Assessment of origin and relative contributions of various plant species as honeybee (*a. Mellifera*) pollen sources

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Abstract

Survival of honeybee colony is totally linked to its ability to collect sufficient quantities of pollen and nectar from plants flower. This study analyzes the origin and relative contributions of various floral sources around Utrecht University, Netherlands through pollen collection from honeybees (*A.mellifera* L.) during seven months of 2003. The study identified 50 different plant families comprising 105 different species in the whole collections with an average of 10.3 species per collection week. There was a significant variations ($\chi 2$ =6519.622, df=26, $p<0.01$) in the amount of pollen collected between the weeks with an average of 140.6g. Only 13 families contributed to more than 95% of the collection in which the four most important contributors Rosaceae (39.59%), Legumes (18.28%), Oleaceae (11.97%) and Compositae (8.08%)) accounted for more than 77%. Shannon Weiner index indicated low pollen source species diversity in the first weeks and at climax in the middle with subsequent declining as season advanced. Four different plant life forms (shrubs, herbs, trees and grasses) were identified in the total collections and shrubs and herbs alone contributed for 95% of the total pollen collections. Similarly, Pearson product-moment correlation coefficient showed a strong negative correlation (r= -532, n=27, $P<0.05$) between the amount of pollen and number of species collected, suggesting bee's pollen collection behavior is largely influenced by pollen availability than the species diversity.

Keywords: *Honeybees; pollen; herbs; diversity; trees*

Introduction

The ecological relationship between bees and the flowering plants (angiosperms) of the world is long lasting in that flowers provide different kinds of resources to bees and the bees provide pollination to the plants. Bees collect pollen and store it in the comb cell to provide as food for the brood and newly emerging bees. Including the full development of their hypopharyngeal glands, pollen provides bees with amino acids, fats and vitamins to achieve maturity (Ribbands 1953). Fresh pollen brought to the beehive stimulates brood rearing and hence, an average bee colony requires about 20 - 50kg pollen per year (Butler 1949, Winston 1987 and Seeley 1995). The number of plant species honeybee uses to collect food to make all hive products is enormous. In due course of collecting and transporting pollen as food sources, the types and amount transported by honeybees

varies from place to place (Seeley 1995). It has been documented that honeybee forage choice is overruled by the variations in the date and duration of the flowering periods of plants, and these in turn depends on the season, the area in which an apiary is situated, its soil conditions and climates (Hodges 1984 and Roubik 1989). In other words, the origin and pollen amount contributed by each plant species varies according to different localities and it is difficult to draw conclusions based on the result recorded for one specific area. Owing to this, there is a large gap in our knowledge on the varieties and quantity of pollen collected from each plant species for a given area. Therefore, the main purpose of this study is to assess the pollen source plants and determine their relative pollen contributions as honeybee food sources around Utrecht University, The Netherlands.

Materials and Methods

The study was conducted at the Utrecht University, The Netherlands, Botanical Garden compound [(zero meter above sea level, Rainfall (236mm), Temperature (-4.4 to 35^{0}C)].

Pollen samples were collected from a strong honeybee colony (*A. mellifera* L.) housed in 40 framed langstroth hive placed under the roof. Pollen collections were performed once a week from April 2003 until October 2003 using conventional pollen trap (18% efficiency) fitted to the hive entrance. Until the date of the analysis, the collected pollen samples were stored in a deep freeze according to Doull (1966) and 24hrs before running the analysis; it was allowed to dry at room temperature. The total weights of the dried pollen were taken to obtain information on the foraging intensity of the bees and to infer the relative contribution of each botanical species. Subsequently, 20g bee collected pollen was randomly sampled from each collection round, weighed and sorted into homogeneous colour groups using standard colour charts according to Hodges (1984) and Krik (1994). From each homogeneously sorted pollen loads, 1-2 loads were placed on microscopic slide, mixed with distilled water, smeared across the slide, cover with rectangular coverslips (17x17mm), stain either side of the coverslips with coloured and uncoloured glycerine jelly to differentiate between the pollen components and make them more visible and carefully dried on a warm plate to evaporate extra water (Sawer 1981). Subsequently the identity of the pollen to its plant type and morphology were confirmed by measuring the pollen sizes using 40x magnification of compound microscope (Louveaux, *et al*., 1978 and Sawer 1981). In addition, the identification processes were assisted with keys, diagrams and photographs in the books and other previous publications (Sawer 1988, Ricciardelli 1997, Van der Ham *et al*, 1999).

The contributions of each coloured sorted pollen loads in the 20g sample to the total collection were calculated from the total weight of weekly collected pollen. The levels of importance as honeybees' pollen source were determined based on their proportions in the total weight (Silveria, 1991). The relationships between the amount of pollen

collected per week (measured as the weight of pollen collected) and number of species collected per week (measured as number of species occurring in the weeks sample) was investigated using Pearson product-moment correlation coefficient. The dominance of each taxon in the collection sample and hence in the whole collections were analyzed based on their relative weight in every sample. Shannon Wiener diversity index were run to estimate species diversity in each weekly-collections. Honeybee foraging intensity (amount of pollen collected) from each taxon was correlated over the week collections.

Results

Pollen spectrum and major pollen sources

The result indicated that the number of plant families and species of floral sources of pollen foraged by honeybees were diverse and varied during the collection periods (Figure 1). Totally, 50 families and 105 species pollen was represented in the collection as honeybee pollen sources. Even if the pollen collected spectrum is high, only limited families contributed largely to the total collections and about 95% of the collection came only from 13 families (Table 1). Honeybees collected pollen from an average of 23 plant families monthly, with highest species diversity in August (Figure 1). The collected pollen spectrum included entomophilous, anemophilous, native and introduced plant species (Table 1). Collections of pollen from introduced plants were evidenced, in June and July from Anacardium spp and in October from Palmeae. Likewise, it was recognized that bees included pollen from stimulant plant Canabiaceae (Canabis sativa) and fungus spore as a pollen sources into their collections. The identified anemophilous plants pollen (Avena sativa, Zea mays, and Dactylis glomerata) contributed nearly 3% of the total collection (Table 1).

Table-1. Plant families and their pollen weight collected in percent

Family	% collected	Plant family	% collected	Plant family	% collected
Rosaceae	39.59	Anacardiaceae	0,26	Lbiatae	0.05
Legunimosae	18.28	Corniaceae	0.26	Polmoniaceae	0.05
Oleaceae	11.95	Locinicer aceae	0.23	Rutaceae	0.05
Compositae	8.08	Liliaceae	0.21	Crassulaceae	0.04
Bracicaseae	2.92	Simarcubaceae	0.20	Fagaceae	0.04
Gramineae	2.85	Ericaceae	0.18	Canabiaceae	0.03
Aralianceae	2.53	Balsaminaceae	0.12	Nymphaeaceacae	0.03
Hippocastanaceae	1.95	Chenopodiaceae	0.12	Valerianaceae	0.02
Unidentified	1.76	Apiaceae	0.11	Cyperaceae	0.01
Plantaginaceae	1.66	Malvaceae	0.10	Hydrophyllaceae	0.01
Scrophalareacae	1.60	Graniaceae	0.08	Polygalaceae	0.01

Family	% collected	Plant family	% collected	Plant family	% collected
Ranunculaceae	1.31	Mangoliaceae	0.08	Saxiferagaeceae	0.01
Oenotheraceae	1.13	Onagraceae	0.08	Solanaceae	0.01
Cucurbitaceae	0.73	Palmeae	0.08	Tropeolaceae	0.01
Fungus	0.54	Crucuferae	0.07		
Polygonaceae	0.46	Papaveraceae	0.07		

The number of plant families and species identified in the collection varied over the collection periods and ranged from 4-22 and 6-33 with an average of 16.14 and 23.86, respectively (Figure-1). The diversity increased with season and reached maximum in August. More specifically, pollen from Compositae, Leguminosae and Rosaceae families were more divers and collected for long periods (for about six months).

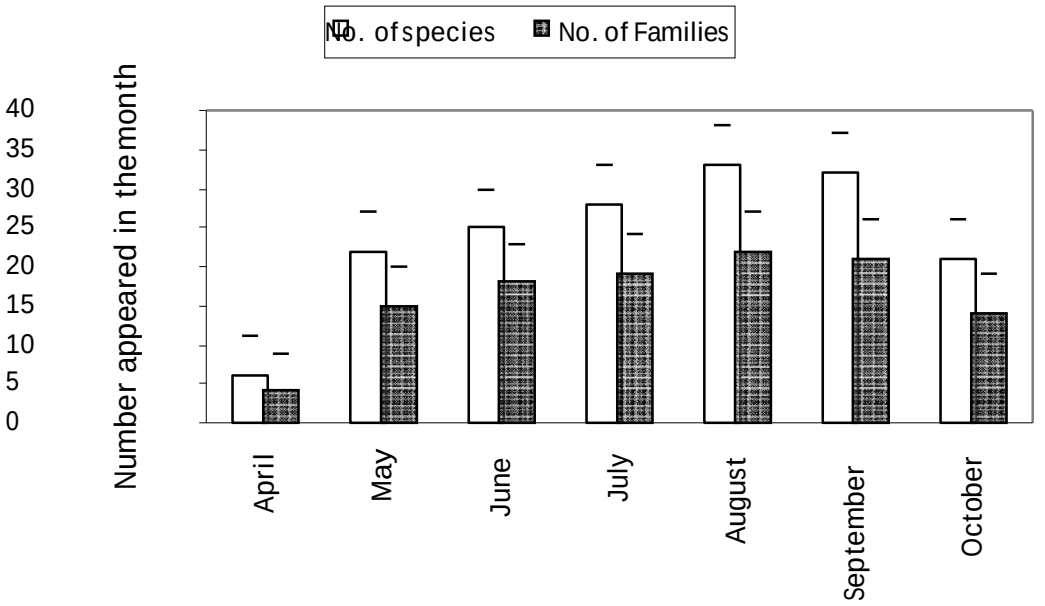

Figure 1. Number of plant families and species identified over the collection months

Pollen foraging intensity

With 18% efficiency pollen trap, a colony collected a total of 3794.52g of pollen during the whole collection periods and this averagely equals to about 21kg of pollen for the season without pollen trap. However, there is big variation in terms of the amount collected (16-668.6g) between the weeks ($\chi 2$ =6519.622, df=26, $p<0.01$) with an average of 140.6g per collection week and bees have collected maximum amount in the first three weeks and minimum in the 21st and 22nd weeks (Figure 2).

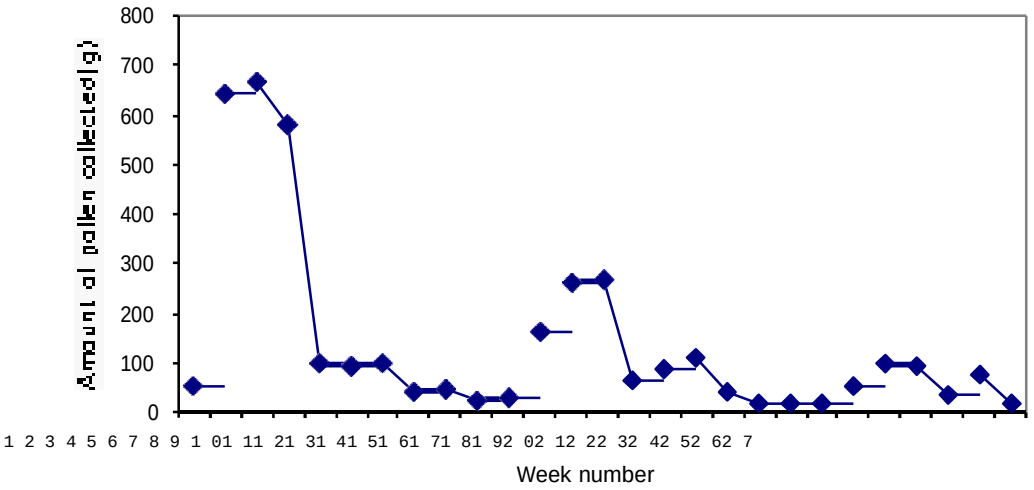

Figure 2. Amount of pollen collected by the honeybees over the collection weeks

Species diversity over the collection weeks were evaluated using Shannon Weiner diversity index and have shown low species diversity in the first weeks and increased as season advanced and attained climax at the middle of the season, and started to decline as season advanced (Figure 3). From the total 105 plant species identified during the collection periods, to the minimum one species is collected only once and to the maximum 12 with an average of 10.3 per collection week.

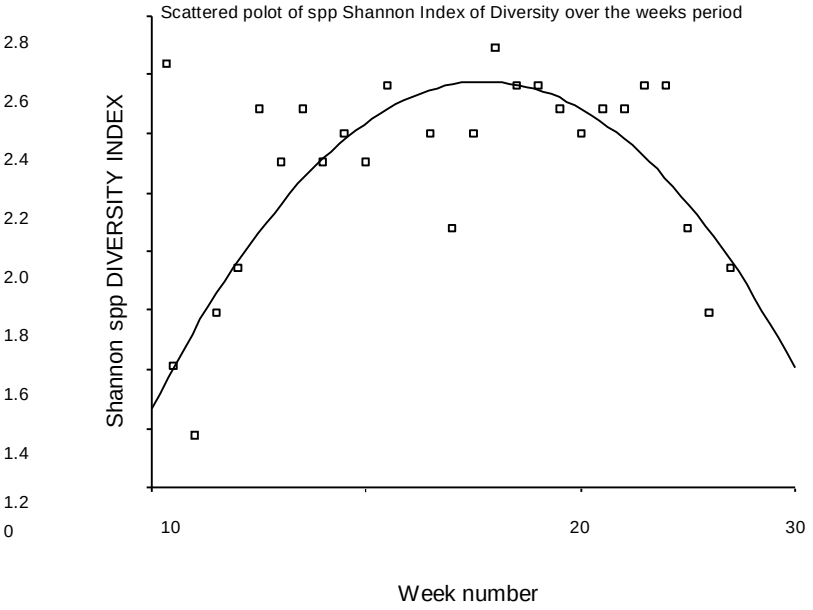

Figure 3. Shannon species diversity index over the collection weeks

Therefore, the species Diversity Index out put related to the collection periods (in this case week numbers) can be explained in a general equation of $A=d+am+bm^2$, where, A= Shannon Diversity Index out put; m= time in weeks, d, a and b, values of linear regression analysis. Hence, the relationship between the Shannon Diversity Index out put and the time of collection in this study case can be calculated as:

$A= 1.464+0.144m-1.005m^2$ (Figure 3)

Pollen amount versus species number

The relationships between the amount of pollen collected per week and the number of species collected were investigated using Pearson product-moment correlation coefficient. Accordingly, there was strong negative correlation between these two factors ($r = -532$, $n = 27$, $P< 0.05$), i.e., high pollen weight record associates with low species diversity (Figure 4).

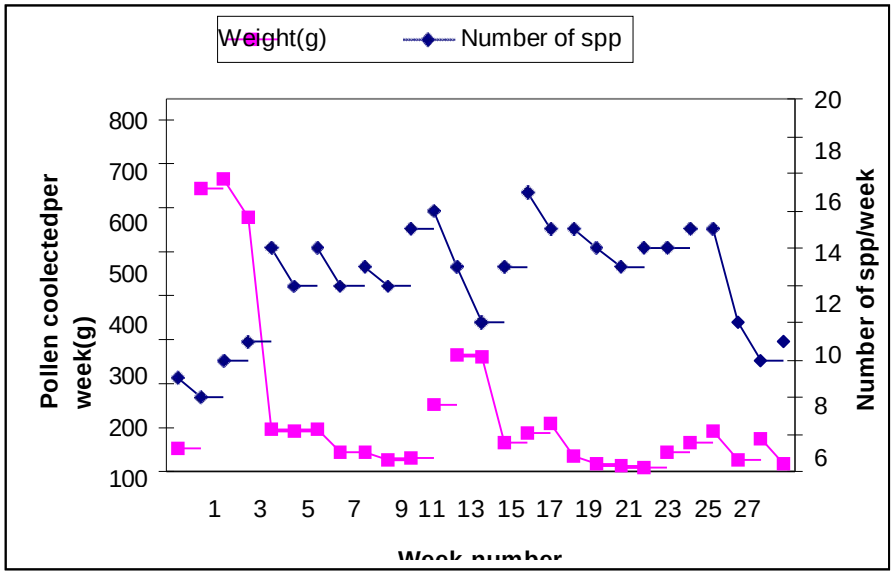

Figure 4. Amount of pollen collected and number of species over the collection weeks

Relative pollen contribution of plant forms

There were four different plant life forms (trees, shrubs, herbs and grasses) identified which contributed to the collected pollens. Comparing their species abundances and frequency of pollen collections from each life forms, there is significant variations ($\chi 2 = 131.165$, $df = 3$, $p<0.01$) and ($\chi 2 = 351.420$, $df = 3$, $p < 0.01$), respectively for trees, shrubs, herbs and grasses. Similarly, each plants life was evaluated in terms of pollen contribution to the total collection. Hence, there is significant difference between them

($\chi 2$ = 3102.197, df = 3, $p<0.01$) and large amounts of contribution were done from shrubs (54%) and herbs (41%). Whereas, the contributions from grasses and trees were very minimal and accounted for 3% and 2%, respectively (Figure 5).

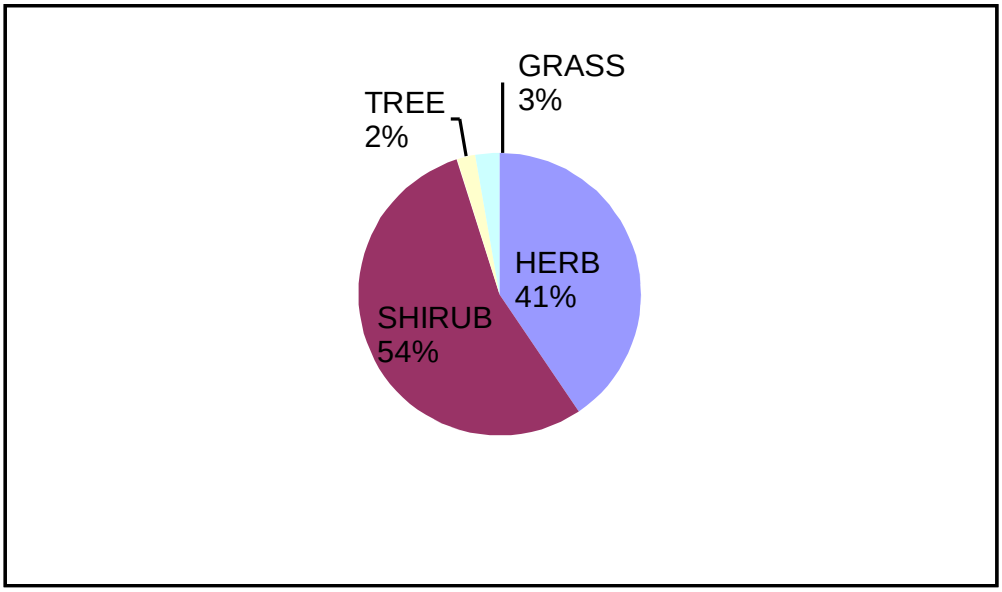

Figure 5. Relative pollen contributions of different plant life forms

Discussion

During the study period, honeybees collected pollen grains from 105 plant species under 50 plant families. However, only 13 plant families were found as main contributors in terms of pollen amount and pollen collection time span. The bees have collected about 21kg of pollen in the season and this accord with the previous records (Butler 1949, Winston 1987 and Seeley 1995). There is a considerable fluctuation in terms of the collected pollen amount over the months. This might be due to the interaction of the climatic and floral factors and the colony demand which in turn is governed by the amount of brood in the hive. Large amounts of pollen collections were done during the first weeks of the season. This might be because of large pollen requirement by the bees to initiate and enhance brood rearing that was suspended as a result of unfavorable winter season. However, the declining of the pollen collected after the third week might be attributed to the temporary pollen optimal collection by the bees and lack of storage space, which might insist the bees to shift their mind to collect nectar. Collections of large amounts of pollen from few species were done at the beginning of the season. This might be due to the fact these plant species were either preferred by the bees as pollen source or because they were the copious species, as pollen collection is often done from the most abundant species.

Contrarily, small pollen amounts were collected from relatively large variety of plant species towards the end of the active season. This most probably indicates the existences of diversified plant species but sparsely populated to be plenty pollen sources.

The anemophilous pollen collections were done by the honeybees in almost all the collection months and this fact is recorded (Soderstorm & Calderon 1971, Pojar 1973, Severson and Parry 1981, Cortopassi-Laurino and Ramalho 1989 and Suryanarayana et al 1992). Although the reason and its side effects were not stated, bees collection of pollen from stimulant plant Canabiaceae as a pollen source were also recorded in India (Suryanarayana et al. 1992). Similarly, honeybees' inclusion of fungal spores and other none pollen materials in their collection is also reported (Chapman 1964 and Roubik 1989). Even if the motive is not yet clear, it is speculated that bees collect fungus due to lack of sufficient or quality pollen (Roubik 1989). But, this speculation didn't hold true in this study as the bees did fungal collection when there are plenteous of natural pollen to collect. Collections of pollen by the bees from introduced plants of the adjacent green house suggests wide adaptations of honeybees to collect pollen from any available pollen sources regardless of their co-evolution/co-existences with pollen source species, as it is demonstrated on artificial bioassay (Pernal and Currie 2002).

Conclusions

The study identified 50 different plant families comprising 105 different species with an average of 10.3 species per week. Of the so different plant families occurring in the total collection, only 13 families contributed to more than 95% of the collection. Among these 13 families, the four most important plant families namely Rosaceae (39.59%), Legumes (18.28%), Oleaceae (11.97%), and Compositae (8.08%)) contributed more than 77% of total pollen collection. Four different plant life forms (trees, shrubs, herbs and grasses) were identified in the collected pollens and shrubs and herbs alone contributed 95% of the pollen. There was high foraging intensity at the beginning of the season as a sign of commencing brood rearing and colony build up after long time wintering. In addition to the origin and relative contributions of pollen source plants this study showed strong negative relation between the amount of pollen collected and the number of pollen source plant species and this suggests the largely influence of pollen availability on pollen collection behavior of the bee than the species diversity.

Finally, this study not only gave an insight into the origin and relative pollen contributions of the surrounding plants as honeybee food sources, but also highlighted major pollen source plants for further plantation, conservation and characterizations.

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