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Technical note

Corbicular pollen spectrum (*Apis mellifera*) of samples from Huejotitan, Jalisco, Mexico

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Abstract:

This study examines the different plants visited by the honeybee (*Apis mellifera* L.) during the honey harvest season (August to November) 2012. The work consisted in identifying the corbicular pollen pellets collected by the bees in one apiary in the village of Huejotitan, municipality of Jocotepec, state of Jalisco, Mexico. Three hives were selected and sampled monthly by means of Ontario modified pollen traps. The samples were tagged and frozen and later processed by acetolysis technique to remove the exine; permanent glycerine slides were made for the preservation and analysis. Identification and counting of pollen grains was performed using an Olympus BH-2® upright microscope equipped with a 100X ocular micrometer to measure each individual species pollen grain, using immersion oil. Wild plants in bloom were also collected monthly, tagged, pressed and taken to the herbarium for identification; the pollen was extracted, processed and identified for a reference collection that served as an ancillary means of identification and as a seasonal reference to the blooming species. In the corbicular pollen, 23 types of plants were identified: 13 at species level, five at genus level and five at family level belonging to 17 plant families. Myrtaceae resulted the most frequently represented family followed by Asteraceae, Fabaceae and Lamiaceae.

Key words: Bee behavior, Bee foraging, Apipalynology, Apibotany.

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Despite their role as key pollinators among insects[1], the biological fundamentals for pollen source selection by honey bees (*Apis mellifera*) in Mexico are still mostly unknown. Botanical studies with apicultural interests are not particularly abundant if is considered that Mexico is a large and mega diverse country, classified in the first places in apicultural production and exports in the world[2]. Since bees depend entirely on the vegetation for their survival, it is crucial to understand their feeding preferences as well as the specifics about pollen availability throughout the year. Pollen contains the nutrient protein[3] needed for the brood and young workers survival and proper development. It also contains lipids, vitamins and minerals[4] as incidental components.

Foragers collect pollen in a trend and proportion that vary greatly according to the availability of the resources, distance to the source, nutritional value[5], needs of the hive, *i.e*. life cycle and physiology of workers, queen and drones and weather conditions[6]. This is of particular interest to beekeepers and researchers because the gathering behaviour of the bees does not seem to have fixed patterns. Each season bees will collect pollen in regards to different variables and even in arbitrary ways, *i.e*. without regard to its nutritional value or from resources that are not as close or highly available as others[7]. This type of information is fundamental to beekeeping and to assess the potential of any determined area, for the production of pollen and for all efforts related to the conservation of biodiversity[8], particularly where the populations of bees are declining.

Analysis of the pollen collected provides information about its botanical origin, the preferred plant species and aids in understanding the foraging behaviour of the bees. The objective of this study was to find what pollen types were collected by honey bees during the honey production season.

With this in mind, a sampling project was designed to collect and analyze corbicular pollen to determine the spectrum of the pollen used by *A. mellifera*. The pollen grains were primarily identified by means of a special pollen reference collection made from plants in bloom in the locality. These plants were identified by botanical specialists from the University of Guadalajara, and the voucher specimens remain at the Botanical Institute Herbarium of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA). A site was selected within an area of importance for beekeeping, in one apiary in the village of Huejotitan, municipality of Jocotepec, state of Jalisco. The experimental site was located at 20°21’13.45’’N, 103°29’6.97’’W. The elevation at the site is 1,597 m asl. around the apiary, the land cover is dominated by seasonal cultivated crops, pastures and secondary vegetation interspersed with tropical deciduous forest.

Among 23 bee hives in the apiary, three were chosen for their strength to be sampled once a month for four months with modified Ontario pollen traps[9]. From August to November 2012, traps were installed and kept in place for 24 to 48 h and then removed. This period corresponds to the honey preharvest and harvest season. The corbicular pellets were gathered from the trays, cleared of debris, put in plastic containers, tagged and frozen. At the laboratory, 1.5 g of pollen were taken from each of the three samples corresponding to one given month and mixed together to form one single larger sample of the pollen collected from the three hives together. In the end there were four samples from the original 12, one for each month.

Before processing, the pellets were carefully and softly mashed in a mortar. The pollen grains were processed by acetolysis technique to remove the exine; permanent glycerine jelly slides were made for the preservation and analysis; the pollen grains were identified by their size and shape, with an Olympus BH-2® upright microscope equipped with a 100X ocular micrometer to measure each individual species pollen grain, using immersion oil; volume of the individual pollen grain was calculated with the formula: $V=4/3\pi a^2 b$ where "V" is volume, "a" is the major axe of the pollen grain and "b" the minor axe[(10)]. Identification was made by comparison with the pollen reference collection of the Institute of Geology, Universidad Nacional Autónoma de México. In order to obtain the relative percentage, all the pollen grains were counted in each slide. A reference collection with the pollen grains of local plants in bloom was prepared as an ancillary means of identification.

Every month for 4 mo a circuit between 3 and 5 km long in the surroundings of the apiary was walked to sample all blooming plants. The pollen grains where obtained by extracting the anthers from the flowers and then processed for acetolysis according to the same technique[(11)]mentioned above. The information was used to determine whether a plant was a source of nectar, pollen or both, as well as their migratory status. Information was also taken from the available domestic bee flora publications[(11-15)]. From the pellet samples, 23 different pollen types belonging to 17 plant families were recorded (Table 1) and from these 13 were identified at species level, 5 at genus level and 5 at family level. In August there was no dominant pollen type, however there were three secondary types, *Aster* sp., *Eucalyptus citriodora* and *Ricinus communis*, one important minor, Cyperaceae, and traces of other ones. Thus the four types were significant, with percentages above ten. In September *E. citriodora* was the dominant type with Poaceae and *Psidium guajava* as secondary types and traces of others. In this month three types were significant, with percentages above ten. In October no dominant type was obtained but there were again three secondary types, *E. citriodora*, *Hyptis albida* and *L. leucocephala*, all significant, with percentages above ten, and traces of others. In November *E. citriodora* was considerably dominant over the two secondary types, Asteraceae and *Pseudosmodingium* sp., but the three were significant, with percentages above ten, and traces of others. *E. citriodora* was significant in the four samples and Asteraceae and *L. leucocephala* were found in three. *R. communis*, *Sicyos angulatus*, *Citrus* sp., *Pseudosmodingium* sp. and Poaceae appeared in two samples each.

Table 1: Pollen types from pollen pellet samples, represented by taxa in percentages in the Huejotitan, Jalisco region during August-November 2012

Taxa	**Family**	**Aug (%)**	**Sep (%)**	**Oct (%)**	**Nov (%)**	**Migratory status**
Acacia farnesiana	Fabaceae			2.4		native
Aster sp.	Asteraceae	34.1				unknown
Asteraceae	Asteraceae		5.3	5.8	14.5	unknown
Betula sp.	Betulaceae		5.5			unknown
Citrus sp.	Rutaceae	1.7		1.4		exotic
Cyperaceae	Cyperaceae	14.0				unknown
Dodonaea viscosa	Sapindaceae		1.0	1.2		native
Eucalyptus citriodora	Myrtaceae	20.5	47.2	34.6	65.6	exotic
Fabaceae	Fabaceae	2.1				unknown
Fragaria vesca	Rosaceae	4.5				exotic
Fraxinus uhdei	Oleaceae				3.0	native
Heliocarpus terebinthinaceus	Malvaceae			2.1		native
Hyptis albida	Lamiaceae			18.2		native
Leucaena leucocephala	Fabaceae		2.9	16.1	2.0	native
Poaceae	Poaceae		17.5	1.0		unknown
Pseudosmodingium sp.	Anacardiaceae			8.2	11.9	unknown
Psidium guajava	Myrtaceae		17.0			native
Psittacanthus calyculatus	Loranthaceae	2.1				native
Ricinus communis	Euphorbiaceae	17.1	1.9			exotic
Rubus idaeus	Rosaceae			1.6		exotic
Salix sp.	Salicaceae	3.9				native
Sapindaceae	Sapindaceae					unknown
Sicyos angulatus	Cucurbitaceae			5.8	1.3	native
Others			1.7	1.6	1.7	unknown

Each month the represented families changed, however, there was a consistency in their overall presence and percentages of representation (Table 2).

Table 2: Pollen types from pollen pellet samples, represented by families and percentages in the Huejotitan, Jalisco region during August-November 2012

August 2012		**September 2012**		**October 2012**		**November 2012**	
Asteraceae	34.1	Myrtaceae	64.2	Myrtaceae	34.6	Myrtaceae	65.6
Myrtaceae	20.5	Poaceae	17.5	Fabaceae	18.5	Asteraceae	14.5
Euphorbiaceae	17.2	Betulaceae	5.5	Lamiaceae	18.2	Anacardiaceae	11.9
Cyperaceae	14.0	Asteraceae	5.3	Anacardiaceae	8.2	Oleaceae	3.0
Rosaceae	4.5	Fabaceae	2.9	Asteraceae	5.8	Fabaceae	2.0
Salicaceae	3.9	Euphorbiaceae	1.9	Cucurbitaceae	5.8	Cucurbitaceae	1.3
Fabaceae	2.1	Sapindaceae	1.0	Malvaceae	2.1	Others	1.7
Loranthaceae	2.1	Others	1.7	Rosaceae	1.6		
Rutaceae	1.6			Rutaceae	1.4		
				Sapindaceae	1.2		
				Poaceae	1.0		
				Others	1.6		
Total	100	Total	100	Total	100	Total	100

Asteraceae was present in the four samples, Myrtaceae in three, Anacardiaceae, Fabaceae and Rosaceae in two, and Betulaceae, Cucurbitaceae, Cyperaceae, Euphorbiaceae, Lamiaceae and Oleaceae in one. 78 different species of plants in bloom, belonging to 30 families and 71 genres, were documented during the 11 mo (Table 3) in order to have as many as possible species of pollen grains documented for reference. The five best represented families were Asteraceae with 33.33 %, Fabaceae with 8.97 %, Solanaceae with 6.41 %, Lamiaceae with 5.12 % and Verbenaceae with 3.84 %. These five families represent 29.41 % of the total number of families and 57.67 % of the total number of species. 17 of all the species have been reported to be nectar producers, seven pollen producers, 17 nectar and pollen producers and 37 are not documented in terms of their importance for honey bees; 50 % were forbs, 30.77 % shrubs and 19.23 % trees. Considering all the species, 88.46 % were native and 11.54 % were exotic. Twenty-six (26) species were documented to be visited by honey bees.

Table 3: Species of plants sampled for the reference collection

Species	Family	Source	Form	Migratory status
Acacia farnesiana	Fabaceae	N-P	shrub	native
Adenophyllum cancellatum	Asteraceae	x	forb	native
Argemone mexicana	Papaveraceae	P	forb	native
Asclepias glaucescens	Apocynaceae	N	shrub	native
Bidens odorata	Asteraceae	N-P	forb	native
Bidens pilosa	Asteraceae	N-P	forb	native
Bocconia arborea	Papaveraceae	x	tree	native
Brassica rapa	Brassicaceae	N	forb	exotic
Buddleja sessiliflora	Scrophulariaceae	N-P	shrub	native
Casimiroa edulis	Rutaceae	N	tree	native
Castilleja tenuiflora	Orobanchaceae	x	forb	native
Chromolaena collina	Asteraceae	x	shrub	native
Cissus verticillata	Vitaceae	N	forb	native
Clematis rhodocarpa	Ranunculaceae	x	forb	native
Conyza canadensis	Asteraceae	x	forb	native
Cucurbita foetidissima	Cucurbitaceae	P	forb	native
Dicliptera peduncularis	Acanthaceae	x	forb	native
Diphysa puberulenta	Fabaceae	N-P	shrub	native
Dyssodia tagetiflora	Asteraceae	x	forb	native
Ehretia latifolia	Boraginaceae	x	tree	native
Erythrina coralloides	Fabaceae	x	tree	native
Eucalyptus citriodora	Myrtaceae	N-P	tree	exotic
Eupatorium odoratum	Asteraceae	N-P	forb	native
Flaveria trinervia	Asteraceae	x	forb	native
Fleischmannia sonorae	Asteraceae	x	forb	native
Fraxinusuhdei	Oleaceae	N-P	tree	native
Gronovia scandens	Loasaceae	x	forb	native
Guazuma ulmifolia	Malvaceae	N-P	tree	native
Heimia salicifolia	Lythraceae	x	shrub	native
Helianthus annuus L.	Asteraceae	N-P	forb	native
Hyptis albida	Lamiaceae	N	shrub	native
Ipomoea hederifolia	Convolvulaceae	x	forb	native
Ipomoea murucoides	Convolvulaceae	N	tree	native
Ipomoea purpurea	Convolvulaceae	x	forb	native
Iresine diffusa	Amarantaceae	x	forb	native
Jacaranda mimosifolia	Bignoniaceae	P	tree	exotic
Lantana camara	Verbenaceae	P	shrub	native

Leonotis nepetifolia	Lamiaceae	N-P	shrub	exotic
Licopersicum esculentum var. cerasiforme	Solanaceae	N	forb	native
Lippia umbellata	Verbenaceae	N	shrub	native
Mandevilla foliosa	Apocynaceae	x	shrub	native
Melampodium perfoliatum	Asteraceae	x	forb	native
Melia azedarach	Meliaceae	N-P	tree	exotic
Mimosa galeottii	Fabaceae	N	shrub	native
Montanoa karwinskii	Asteraceae	N-P	shrub	native
Nicotiana glauca	Solanaceae	x	shrub	exotic
Olivaea tricuspis	Asteraceae	x	forb	native
Parthenium hysterophorus	Asteraceae	P	forb	native
Perityle microglossa	Asteraceae	x	forb	native
Phytolacca icosandra	Phytolaccaceae	N	forb	native
Pistacia mexicana	Anacardiaceae	x	tree	native
Pithecellobium dulce	Fabaceae	N-P	tree	native
Prosopis laevigata	Fabaceae	N-P	tree	native
Pseudognaphalium chartaceum	Asteraceae	x	forb	native
Psidium guajava	Myrtaceae	N	tree	native
Psilactis asteroides	Asteraceae	x	forb	native
Psittacanthus calyculatus	Loranthaceae	N	forb	native
Ricinus communis	Euphorbiaceae	N	shrub	exotic
Salvia misella	Lamiaceae	x	forb	native
Salvia tiliifolia	Lamiaceae	x	forb	native
Schinus molle	Anacardiaceae	N-P	tree	exotic
Senecio salignus	Asteraceae	P	shrub	native
Senna occidentalis	Fabaceae	x	forb	native
Serjania racemosa	Sapindaceae	N-P	forb	native
Solanum ferrugineum	Solanaceae	x	shrub	native
Solanum grayi	Solanaceae	x	forb	native
Solanum grayi var. grandiflorum	Solanaceae	x	forb	native
Tagetes erecta	Asteraceae	N	forb	native
Thunbergia alata	Acanthaceae	x	forb	exotic
Tithonia tubiformis	Asteraceae	N	forb	native
Tournefortia mutabilis	Boraginaceae	x	shrub	native
Trixis hyposericea	Asteraceae	x	shrub	native
Verbena bipinnatifida	Verbenaceae	x	forb	native
Verbesina barrancae	Asteraceae	x	shrub	native
Verbesina crocata	Asteraceae	x	shrub	native
Vernonanthura cordata	Asteraceae	N	shrub	native

Vernonia bealliae	Asteraceae	P	shrub	native
Viguiera quinqueradiata	Asteraceae	N	shrub	native

The columns show species, family, food source: P= pollen, N= Nectar, x= not documented, form of life and migratory status in Mexico.

The months with more species in bloom were September and November with 16 species each, then October with 13 and finally August with 12. Bees represent the primary pollinators among insects and honey bees are becoming the only ones in areas where intensive crop monoculture is gradually wiping out the wild native insects. One of the reasons is that *A. mellifera* belongs to one of the few bee genera known to have polylectic habits(16). Yield increases are reported to be up to 96% in cultivated crops pollinated by them(17). In terms of the sources used by the honey bees as revealed in this study, Myrtaceae had the second highest percentage in August and by far the first in September, October and November. This family was prominently represented by *E. citriodora*, an introduced species. Originally evolved in the Austro-Malaysian region(18), has been introduced in many countries for its value as timber, fuel wood, wood fiber and ornament(19). The floral phenology of *Eucalyptus* tends to be synchronous among different individuals within one stand but at the same time shows great variation in flowering time and even intermittent flowering periods over the greater part of the year(20); honeybee has been documented to be one of the most prevalent visitors its flowers(21).

In August the Asteraceae family was dominant over Myrtaceae. Asteraceae is the most abundant family in Mexico(22-25) and represents an estimated 10 % of all know plants in the world(26) and its center of diversification is located in Mexico, where it is the largest and most representative group, containing from 7 to 32 % of the country's flora and 12.5 % of Jalisco´s(27). In September, the second most abundant family was Poaceae, but neither species nor genre were determined. This is also an extensive group, with more than 500 species worldwide, which includes the cereals humans consume and the grasses for cattle feed(26). In October, the second most important families, in equal percentages were Fabaceae, represented by *L. leucocephala* and *A. farnesiana*, and Lamiaceae, by *H. albida*. Although the percentages in this study refer to the number of pollen grains and not to their volume, the sizes are still relevant because the ratios change when analyzed in terms of a different variable. *E. citriodora* pollen grains are small, 25 µm average, and have the shape of a flattened triangular prism. The volume of one these grains averages approximately 3,125 $µm^3$. Contrastingly, the pollen grains of *A. farnesiana* are 60 µm average, and ellipsoid in shape. Their volume is approximately 78,539.8 $µm^3$. *E. citriodora* represented 34 % of the sample and *A. farnesiana* (Fabaceae) 2.4 % in terms of number of grains. Nevertheless, if their total volumes compared, the proportions change radically: 106,250 $µm^3$ for *E. citriodora* and 196,349.5$µm^3$ for *A. farnesiana*, almost twice the volume of *E. citriodora*.

In November Asteraceae was the second most important family; some pollen species, regardless of their frequency, were present in at least two of the pollen load samples indicating their presence for longer than a single month thus representing a long-term food resource during the year. Such is the case with *E. citriodora*, present in the four samples, and Asteraceae and *L. leucocephala* (Fabaceae), present in three. Of the 23 pollen types found in the samples, seven have been reported as pollen sources for honey bees in other states *A. farnesiana*, *Fraxinus uhdei*, *Heliocarpus terebinthinaceus*, *L. leucocephala*, *P. guajava*, *R. communis*, and *S. angulatus*(11-15,27,28).

Of the total number of plant species in bloom observed in the area throughout the year only 34.21% have been reported to be used by the honeybees(22-26,29,30). This might be explained by the selectiveness of honey bees depending on the relative abundance and quality of nectar, pollen and distance to the sources.

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