.2%). There is no pollen type common to all samples but *Schinopsis balansae*-type and *Anadenanthera columbiana* were found in the 73.9% of them, *Leonurus sibiricus* and *Salix humboldtiana* in 68.4%, *Sapium haematospermum* and Brassicaceae sp. in 63.2% and *Prosopis* sp. in 57.9%.

This work is a contribution to a more extensive research programme that pretend to know the melliferous resources and the botanical origin of honeys produced in the northwest of Argentine. Until now, studies about pollen characterization of native honeys in this region are scarce or none existent at all.

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### The importance of endemic species of *Echium* and *Micromeria hyssopifolia* as source of nectar in honey from El Hierro (Canary Islands, Spain)

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The pollen content of thirty-one honey samples from nineteen different apiaries on El Hierro (Canary Islands) were subjected to qualitative and quantitative melissopalynological analysis.

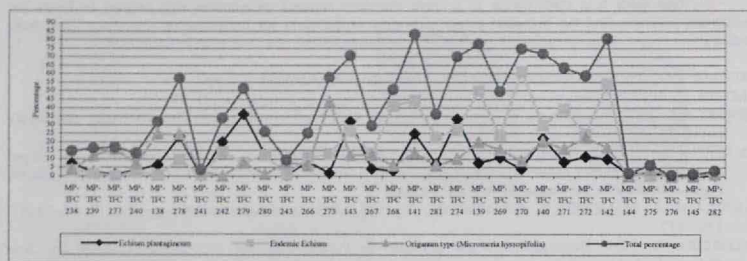
For the quantitative analysis natural honey was used, following the methodology proposed by La Serna et al. 1999. The qualitative analysis used acetolysed honey and was based on the acetolysis method of Erdtman (1969) slightly modified by Hideux (1972). Nevertheless, permanent slides of natural honey were prepared (Louveaux et al. 1978) in order to confirm the presence or absence of honeydew elements and fragile grains that cannot withstand acetolysis.

The quantitative analysis showed that 13% of the honey belonged to Maurizio Class I, 68% to Class II and 19% to Class III. The pollinic density ranges from 1,042 grains/g of honey to 24,478 grains/g with an average of 7,471 grains/g.

According to the qualitative analysis, six honeys samples were typified as unifloral and twenty-five as multifloral. The unifloral honeys samples were broken down as follows: two of heather (*Erica arborea*), two of "tagasaste" (*Chamaecytisus proliferus*-type), one of Fabaceae (Genistaceae sp.) and one of Lamiaceae *Origanum vulgare* ssp. *viridifolium*-type (thyme: *Micromeria hyssopifolia*).

Honeydew elements were practically absent. 69 pollen types were identified belonging 42 different families. This number ranges from 18 to 39 (mean of 27.42). *Foeniculum vulgare* type pollen is present in all the samples. *Galactites tomentosa*-type, *Echium plantagineum*, *Echium* sp. (endemic *Echium*), *Bituminaria bituminosa*, *Chamaecytisus proliferus*-type and *Origanum vulgare* ssp. *viridifolium*-type pollen was found in 96.8% of the samples.

In 14 of the 25 multifloral honeys (56%) the sum of the *Echium* and thyme pollen exceeded 45%, and 11 of them (44%) may be regarded as mixed honeys of "tajinaste" (*Echium* sp.), "sonaja" (*Echium plantagineum*) and "thyme" (*Micromeria hyssopifolia*).



The sensorial analysis indicated that they are generally of good quality, for 62% of them were evaluated as very high, high and good (16%, 23% and 23% respectively).

ERDTMAN, G., 1969. *Handbook of Palynology*. Munksgaard, Copenhagen. 486 pp.

HIDEUX, M., 1972. Techniques d'étude du pollen au MEB: effets comparés des différents traitements physicochimiques. *Micron*, 3: 1-31.

LA-SERNA RAMOS, I., MÉNDEZ PÉREZ, B. & GÓMEZ FERRERAS, C., 1999. *Aplicación de nuevas tecnologías en mieles canarias para su tipificación y control de calidad*. Ed. Confederación de Cajas de Cajas de Ahorros. Caja Canarias, Tenerife. 267 pp.

LOUVEAUX, J., MAURIZIO, A. & VORWOHL, G., 1978. Methods of melissopalynology. *Bee World*, 59 (4): 139-157.

<sup>1</sup>Work included in the PI 2001/057 Project financed by the General Directorate of Universities and Research (Education, Culture and Sports Dept.) of the Canarian Government.

### Palynological and geographical characterisation of Spanish thyme honeys

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In this work we present the results of the pollen analysis of 25 samples of thyme honey selected among 60 Spanish honeys collected in 2002. These honeys show sensorial and palynological characteristics that allow them to be classified as unifloral of thyme. A minimum of 8% pollen from *Thymus* sp. (considering the pollen

grains from anemophilous and nectarless plants) was required to consider these honeys as unifloral from a melissopalynological point of view. The samples were directly collected from professional beekeepers; the extraction of the honeys was made by centrifugation. The quantitative analysis showed that nectar is the main honey source in the samples studied.

The quantitative analysis of the samples was carried out using the light microscope (LM) on slides prepared without any chemical treatment, according to the method described by MAURIZIO (1979). The qualitative analysis was carried out using acetolysed slides prepared according to the method described by ERDTMAN (1960), slightly modified, and always using a subsample of 10 g of honey. Following the results obtained by BEHM, VONDEROHE & HENRICH (1996), at least 500 pollen grains were counted among four different slides for each honey sample. The different pollen types were identified using the key published by DÍEZ (1987) as the main source, but reference slides from the collection at the Department of Botany, University of Seville, were also used. When possible, the identification to a specific level (e.g. *Ridolfia segetum* gr.) was made; in other cases, to a generic level (e.g. *Eucalyptus* f.) or to the pollen type (e.g. *Echium plantagineum* gr.). The types are named according to PERSANO ODDO & RICCIARDELLI D'ALBORE (1989). Field observations made in situ while collecting the samples were taken into account and, in these cases, the most likely species are quoted in brackets after the pollen type (e.g. *Ononis* f. (*Ononis natrix*)).

The results from the quantitative analysis show that one sample was rich in pollen sediments (Class IV), 23 showed a medium content (Classes II and III) and the remaining sample was quite poor in pollen sediments. The number of pollen grains (NPG) in 10 g of honey ranges between 9 500 (sample 25) and 640 000 (sample 21). Although all the samples show the presence of honeydew elements (HDE; fungic mycelium and spores), generally speaking they are few, ranging from 1 300 (sample 25) to 24 800 (sample 21); and in the qualitative we have found 53 pollen types corresponding to 27 families.

The Spanish thyme honeys of the studied region are characterised by their medium-high content in pollen grains (NGP; mean= 155 000) and their medium-high honeydew indicator elements content (HDE; mean= 8 300). *Genista* f. (present in 100% of the samples), *Echium plantagineum* gr. and *Reseda luteola* (90%), *Eucalyptus* f. (80%), *Calendula* f. and *Olea europaea* (75%), and *Cistus monspeliensis* (70%) could be mentioned among the characteristic accompanying species of this honey type.

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### Pollen characterization of honeys from the Spanish market: Eucalyptus, rosemary, orange blossom and heather

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In this work, we studied the pollen spectrum of 28 monofloral honeys commercialized in Spain with the denomination of Eucalyptus (9), rosemary (8), orange (3) and heather (7).

The melissopalynological method used in this project was proposed by the International Commission for Bee Botany (ICBB) (LOUVEAUX et al., 1978). The fractions were analysed without acetolysis. The pollen types were determined following the atlas of VALDÉS et al., (1987) and we also used as reference slides of Spain flora from the University of León (Spain).

The pollen richness is medium-high, 89% of the samples have more than 10,000 grains per honey gram. According to the types established by MAURIZIO (1939), 3 samples are included in type II, 16 samples belong type III and 9 samples to type IV.

Spores and honeydew elements were found in the 28 honeys samples, in general, they had very scarce values.

Of the 28 samples, 13 were of the type indicated in the label (3 of Eucalyptus, 4 of rosemary, 2 of orange blossom and 4 of heather) and the other were: multifloral (5), Echium (3), sunflower (1) and mixed (6).

Honey characterization is essential when its commercial quality must be assessed. This study reflects the importance of palynological analysis in the control of quality of this product.

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### A comparative study of the pollinic spectrum of homemade and commercial honeys produced in the NW of the Iberian Peninsula

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Policlinic analyses were performed on 38 samples of honey produced in Galicia. Twenty-two were homemade and sold directly to the consumer and sixteen had a commercial label, some protected by the Quality Denomination "Mel de Galicia".

The commercial honeys were: eight monoflorals, five of *Rubus* sp and three of *Castanea sativa* and another eight multiflorals; five with *Castanea sativa* predominant; two with *Rubus* sp. predominant and one multifloral with *Erica* predominant.

Of the samples of homemade honeys, two were monoflorals of *Castanea sativa*; three were monoflorals of *Rubus*; two were multiflorals with *T. Cytisus scoparius* dominant; six with *Castanea sativa* dominant; one with *Erica* dominant and eight with *Rubus* sp. dominant.

It was discovered that the pollinic spectrum of the commercial honeys had characteristics different to the homemade honeys from the same floral origin. These differences were the following:

None of the honeys with *T. Cytisus scoparius* or with *Erica* as dominant were multifloral.

Each commercial honey showed a different pollinic combination in "Dominant", "Companion" and "Important" (according to the denomination of Louveaux *et al.* 1978).

The pollens of the least interesting apian plants found in Galicia, such as *Reseda alba*, *Frangula alnus*, *T. Ononis repens* and *T. Echium plantagineum*, reach relatively high values in their spectrum.

### A study of the mellisspalynological and organoleptic variations in monofloral and multifloral mixtures of honeys from the NW of the Iberian Peninsula

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A study was made to discover what amount of multifloral honey could be mixed with the respective monofloral honeys of *Castanea sativa* and *Rubus* sp. produced in the same geographical municipality, so that they can be commercialised as monofloral honeys. For this, pollinic spectrums of the original multifloral and monofloral honeys were made and compared to the pollinic spectrum of the mixed honeys using organoleptic tests. The honeys were mixed in proportions monofloral/multifloral of: 9/1, 8/2, 7/3 and 6/4

The results show that, when related to the pollinic spectrum variations, all the monofloral honeys of *Rubus* sp. conserve their monofloral condition when mixed with the locally produced multifloral honeys in a proportion of 8/2. This monofloral condition is also maintained at proportions of 7/3, if the multifloral honeys contain more than 16% of *Rubus* sp. pollen. Eighty percent of the monofloral honeys of *Castanea sativa* conserve this condition when mixed at a proportion of 9/1 with locally produced multifloral honeys.

With relation to the organoleptic tests, in the locally produced *Rubus* sp. and *Castanea sativa* honeys, the mixture of 9/1 (monofloral/multifloral) was the only mixture that the tasters perceived as monofloral.

### Variability in pollen spectra of chestnut honeys from different sites and years

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Chestnut honey is an important production in various EU countries, and all Italian regions are very good producers of this kind of honey (RICCIARDELLI D'ALBORE, 1998).

Pollen in chestnut honey is overrepresented, so only when the percent of *Castanea sativa* Miller grains is higher than 90 %, the honey can be classified as unifloral. Accompanying pollen species, although poorly represented, play an important role for the identification of the geographical origin of the honey. In different regions, chestnut woods can be associated with the presence of various species. The knowledge of the species occurring in and around the chestnuts woods, as well as their flowering phenology, is critical to link the honey to the area where the bees have collected the nectar.

The control of the geographical origin is necessary to individuate the site of production; this is basic to request a PGI (Protected Geographical Indication) mark, and to certify the real geographical source. In some cases, certified origin reported on the label of the honey is considered an added value and the final consumer accepts to pay an higher price for it. For this reasons, to avoid mislabelling, an objective method for the determination of geographical origin would be helpful.

In some areas, the pollen spectra of chestnut honey have been defined and single samples can be compared to them to evaluate the correspondence of occurrence and frequency of the species. The differences between the honeys produced in distinct sites, are due both to flora composition and seasonal variability, which affect flowering phenology.

The aim of this study was to evaluate the variability of pollen spectra in honeys produced within and between sites in consecutive years. Chestnut honeys were sampled in various sites from Southern Italy and were analysed according to LOUVEAUX *et al.* (1978). Pollen spectra were subjected to multivariate statistical analyses (principal component analysis and cluster analysis) in order to compare all samples at a time. Results showed that for unifloral productions a one-to-one correspondence between honey and its geographical origin is not always easy and straightforward. As supposed the more similar is the vegetation of the sites the higher are the similarities between the pollen spectra. However, this methodological approach seems a powerful tool to discriminate objectively among numerous similar samples belonging to different areas.

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### Pollen analysis of mint and white annual *Stachys* honeys from Serbia

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Pollen analyses are an essential tool for honey standardization and certification. There are variety of honey types offered at honey market in Serbia. The most of them are often declared as unifloral in order to

increase their value. Law requirements in Serbia classifies honey as unifloral if the most pollen grains belongs to one plant species but methodology for conducting such analysis is rather scarce and there are no instructions for interpreting pollen analysis results, especially for honeys that have under-represented pollen types such as pollen of Lamiaceae family.

The aim of this study was to conduct qualitative and quantitative pollen analysis of mint and white annual stachys honeys which are among the most expensive honeys on the honey market. This is preliminary study and it should provide basis for certification of botanical origin of Serbian honeys, especially those that are the most exotic.

The samples were donated by beekeepers from Novi Sad region (Panonian valley) and were said to be from mint and white annual stachys. Two samples from each honey type were taken. Preparations were made according to procedure given by SAWYER (1988). Pollen analysis was performed with an optic microscope. For qualitative analysis pollen was counted in 6 horizontal rows at magnification of 200x (25% of total slide area) and the result was transformed to total pollen amount per 10g of honey (pg/10g of honey). Qualitative analysis considered determining and counting first 1000 pollen grains, in random microscope fields, at magnification of 400x, and making the frequency classes (according to RICCIARDELLI D'ALBORE, 1997).

Quantitative analysis showed that only one sample of mint honey had between 20000 and 100000 pg/10g of honey, which corresponds to honey made from normal floral sources (JONES & BRYANT, 1996). Other honey samples have less than 20000 pg/10g of honey. It is difficult to say, without chemical analysis, if extremely low value (1764 pg/10g of honey) in one sample of white annual stachys honey was result of adulteration or difficulties in pollen extraction during preparation, since this sample was very viscous after dissolving in water.

Qualitative analysis of mint honeys showed that they have 5.9% and 7% pollen of anemophilous and nectarless plant species. Both samples of mint honey were characterized with predominance of *Mentha pulegium* pollen (66.5% and 66.8%) and there were no accompanying pollen types.

First sample of white annual stachys honey had 22.2% pollen of anemophilous and nectarless plant species, sunflower pollen was predominant (55.1%) and there were only 4.9% of *Stachys* sp. pollen. Second sample of white annual stachys honey had 3.4% pollen of anemophilous and nectarless plant species and did not have predominant pollen type. *Stachys* sp. pollen reached 36.4% but qualitative analysis results of this sample is difficult to interpret since it had extremely low total pollen number per 10g of honey. Accompanying pollen types were absent in both white annual stachys honey samples.

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### Pollen analysis of natural honey samples from Sikkim and Darjeeling Himalayas, India

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Pollen analytical data of forty-two natural honey samples collected in different seasons during the period of June 1995 – December 1998 from different rural areas of Darjeeling, Jalpaiguri districts of West Bengal and Sikkim, India, are presented here. Twenty-five honey samples are collected from Darjeeling region among which twenty-three are of *Apis cerana indica* F. and two are of *A. florea* F. Eighteen honey samples are found to be unifloral with *Rosa* spp., *Prunus* spp., *Fragaria* sp., *Rubus ellipticus*, *Spiraea* sp., *Ageratum conyzoides*, *Primula* spp., *Melilotus pulchra*, *Trifolium repens* (during summer); *Rosa* spp., *Aristolochia* sp. (during winter) and *Sehima wallichii*, *Sedum multicaule*, *Ageratum conyzoides* (during autumn) constituting the predominant pollen types. Seventeen honey samples foraged by *Apis cerana indica* F. are collected from Sikkim among which

nine are found to be unifloral and the rest eight multifloral. Predominant pollen types encountered in the unifloral honey samples are *Rosa* spp., *Prunus* spp., *Brassica* spp., *Citrus* spp., *Calendula officinalis* (during summer), *Michelia* spp. and *Buddleja asiatica* (during winter).

Three honey flow periods (HFP I-III) have been recognized in this part of eastern Himalayas. HFP II (March-June) is proved to be the principal flow period, HFP I (November – February) and HFP III (July-October) are the secondary flow periods. Quantitatively Group I, II and III honeys are the characteristics of sub-Himalayan West Bengal region whereas Group I and II characterize Sikkim Himalayan region.

Present study proves that Darjeeling and Sikkim Himalayan region endowed with rich and varied floristic complex has the natural potential for establishing organized apary industry and production of commercial single source honeys. Honeybees being the effective pollinators will also enhance the crop productivity in this area. This may improve the socio-economic set up of the local tribal communities of the area.

### Melissopalynological investigation in Sunderbans, West Bengal, India

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The present study deals with the pollen analysis of 34 honey samples (squeezed and extracted) from Sunderbans, the largest delta in the world and is constituted by characteristic tidal mangrove forest. The total area is 9360sq Km of which mangrove forest extends 4264 sq Km and 2585 sq Km under reserve forest areas. More than one thousand Mouli (honey hunter) families depend on forest honey and harvest more or less 60,000 kg of *Apis dorsata* honey per annum. A large number of *Apis dorsata* swarms make thousands of honey comb during summer (March-June) every year.

Of the 34 honey samples 14 were collected from the tiger reserve forest areas and the rest were from the inhabited and cultivated zones of Sunderbans. Among these only 4 samples (2 samples each) were taken from the hives of *Apis mellifera* and *A. cerana indica* and rest 28 samples were extracted honey of *Apis dorsata*.

The methodology recommended by the International Commission for Bee Botany (Louveaux, et al., 1978) was employed for the quantification of the pollen contents of honeys. A total of 23 pollen types referable to 18 families were recorded from the honeys of the reserve forest areas and 46 pollen types belonging to 32 families were recovered from the honey samples of the rest part of Sunderbans.

Out of 34 honey samples 25 were found to be unifloral and 9 multifloral in nature. *Sonneratia apetala*, *Aegiceras corniculatum*, *Ceriops decandra*, *Bruguiera gymnorhiza*, *Phoenix sylvestris* constituted the predominant pollen types recorded in the unifloral honeys studied. Pollen grains of tree taxa were better represented. *Avicennia* sp., *Excoecaria agallocha*, *Xylocarpus mekongensis*, *Nypa fruticans* were also recorded as secondary and important minor types. *Heritiera fomes*, once which was the most predominant plant in Sunderbans, was poorly represented as minor pollen types in a few samples. *Sonneratia apetala*, *Ceriops decandra*, *Phoenix sylvestris* were considered as very frequent types in the honey samples of mangrove forest. It was also noted that microscopic analysis of honey samples of the core areas of Sunderbans showed the pollen grains of some species which do not even occur in the reserved forest area or in the vicinity. Like *A. dorsata* honeybees the domestic honeybees *A. cerana indica* and *A. mellifera* also preferred mangrove flowers as chief source of nectar. The fact indicates the possibility of exploiting the vast nectar pool of tidal mangrove forest for beekeeping ventures using domestic hive bees. The honeys collected outside the reserve forest areas exhibited a mixed assemblage of mangrove and cultivated crop plants. A nectar calendar has been prepared recognising the chief nectar sources in different months. It will help the local honey hunters to seek their suitable area of honey harvesting. The study further suggests that *Sonneratia apetala*, *Aegiceras corniculatum*, *Ceriops decandra*, *Bruguiera gymnorhiza*, *Phoenix sylvestris* can be propagated under afforestation and also in social forestry programmes for commercial beekeeping ventures involving *A. cerana indica* and *A. mellifera* in this deltaic region of West Bengal, India.

### Pollen analysis of a honey sample from the Iranian domestic market

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Based on study of palynomorphs encountered in a honey, 300 pollen and spores counted. The pollen analysis indicates that the honey appears to be multifloral in origin. Pollen of 20 angiosperm families identified. The families in ascending order, are as follow: Apiaceae, Asteraceae, Betulaceae, Cannabaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae, Ericaceae, Fagaceae, Juglandaceae, Oleaceae, Onagraceae, Plantaginaceae, Poaceae, Polygonaceae, Primulaceae, Rosaceae, Salicaceae and Verbenaceae. Of these, pollen of Brassicaceae, Fagaceae, Asteraceae, Primulaceae and Betulaceae are the most abundant composed to the others. The percentage of them is 61.3% of total percentage.

According to considerable presence of Betulaceae pollen, it is probable that the study honey belongs to forests of Arasbaran-Ardebil Area, northwest of Iran.

### Importance and development prospects for melissopalynology in Ukraine

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Ukraine was and remains a country with a developed bee-keeping sector. Until recently, it accounted for one ninth of the world number of bee families and for one eighth of the honey output.

It is predicted that the number of bee families will be increased to 6Ml in 2005. While small volume of Ukrainian production arrived to the foreign market in the 90's of the 20th century, the honey export has greatly risen over the recent years, reaching 3380 ton in 2002. Other bee products, in particular pollen load, are export items as well.

The evaluation of environmental conditions through perfect pollen analysis becomes not less important now. This statement is based on the fact that in adverse conditions plants produce a lot of outwardly degenerated pollen-grains, whereas in areas with insignificant environmental tension pollen-grains are normally developed in the absolute majority of cases.

The European Commission recommends to countries exporting apiary products to develop criteria for evaluation of their quality and to ensure their environmental monitoring. Therefore, for Ukraine, as a regular exporter of apiary products and a potential member of the European Union, there becomes exceptionally urgent the problem of harmonization of current state legal documents, as well as drawing up new ones, determining the quality of bee products according to requirements of world and European standards.

Melissopalynology revives on the modern scientific basis in Ukraine, which has a great potential for bee keeping development though is in a very difficult environmental situation.

The above-mentioned statements in our studies are evaluated with regard to areas of high environmental, especially radioecological, tension due to the Chernobyl disaster, consequences of which are especially perceptible in the Forest zone of Ukraine. Its point is that overall increase in radionuclide concentration often concurs with the flowering stage of the plant phenological development. Wind-pollinated plants produce a lot of pollen that results in formation of a biological way of the secondary aerial transfer of radionuclides. Thus, the radionuclide factor is present in the apiary ecological system as well.

The pointed out circumstances determine directions in the implementation of the national strategy and international cooperation with respect to the quality and standardization of bee products. The pollenological studies are carried out in the context of establishing specific peculiarities of morphological structure and its correlation with biochemical content (total lipids, polyunsaturated, monounsaturated and saturated fatty acids, aminoacids, mineral elements) as an integrated factor of nutritive, biological and pharmacological value, attractive properties and antimicrobial activity of plant pollen and bee load.

As a result of the Ukrainian-Austrian cooperation, a plant pollen data bank is being founded as a fundamental basis for further mutual studies for evaluation of apiary flora, determining of physical and chemical properties of bee load, pollination and protection of plants. At the same time, special attention is paid to ecological contamination of pollen loads and honey in different regions of Europe.

Just because of the above, the pollen analysis of bee products gains increasing importance, as naturalness, botanical identification and geographical origin of honey and bee load is determined by it with high reliability. Also, nutritive and apitherapeutic properties of these products are evaluated, the environment's impact is established, the risk degree of bee keeping in tense environmental conditions is found out.

In different times, researchers of Ukraine made studies of specific content of bee products (honey, cerago, bee load) using pollen analysis (V.N. Andreev, 1927; A.I. Cherkasova, 1972; M.K. Kolomijets, 1973). These studies grounded a new stage in the investigation of the melliferous feed basis of Ukraine's present bee keeping.

Owing to its favorable natural and geographical location, the feed basis of bee keeping in Ukraine is distinguished by its diversity. Ukraine's flora consists of about 5,000 species of flowering plants, 900 of which are used by bees as nectar sources. This diversity of species is explained by Ukraine's location in five different natural and climatic zones: Forest, Forest-Steppe, Steppe, mountainous part of the Carpathians, and Crimea. Due to these unique geographical conditions, plants of both European and Asian floras are present here. All this enables obtaining bee products of various floristic compositions and qualities.

Ukraine's prospective joining of the European market obligates the country to ensure high quality of production control. The pollen analysis is the basis for ensuring this quality with respect to bee products. Investigations in this field are carried out by joined efforts of several institutions of the Ukrainian Academy of Agrarian Sciences along with the apiculture chair of the National Agricultural University of Ukraine. Charting an atlas of melliferous plant pollen is one of fields of this work.

### The melliferous plants of the Algerian northeast

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In the extremity of the northeast of Algeria, vegetation forms a melliferous potential e where product a big variety of honeys during all year round. Many middle factors, ecological conditions and anthropological actions exercise a determining influence on the melliferous potentialities. The main objective of our contribution is to succeed to a knowledge of the melliferous flora in the zone of survey through land inspections.

The results permitted the identification of 280 entomophiles melliferous species belonging to 34 plant families.

We noted that the family of the Asteraceae contains the biggest number of melliferous species, consistent of the family of the Brassicaceae and the family of the Lamiaceae. Period of flowering, morphological features of pollen grains and production honey value have been noted for each pollen species. The distribution of the melliferous plantations was cartographed.

**Key words:** Melliferous Plants, honeys, pollens, cartography, northeast of Algeria.

### Melissopalynological study of five honey samples of the semi arid areas of Algeria

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With an aim of knowing the botanical origin of honeys of the semi arid areas of the Algerian (areas intern with dry climate), five samples of various zones were collected.

These honeys were prepared according to the methods of the melissopalynology.

Qualitative analysis and the estimate of the frequency of pollen starting from the counting of 200 - 500 pollen grains by sample reveal that:

- Studied honeys are poly floral except for only one honey.
- The presence of 55 botanical species in the various zones of study were visited by the bees.
- The pollen of Eucalyptus has found in all honeys without exception, whereas it is a plant exotic which could well adapted in Algeria.

**Key words:** Pollen, plants mellifère, semi arid, floral honey poly.

### Pollen characterisation of honeys produced in Viana do Castelo, Aveiro, Porto, Braga (N.W. Portugal)

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The qualitative and quantitative melissopalynological analysis of twenty honey samples produced in Viana do Castelo, Aveiro, Porto and Braga is presented. According to the qualitative analysis the honeys were classed as multifloral, unifloral of *Erica* and unifloral of *Eucalyptus*.

The most abundant taxa in unifloral honeys of *Erica* were *Cytisus scoparius*-type and *Castanea sativa*; the pollen of *Eucalyptus* and *Olea* were frequent too but, with representations less than 15%. The most abundant taxa in unifloral honeys of *Eucalyptus* were *Cytisus scoparius*, *Erica* and *Castanea sativa*; the pollen of *Olea* were frequent in this honeys.

The multifloral honeys were clustered in: multifloral *Echium* predominant and multifloral honey *Castanea* predominant. The pollens of *Castanea*, *Cytisus scoparius*-type, *Eucalyptus*, *Rubus* and *Echium* were important in this honeys.

The quantitative analysis showed that this honeys belonged to Maurizio's Classes I and II.

### Pollen characterisation of honeys produced in Setúbal, Santarem, Lisboa and Leiria (Portugal)

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We analysed twenty-three honeys produced in the Setúbal, Santarem, Lisboa and Leiria.

The qualitative analysis showed that 70% of the honeys were multifloral and 30% were unifloral of *Lavandula latifolia*, *Echium* and *Trifolium*.

The most frequent taxa in multifloral honeys were *Echium*, *Eucalyptus*, *Castanea sativa*, *Rubus*, *Cytisus scoparius* type, Ericaceae and, Cistaceae.

The pollen of Cistaceae, *Cytisus scoparius*-type, *Lavandula latifolia* and *Eucalyptus* were frequent in unifloral honeys of *Echium*; *Echium*, Ericaceae, Cistaceae and *Cytisus scoparius*-type were frequent in unifloral honeys of *Lavandula latifolia*; and *Cytisus scoparius*-type, *Rosmarinus officinalis*, *Rubus* and *Crataegus monogyna*-type were frequent in unifloral honeys of *Trifolium*.

The quantitative analysis showed that this honeys belonged to Maurizio's Classes II and III.

### The pollinic spectrum of honey from Alto Adige

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The pollen spectrum of the honeys from South Tyrol (North-Italy)" is a recent publication issued by the Biological Laboratory (Environmental Protection Agency - Province of Bolzano, Italy).

In an extensive research the authors analyzed honey samples from all parts of South Tyrol to characterize the pollen flora typical for the region. Important information about the type of apiculture (stationary or nomadic) was collected with a questionnaire compiled by the beekeepers. All results of the microscopic analyses were introduced into a

database. The study is not only the base for a future geographical origin control on local honeys but also gives information about the importance of different local plants species as nectar and pollen sources.

Besides the description of the scientific study, large part of the book is dedicated to a pollen atlas, containing 660 excellent microscopic pollen photos of 165 plant species. The book is therefore not only of interest for melissopalynologists, but also a useful reference book for all people working with pollen in their different application fields.

The book has a limited edition and is available in Italian or German language. You can request the book by email

### Honey sources of *Melipona beecheii* (Apidae: Meliponinae) in the Northern part of Quintana Roo

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The present study was done Puerto Morelos, in the Northern part of Quintana Roo state, Mexico. There is a medium stature tropical forest present in the study area, this type of vegetation is very common in the rest of the Yucatán Peninsula. We also can find secondary vegetation in different successional stages in the study site. A palynological analysis was made in order to determine the most important nectar sources of *Melipona beecheii* ("xunucab" in Mayan language).

This study took place from 1995 to 1999, 15 *Melipona* colonies were selected. Honey samples were obtained from the honey pots of the colonies for determination and quantification of nectar sources for this stingless bee. Overall, a total of 90 honey samples were obtained from *Melipona beecheii* colonies during the wet (May to October) and the dry (November to April) seasons. Six colonies were lost through the attack of predators like the tayra or "San hool" (*Eira barbara*) and the "Xulab" ant (*Eciton burckhelli*). Ten grams of honey from each sample were dissolved in 10 ml of distilled water. The pollen was precipitated by centrifugation, acetolysed using the Erdtman technique (1943); *Lycopodium clavatum* spore tablets were added (13,000 spores per tablet approximately) to serve as an internal calibration standard. Then the pollen sample was mounted on slides with glycerine jelly. After the palynological identification, pollen grains were randomly counted (600 grains for each sample) in order to estimate the pollen percentage frequency of each pollen type found and the period in which the bees visited the flowers.

A total of 75 pollen species were found in the honey samples of *Melipona beecheii*. These species belong to 65 different genera and 32 family taxa. We obtained the pollen percentage frequency of these pollen species. During the wet season, the most frequent pollen species were *Cydistia potosina*, *Bursera simaruba*, *Platymiscium yucatanum*, *Eugenia axillaris*, *Torrubia linearibracteata*, *Smilax spinosa* y *Thrinax radiata*. During the dry season the most frequent pollen species were *Althermanthera ramossissima*, *Bignoniaceae* 1, *Bignoniaceae* 2, *Cecropia peltata*, *Conocarpus erecta*, *Terminalia catappa*, *Corton campechianus*, *Corton cortesianus*, *Mimosa pudica*, *Swartzia cubensis*, *Tilisia olivaeformis*, *Physalis pubescens*, *Solanum lanceifolium*, *Eugenia boxifolia*, *Myrcianthes fragans*, *Eugenia axillaris*, *Smilax spinosa* y *Thrinax radiata*, the three latter ones appeared in the honey samples of the wet season as well. Although *Cecropia peltata* is a nectarless plant, their pollen grains appeared in the honey samples, probably the bees contaminated the honey within the colony. The families with the largest number of species were Fabaceae, Myrthaceae and Sapindaceae. It is important to protect and conserve the forest, most of these species are shrub and trees of the medium stature forest, we also recommend to plant these species nearby the domesticated *Melipona* colonies.