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No acute mortalities in honey bee colonies (*Apis mellifera*) after the exposure to sunflower cultures

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Three groups of 10 homogeneous honey bee colonies were placed before sunflower blooming in different surroundings within a 20 km-diameter area. Two groups were located in an area with a large surface of cultivated sunflowers, the last one being exposed to a forest environment. Exposure to sunflower culture was assessed by the acreage of cultivated plants within a radius of 1.5 km around each apiary. Mean adult and brood honey bee population decreased over time and were not statistically different between groups. At the end of the sunflower honey flow, colonies placed in the forest environment had statistically higher populations. When exposed to sunflower, honey production was low compared to historical figures (around 13 kg per colony in both sites), and was highly variable depending on field conditions. No residues of imidacloprid, fipronil and metabolites were found in honey bee, pollen loads, honey and beebread.

Spores of *Nosema sp.* were found in honey bees collected at hive entrances before and after sunflower flowering. No symptoms was recorded at hive entrance at any time, neither honey bee mortality. Foraging activity was irregular and highly dependent on time in all of the three groups of hives.

Small quantities of sunflower pollen were found stored in beebread as reserves although quantities of beebread were high, which means that sunflower pollen had been consumed by honeybees during the sunflower honey flow. Bibliographic references indicate that the poor quality of sunflower pollen and the low pollen diversity in collected pollens could have been the key factors in honey bee population development. Conservation, restoration and management of diversified melliferous habitats are crucial for beekeeping and to maintain wild fauna. It was suggested that more work should be done on nectar and pollen production as cost for new sunflower cultivars selection, particularly on attractiveness and accessibility towards pollinators (nectar and aroma composition, floral anatomy). These possible costs for selection should be tested before marketing new plants lines.
Keywords: *Apis mellifera,* mortality, honey production, pesticide residues.

Des colonies d'abeilles (*Apis mellifera*) n'ont pas montré de mortalités aiguës après l'exposition à la miellée de tournesol
Trois groupes de 10 colonies d'abeilles ont été placés avant la floraison du tournesol dans des milieux culturaux différents, à l'intérieur d'une zone de 20 km de diamètre. Deux des groupes étaient situés dans une zone avec de grandes surfaces de cultures de tournesol, le dernier groupe étant placé dans un environnement forestier. L'exposition à la culture de tournesol a été évaluée par les surfaces de tournesol cultivées à l'intérieur d'une zone de rayon de 1.5 km autour de chaque rucher suivi. Les populations moyennes d'abeilles adultes et de couvain ont diminué au cours de l'étude et n'étaient pas statistiquement différentes entre les lots. A la fin de la miellée de tournesol, la production de miel était faible (environ 13 kg par colonie pour chaque site) comparée aux résultats historiques, et était très dépendante des conditions rencontrées sur le terrain. Aucun résidu d'imidaclopride, de fipronil ou de leurs métabolites n'a été retrouvé dans les pelotes de pollen, le miel ou le pain d'abeille.

Des spores de *Nosema sp.* ont été trouvés dans des abeilles échantillonnées à l'entrée des ruches avant et après la floraison des tournesol. Aucun symptôme n'a été relevé à l'entrée des colonies à aucun moment de la miellée, ni aucune mortalité d'abeilles.

De petites quantités de pollen de tournesol ont été stockées sous forme de pain d'abeille, bien que les réserves en pain d'abeille étaient correctes, ce qui signifie que le pollen de tournesol a été consommé par les abeilles pendant la miellée. Des éléments bibliographiques montrent que la piètre qualité du pollen de tournesol, associée à la faible diversité en pollens des pelotes peuvent être les facteurs clés du développement limité des colonies d'abeilles. La conservation, la restauration et la gestion d'habitats diversifiés et mellifères sont cruciales pour le maintien d'une apiculture de qualité et pour garder la faune entomophile sauvage. Les auteurs suggèrent que des études soient entreprises sur la production de nectar et de pollen des nouveaux cultivars de tournesol. Une attention particulière devra être portée sur l'attractivité et l'accessibilité du nectar pour les pollinisateurs (composition aromatique et anatomie des fleurons). Ces possibles coûts de sélection devraient être testés sur l'abeille domestique en laboratoire avant la commercialisation des nouvelles lignées de plantes.
Mots clefs: *Apis mellifera*, mortalité, production de miel, résidus de pesticides.

1. INTRODUCTION

Modern agricultural practice attempts to maximize profit by increasing yield against costs, leading to large agricultural parcels that result from the destruction of woodlands, hedgerows, ditches and marginal grassland. Consequently to the set up of these intensive agricultural landscapes, the habitat diversity decreases which, in turns, has an impact on pollinator populations (Richards, 2001). These insects have yet been estimated to be responsible for the pollination of 84% of the 264 species grown as crops in the European Union (EU) (Richards, 2001). Recently, the total economic value of pollination worldwide amounted to €153 billion for the 100 crops used directly for human food (Gallai *et al.*, 2008). However, intensive agricultural landscapes are not devoid of pollinators, so that large bees, hoverflies and some butterflies visit crop flowers in intensive agricultural landscapes located far from semi-natural habitats. However pollinators become noticeably less diverse and numerous when safe nest sites are absent. Consequently, farmers often import hives of honey bees (*Apis mellifera* L., Hymenoptera: Apidae) into a crop which requires pollination, particularly orchards. This is however different in the case of sunflower culture: beekeepers are eager to install their hives near the cultivated plants as the reward of honey flow ensured good quality harvests.

Since the early 90's, some French beekeepers have reported losses of honey bee workers during sunflower honey flow associated with low honey production (Laurent and Rathahao, 2003; Madelon, 1998). A newly registered insecticide used for seed treatment (Gaucho®; active ingredient imidacloprid) was rapidly incriminated (Pham-Delègue, 2001). In 1999, the combination of the research findings, social pressure and media attention led to the first application of the precautionary principle for an environmental issue in France by the French Minister of Agriculture (Maxim & van der Sluijs, 2007). The use of Gaucho® for sunflower seed coating was suspended in 1999. A different insecticide was then used to control soil pests in sunflower and maize cultures (Regent TS®; active ingredient fipronil). As mortalities continued to be observed after 1999, this seed-dressing insecticide was also immediately accused to be responsible for the troubles (Mary & Mary, 2002). In February 2004, the Minister of Agriculture decided to suspend the marketing of all plant treatments containing fipronil although their use was allowed during some months of 2004 until stock depletion. All these compulsory measures were still of actuality in August 2006, when this experiment was run.

During the summer 2005, a field survey was initiated by our laboratory in the centre of France (Indre *département*) on beekeeping farms where beekeepers were complaining about colony depopulation and weak sunflower honey production. During these field visits, various pathologies were observed in hives together with non-adapted treatments against *Varroa destructor* Anderson & Trueman (Acari: Mesostigmata), a parasite that develops in honey bee colonies resulting in a severe disease (Ritter 1980). Abnormal behaviours were also registered at hive entrances such as occupation of the flight board by several adult bee exhibiting either aggressive or waiting behaviours. Moreover some pesticide residues were detected in pollen loads and living honey bees during and after sunflower flowering. The simultaneous presence of several factors did

not allow the laboratory to conclude on the origin of the troubles, whether they act together or separately. In this context, a controlled experimentation was set up in order to study the impact of sunflower culture on honey bee colonies in 2006. This survey aimed at determining whether sunflower honey flow, on its own, could lead to deleterious effects on colonies, such as the decrease in honey bee populations and consequently low honey productions.

2. MATERIAL AND METHODS

2.1. General protocol

All the surveyed colonies originated from two apiaries: half of the colonies originated from a professional beekeeping farm. The other half was transported from our laboratory livestock (AFSSA, Sophia Antipolis, France). During a preliminary visit, colonies were evaluated according to the brood area, adult bee population, symptoms of pathologies. Selected colonies were homogeneous according to these criteria (standardized groups).

Three groups of 10 colonies were placed before sunflower flowering, during the month of May, in different surroundings. The three locations were chosen in a 20 km-diameter area in order to provide comparable climatic constraints. Hives were randomly assigned to groups designated by S1, S2 and F. S1 and S2 batches were located in an area with a large surface of cultivated sunflowers that should insured the necessary provision for a regular honey production. Exposure to sunflower culture was assessed by the acreage of cultivated plants and the high proportion of sunflower pollen in pollen loads collected from the hives. Within a radius of 1.5 km around each apiary, all sunflower parcels were reported. Particular attention was paid to grown varieties and treatments applied for plant protection. The third group of hives (F) was located in a forest area that offered sufficient nectar resources to maintain healthy populations. This last location was distant from 1.5 km to any sunflower or maize cultures.

A supper was added to each hive before sunflower flowering. In each group, two extra colonies were equipped with pollen traps. In each apiary, a hardware cloth was spread in front of hives in order to facilitate the record of acute honey bee mortalities.

2.2. Data collection

During the experiment, colonies (Langstroth) were visited three times: before (29th June 06), during (17th July 06) and after (26th July 06) sunflower flowering. During these visits, honey bee population was evaluated by counting the number of inter-frames occupied by adult bees observed just after removing the feeder and/or the inner cover. The number of occupied inter-frames in the super was also counted (Faucon *et al*., 2005). Larval bee population was quantified by estimating the surface of frames that was occupied by eggs, open or capped brood. Notations ranged from 0 to 4 for each face of frame (0 was attributed when no eggs, no larvae nor pupae was present, 4 when the totality of the frame was covered by eggs, larvae or pupae) (adapted from Imdorf *et al*., 1987). For each colony visit, population was evaluated in all colonies within the same day. Groups were visited in the same order every time. Days of visits were chosen to minimize weather adverse effect on honey bee population: the sun was shining, temperature was above 20°C.

During the three visits, symptoms in hives of the four following diseases were recorded: American foulbrood, European foulbrood, varroosis characterised by phoretic varroas and adult bees with deformed wings and nosemosis by *Nosema apis* (presence of diarrhoeas and cripple bees in front of colonies) (Borchert, 1970). In case of foulbrood symptoms, brood samples were taken for laboratory analyses (see below). At each visit, adult bees were systematically sampled in two locations of each colony: at hive entrance and within colonies.

Each adult bee sample collected at the entrance was submitted to the systematic detection of *Nosema sp.* spores (Fungi: Microsporidae). Abdomens of 10 bees were triturated in water. After centrifugation, pellets were re-suspended in water. The solution was examined under a haemocytometer (OIE, 2000b). European and American foulbrood clinical symptoms were confirmed through bacterioscopic tests using Gram stain. Both clinical symptoms and bacterium morphology were used for diagnosis (OIE, 2000a).

Honey, pollen loads and beebread were sampled in order to look for residues of imidacloprid, fipronil and their metabolites. Limit of quantification of fipronil, sulfon fipronil and desulfinyl fipronil in honey and in pollen was 0.5 µg/kg, and 0.1 µg/kg in beebread (Kadar and Faucon, 2006). Limits of quantification of imidacloprid and 6- chloronicotinic acid (metabolite of imidacloprid) were 1.0 and 0.6 µg/kg, respectively in pollen and honey and 0.3 and 1.0 µg/kg respectively in beebread. Analyses were performed in the AFSSA laboratory (Sophia Antipolis, France) and in the GIRPA laboratory (Groupement Interrégional sur les Recherches des Produits Agropharmaceutiques, Angers, France).
Before sunflower blooming, reserve honey from each hive was sampled in the brood chamber. On the 31st of July 2006, honey in the supers was weighted for each colony and harvested. Mean samples of honey were made by pooling honey samples from all hives of the same group at the two dates (before and after sunflower blooming). A melissopalynologic analysis was conducted on each mean honey samples (Louveaux *et al.*, 1978).

Pollen loads were collected on the 17th July 2006 (during sunflower blooming) from the two extra hives equipped with pollen traps in each apiary. Pollen loads from the two hives were pooled together in order to make a mean pollen sample for each group of colonies. Mean samples were analysed in order to identify species that were visited by the honey bees (Louveaux *et al.*, 1978). Beebread was collected from one hive from each group and was sampled at two dates for palynological analysis: before and after sunflower blooming period.

During sunflower flowering (from the 9th July to 25th July 2006), all apiaries were daily controlled to record honey bee activity at hive entrance. Any unusual behaviour such as aggressiveness, trembling bees, motionless individuals and honey bee mortality in front of hives was recorded. Foraging activity was daily evaluated at all colony entrance between 10 a.m. and 12 a.m. by counting during one minute the number of honey bees going out of the hive. For each day, the recordings were conducted in same order every time.

2.3. Statistical analysis

Mean adult and larval honey bee populations and mean honey production from each group of hives were compared using one way analysis of variance (ANOVA). Conditions of ANOVA appliance were checked on residues: normality (Shapiro-Wilk test) and homocedasticity (Bartlett test). If a difference was statistically significant, means were compared using a Tukey test. When ANOVA appliance conditions were not fulfilled

Table 1: Characteristics of sunflower parcels cultivated in experimental sites (S1 and S2 = sunflower sites, F: forest site): number of parcels, total surface of sunflower culture (ha), sunflower varieties and plant protection treatments. NR: not relevant.

Tableau 1: Caractéristiques des parcelles de tournesol cultivé dans les sites expérimentaux (S1 et S2 = sites à tournesol, F: site forestier): le nombre de parcelles, la surface totale de la culture de tournesol (ha), les variétés de tournesol et les traitements phytosanitaires. NR: inapplicable.

Sites	Sunflower parcels	Total surface	Sunflower varieties	Seed treatment
S1	3	68	Albena LG 54 12 Pacific Pégasol Rustica	Fludioxonil and Metalaxyl M
S2	6	63	Albena Jolly Pionner PR 64 B 24 Pionner PR 64 H 45 Pomar	Fludioxonil and Metalaxyl M
F	0	0	NR	NR

Table 2: Linear mixed model fitted to foraging activity. The activity was measured in 10 colonies in each group (F, S1 and S2) twice a day.

Tableau 2: Modèle linéaire mixte ajusté à l'activité de butinage. L'activité était mesurée dans les 10 colonies de chaque groupe (F, S1 et S2) deux fois par jour.

Variables	Estimate	S.E.	P-value
Intercept	18.4	4.6	<0.001
Group effect			
F	0	-	-
S1	55.2	6.5	<0.001
S2	11.8	6.5	0.081
Time effect			
F	0.86	0.25	<0.001
S1	-2.48	0.35	<0.001
S2	0.78	0.35	0.027

Kruskal-Wallis non parametric tests were performed.

Linear mixed models in the formulation described in Laird and Ware were used to explain foraging activity variation (Laird & Ware, 1982). According to the design of the study, hive was considered as a random term in the model.

ANOVA statistical calculations were carried out with JMP Statistical Discovery Software (SAS Institute). Linear mixed models were performed with the statistical package R version 2.7.0 (R Development Core Team, 2007), using the nlme library (Pinheiro & Bates, 2000).

3. RESULTS

3.1. Assessment of the environment

In the environment of groups S1 and S2, leguminous plants, oleaginous plants, cereals and maize were the main species grown on large parcels. During the course of the experiment, sunflowers were the only blooming cultivated plant. Although there were some differences in sunflower varieties, the totality of sown surface was comparable in both sites (68 and 63 ha in site S1 and S2, respectively, Table 1). The same two fungicides were applied to sunflower seeds in both sites (fludioxonil and metalaxyl-M).
Natural grasslands, wet meadows and forest composed the environment of the third site (F).

3.2. Honey bee population

Mean honey bee and brood population was graphed against time (Figure 1). Before sunflower flowering (29th June 06) mean adult honey bee populations of the 3 groups of hives (S1 = 12.5; S2 = 13.3; and F = 12.9 occupied inter-frames) were not statistically different (Kruskal-Wallis test: Chi^2 = 0.16; P = 0.92). During flowering (17th July 06) mean adult honey bee population were lower than the ones recorded at the previous visit (S1 = 8.3; S2 = 9.4; and F = 9.6 occupied inter-frames) and were not statistically different between each others (Kruskal-Wallis test: Chi^2 = 4.35; P = 0.11). At the end of the flowering (26th July 06), mean adult honey bee population has decreased again (S1 = 7.1; S2 = 6.6; and F = 9.1 occupied inter-frames). Population evaluated in groups S1 and S2 was statistically different from the one recorded in the forest group (ANOVA: F = 6.73; P<0.01, Tukey test: q = 2.41, P = 0.05).

Before sunflower flowering (29th June 06) mean brood populations (S1 = 7.7; S2 = 7.5; and F = 7.6 occupied quarters) were not statistically different between each others (Kruskal-Wallis test: Chi^2 = 0.16; P = 0.92). During flowering (17th July 06) mean brood population were lower than the ones recorded at the previous visit (S1 = 5.2; S2 = 5.1; and F = 5.1 occupied quarters), and were not statistically different between each others (Kruskal-Wallis test: Chi^2 = 0.18; P = 0.92). At the end of the flowering (26th July 06), mean brood honey bee population has decreased again (S1 = 4.3; S2 = 4.9; and F = 4.5 occupied quarters). Means were not significantly different between each others (Kruskal-Wallis test: Chi^2 = 0.54; P = 0.76).

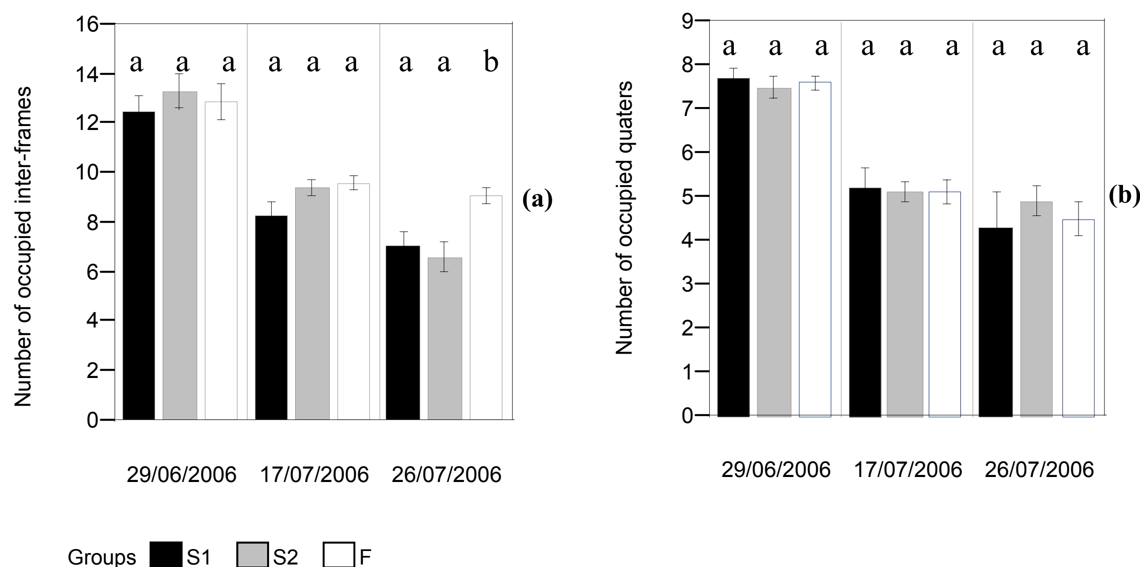

Figure 1: Adult (a) and brood (b) honey bee population ± standard error. Colonies (n=30) were exposed to sunflowers (sites S1 and S2) or placed in a forest environment (site F). Different letters indicate statistical difference within each sampling dates.

Figure 1: Population d'abeilles adultes (a) et de couvain (b) ± erreur standard. Les colonies (n=30) étaient exposées à la culture de tournesol (sites S1 et S2) ou placées dans un environnement forestier (site F). Des lettres différentes indiquent une différence statistique à l'intérieur d'une même date d'échantillonnage.

3.3. Diseases, parasites, pesticides residues and honey bee activity

At the first visit none of the colonies exhibited any disease symptoms. During the course of sunflower flowering (17th July 06), some symptoms (less than 5 cells were affected) of European foulbrood appeared in one colony of S2 group. The disease was subsequently confirmed by laboratory analysis. At the end of the monitoring (26th July 06), European foulbrood was diagnosed in one colony of each group. No other symptoms were recorded in any of the colony, or outside of any colony (acute mortalities, occupied hive entrances).

Spores of *Nosema. sp.* were found in honey bees collected at hive entrances before blooming in two hives of group S2 (3.2 and 6.9 million spores per honey bee) and in two hives of group F (0.04 and 4.2 million spores per honey bee), and after sunflower flowering in five hives of group S1 (0.08 to 3.2 million spores per honey bee) and 1 hive of groups S2 (4.1 million spores per honey bee) and F (3.3 million spores per honey bee).

No pesticide residues was found in honey, in pollen loads or in beebread collected before, during and after flowering.

No symptoms was recorded at hive entrances at any time, neither honey bee mortality. Mean foraging activity was significantly different in groups S1 from group F. In group S2, foraging activity was not statistically different from group F at the significance threshold of $\alpha=0.05$ although the calculated p value was relatively close ($p=0.08$). Foraging activity was irregular and highly dependent on time in all of the three groups of hives (Figure 2, Table 2). Honey bees from group S2 and group F colonies exhibited opposite trends: foraging activity increased with the season in group S2 and F whereas the activity decreased with time in colonies from group S1.

3.4. Honey production and palynological analysis

Mean honey production was 13.0 ± 2.5 kg, 14.1± 1.6 kg and 1.1± 0.5 kg in S1, S2 and F, respectively. The difference was statistically significant between the production of groups S1 and S2 in one hand and F in the other hand (Kruskal-Wallis test: $Chi^2 = 18.2$; $P < 0.001$).

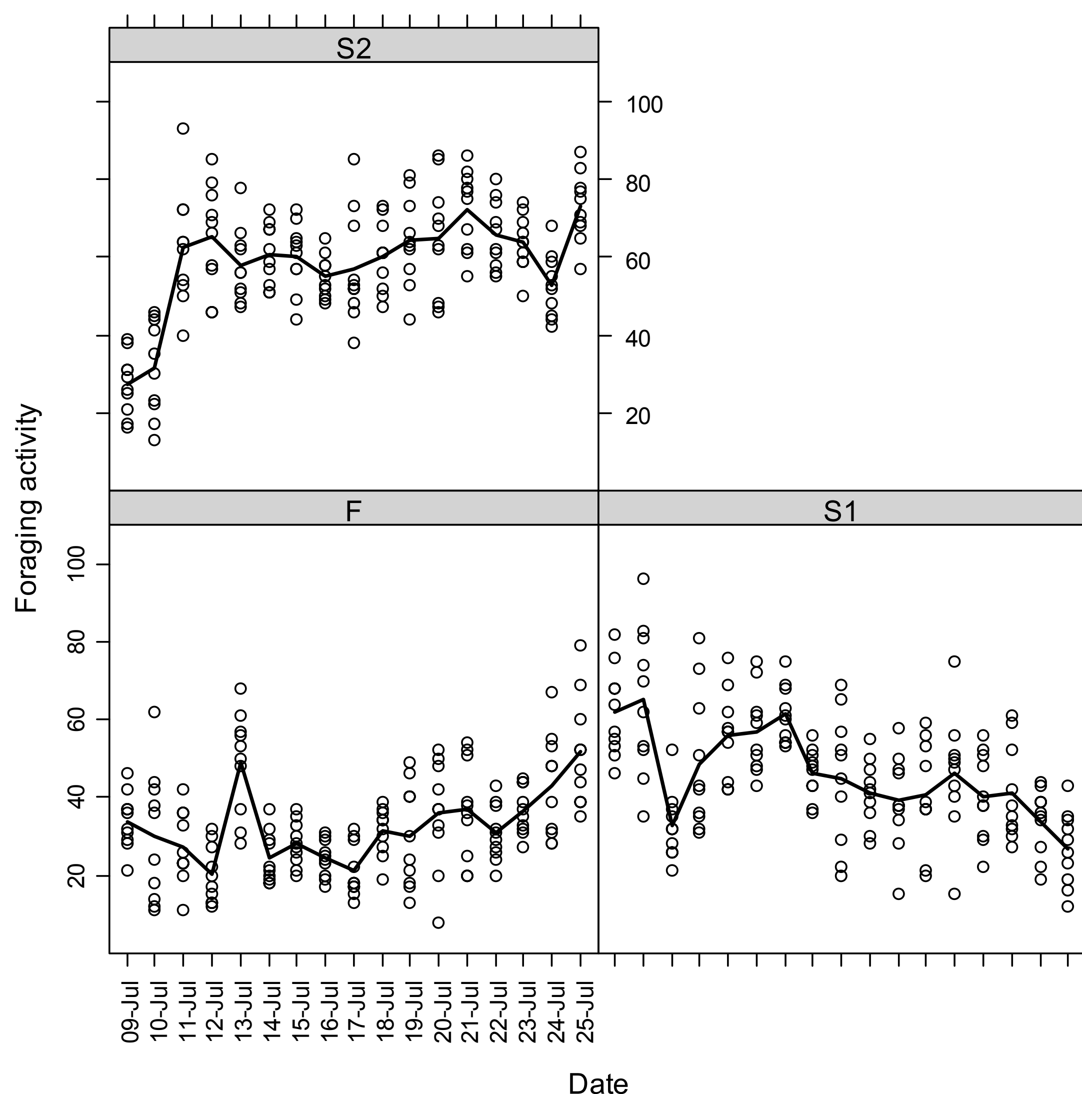

Figure 2: Foraging activity at colony entrances (n=30) when hives were exposed to sunflowers (sites S1 and S2) or placed in a forest environment (site F). Activity was daily recorded. Line = mean value for each time.

Figure 2: Activité de butinage au pas de vol des colonies (n=30) quand les ruches étaient exposées à la culture de tournesol (sites S1 et S2) ou placées dans un environnement forestier (site F). L'activité a été enregistrée quotidiennement. La ligne correspond à la valeur moyenne de chaque série de mesures.

Pollen grains contained in honey were identified in order to know the origin of reserve honey contained in hives before sunflower blooming, and the origin of honey harvested in supers after sunflower honey flow exposure (Table 3). Before sunflower blooming, reserve honey contained mostly oilseed rape pollen (55.2, 59.4 and 86.2% in groups S1, S2 and F, respectively). The second most important pollens were coming from forest plants: chestnut (30.4 and 14.3 % in groups S1 and S2, respectively, and buckthorn: 5.8% in group F). These features were typical of hives that were used for honey production during oilseed rape blooming. After sunflower blooming, pollen grains from honeys collected in supers from group S1 and S2 were distinctive of foraging activity completed on chestnut (70.6 %) and on bramble (23.0%) in group S1, and on buckwheat (70.0%) and oak (11.5%) in group S2. These honeys were typical of the local agricultural landscapes: a mixture of cultivated species together with wild species. In the honey collected in hives placed on a forest environment, the major pollen was bramble (44.4%), the second was oak (22.8%) and the third most frequent pollen was buckthorn (10.9%). This profile was typical of the local environment. In this last group of hives, the total number of pollen taxa found in honey was higher (20 taxa) than the total number of taxa found in honey collected from the other groups of hives (7

and 11 for groups S1 and S2, respectively) (Table 3).

3.5. Pollen analysis in pollen loads and beebread

During sunflower blooming, sunflower pollen was highly predominant (80.7%) in pollen loads collected in group S1 (Table 4). The proportion of this species was lower in pollen loads collected in group S2 (37.3%). In this group, the Virginia creeper had the highest proportion of all species (48.8%). Proportion of maize grains in loads collected from both groups of hives was low (5.8% in groups S1 and S2). Loads collected from hives located in the forest environment showed a higher diversity in species (11 taxa). Virginia creeper and water-lily were the dominant species (45.1% and 26.0%, respectively). Proportion of maize, bramble and plantain pollens ranged from 9.7 to 6.5% in pollen loads collected at the forest site.

Before sunflower blooming, the most frequent pollen found in beebread in any of the groups of hives was bramble pollen (87.6, 81.2 and 58.5%, in groups S1, S2 and F, respectively) (Table 5). The second pollen in proportion was white clover in group S1 (5.0%), chestnut pollen in group S2 (15.6%) and fruit-trees pollen in group F (25.4%). After sunflower blooming, the most frequent pollen was still bramble in group S1 (68.1%), myrtle (60.5%) in group S2, and chestnut (47.8%) in group F. All the three groups had comparable total number of pollen taxa found in beebread (9 in group S1 and F, 10 in group S2).

4. DISCUSSION

In our experiment, honey bee colonies exposed to sunflower blooming did not exhibit acute mortality signs.

Our methodology followed standard criteria for good quality experimentation with honey bees: homogeneous apiaries, reduced number of beekeepers, comparable environmental and climatic conditions (Delaplane, 1997). Cultures of sunflower were sufficient to ensure adequate exposure: 68 and 63 ha of *H. annuus* cultures were reported in a 1.5 km-radius from experimental apiaries at both sites. Therefore our protocol allowed to list all relevant food resources for colonies within the mean foraging distance during the experimental period.

Collected pollen loads were analyzed to appreciate cultures on which honey bees have foraged. Results showed that sunflower pollen was differently collected depending on sites (80.7% of pollen grains were *H. annuus* in pollen loads collected in site S1 compared to only 37.3% in pollen loads from site S2). These variable results are consistent with those found in literature (Charrière *et al.*, 2006, Odoux *et al.*, 2004). Sunflower pollen was not present in pollen loads collected in the forest site, confirming that bees were not exposed to the culture.

This experiment has also shown that production of honey from sunflower culture exposure was low (around 13 kg per colony in both sunflower sites) compared to historical figures. Results of palynological analysis of honey have shown that honey bees did not forage only on sunflower cultures which is consistent with results observed elsewhere in Europe (Switzerland and Germany). Production of honey is highly variable and dependant of various factors such as climate, type of soil, honey flow and internal colony conditions (Janssens *et al.*, 2006). In a similar protocol, Charrière *et al.* (2006) have also noticed that honey gathered by honey bee colonies facing sunflower cultures were not only made of sunflower nectar according to pollen and organoleptic analysis.

Insecticides used for sunflower seed coating are regularly accused to be responsible for bee losses whether they would lead to acute or diffuse mortalities (Laurent & Rathahao, 2003; Mary & Mary, 2002). Although the use of imidacloprid and fipronil was banned for sunflower seed dressing in 2006 when the experiment was run, some of the beekeepers claimed – and still claim – that, because of the molecule persistence properties, residues of these active substances could be present in soil and migrate to the upper part of the plant (Bruderer & Hermieu, 2008). Through this mean, honey bees could be exposed to the active substances or the metabolites by eating or gathering pollen and nectar. Although residues were not searched in soil samples in our experiment, our results demonstrated that, in our conditions, no residue of any molecule was detected on pollen, honey, beebread or honey bees.

Our data also showed that only a limited amount of sunflower pollen was stored in hives for future consumption. These results are in contradiction with the hypothesis of some beekeepers assuming that delayed colony poisoning results from feeding from sunflower polluted pollens during autumn and winter (Mary & Mary, 2002).

4.1. Honey bee population

In this experiment we have separated colonies where bee population was measured from those equipped with pollen traps to collect pollen loads. It has been shown that such traps have an impact on honey bee population: less increase of capped brood and less adult bee population at spring has been noted in hives with traps compared to hives without traps (Webster *et al.*, 1985).

In our experiment, the decrease in honey bee population (adult and brood) over time was homogeneous among the groups, with the exception of the adult population in the forest group measured at the end of experiment, which was found significantly higher than the population measured in groups exposed to sunflower blooming. These data support other work conducted in Switzerland in 2006 where only small and statistically non significant differences in honey bee population were noted between hives exposed or non-exposed to sunflower (Charrière *et al.*, 2006).

The quality of sunflower pollen for honey bee diet is poor. Crude protein content in sunflower pollen (14.8%) was found to be medium to poor compared to the one of *Phacelia tanacetifolia* (30.1%) and *Brassica campestris* (25.7%). In cage experiment, diets composed of *Phacelia* or *Brassica* pollen promoted greater hypopharyngeal gland and ovary development in newly-emerged worker bees than diets prepared from *Heliantus* pollen (Pernal & Currie, 2000). The quality of the food received by the brood and the queen has the potential of influence the overall colony rate (Schmid-Hempel, 1993). Many research teams have estimated that honey bee colonies restricted to forage on poor quality of food resources such as *H. annuus* may suffer of a loss of fitness (Pernal & Currie, 2000; Schmidt *et al.*, 1995; Taber, 1996). The period of restricted access to sunflower and maize cultures has been estimated at 7 weeks in certain parts of France (Odoux *et al.*, 2004) which is a long period for honey bee colonies, covering two cycles of brood rearing. However, the colony being a dynamic system, a form of compensation exists when the population is confronted to detrimental conditions. The poor quality and quantity of diet can explain the apparition of European foulbrood symptoms in hives in all the groups. One hypothesis raised to explain the apparition of this disease involves the competition of nutrients between infected larva and the pathogen (Bailey 1983). In these conditions, varroa detrimental effect - reduction of protein content and decrease in content of some sugar in honey bee haemolymph (Weinberg & Madel, 1985; Zoltowska *et al.*, 2007) adds to poor quality of diets. In our experiment, when honey bees were placed in an environment offering sufficient diversity, the total number of pollen taxa was higher in pollen loads and honey than the ones found in matrixes collected in hives located in agricultural landscapes.

4.2. Modern agricultural landscapes stress pollinators

Due to European and national legislation and management strategies, agricultural landscapes has changed to a mosaic of ecosystems with a predomination of arable fields. The 'pollinator force' is generally thought to be most important to plant reproduction (Kevan, 1999). Honey bees, as a pollinating factor, dominates most crops but was not found to fulfill its function with respect to some plants (Banaszak, 1992). Indeed, habitat destruction has a great impact on pollinator populations.
To grow hybrid sunflower seed, pollen-producing male-fertile cultivars and only nectar-producing male-sterile cultivars are planted in separate rows within a field (Greenleaf & Kremen, 2006). During the mid 80's, in order to enhance sunflower pollination, studies were conducted to better understand why some sunflower cultivars were more attractive to bees than others. These literature references report mainly studies on *A. mellifera* and *Bombus terrestris* (Hymenoptera: Apidae), with the exception of the solitary oligophage bee *Melissodes agilis* C. (Hymenoptera: Apoidea) (Tepedino & Parker, 1982). It is interesting to note that the number of studies on nectar and pollen production as cost for sunflower selection decreased when, at the same time, studies on pesticide residues increased. When sunflower varieties were compared under

tunnel conditions or in field conditions, the one that yielded more seeds had the lowest number of honey bee visits (Pham-Delègue *et al*., 1990; Sammataro *et al.*, 1985). Differential distribution of honey bees between parental lines was related to nectar composition, aroma composition and floral anatomy (Etievant *et al.*, 1984; Fonta *et al*., 1985; Vear *et al*., 1990). Significant variations in volume of nectar per floret (from 0.04 to 0.32 µl/floret), number of florets per head, nectar dry matter content, energy value per floret and number of pollen grains produced per plant were demonstrated to be dependent on sunflower cultivars (Tepedino & Parker, 1982; Vear *et al*., 1990). Studies of sunflower floral anatomy have shown that the corolla length of the floret could be highly dependent of hybrids, ranging from 1.15 to 6.23 mm (Cirnu *et al.*, 1976). Indeed, the accessibility of nectar is crucial for pollinators, which is not specific to *A. mellifera* (Pywell *et al.*, 2005).

The need to maintain floral attractiveness to insect pollinators in hybrid systems has not been generally recognized by plant breeders. Rather, their goals have been to improve other factors such as yield, oil content, and disease and insect resistance (Sammataro *et al*., 1985). The cost for the selection of resistant cultivars to *Sclerotionia sclerotiorum* (an important disease attacking sunflowers) or for the selection of cultivars with high oil contents was studied in terms of nectar and pollen production (Vear *et al*., 1990). It has also been suggested that improving resistance to pests could be linked to the production of some terpenes (Gershenzon *et al*., 1981). These substances have insecticide properties and their toxicity or deterrence to *A. mellifera* are variable depending on molecule (Detzel & Wink, 1993; Fassbinder *et al*., 2002). A recent work has shown that this attractiveness is complex and that the responses of bees to second compounds contained in nectar may depend on sugar concentration (Liu *et al*., 2007). New cultivars are marketed and planted every year in different countries.

Given the high number of factors influencing nectar production (temperature, water availability, type of soil, wind, seasonal variations), it is difficult to evaluate *a posteriori* the potential of nectar production of marketed sunflower cultivars. More work should be done before marketing plant lines, to better know their attractiveness to pollinators and particularly to honey bees. Numerous decision-makers (plant breeders, chemical companies and public institutions) could benefit from the availability of these data.

5. CONCLUSIONS

In this study, we have shown that pollen and nectar collection by honey bees from sunflower cultures is highly variable on field conditions. Production of honey was low compared to historical figures, and was not attributed to sunflower honey flow only. Foraging activity was highly dependent on sites of experiment and on dates within the same site. The low production of honey was not related to pesticides as no residue was found in honey bee, pollen loads, honey and beebread. Small quantities of sunflower pollen were found stored in beebread as reserves.

Honey bees and others pollinators' populations are affected in the field by different factors: nectar quality, quantity and accessibility, and habitat destruction. Vast areas of monocultures create particularly stressful conditions for fauna. Conservation, restoration and management of diversified melliferous habitats are crucial for beekeeping and to the maintenance of wild fauna. Reciprocity is also relevant: beekeeping and wild pollinating fauna are essential for an agriculture respectful of human needs. Therefore, more work should be done on nectar and pollen production decrease as cost for new sunflower cultivars selection, particularly on attractiveness and accessibility towards pollinators (nectar and aroma composition, floral anatomy). These possible costs for selection should be tested before marketing new plants lines.

Table 3: Species and proportions (%) of pollen grains collected in mean honey samples harvested from hives before (29th June 2006) and after (26th July 2006) sunflower blooming, in sites exposed to sunflower cultures (S1 and S2) or in a forest environment (site F).

Tableau 3: Espèces et proportion (%) des grains de pollen identifiés dans les échantillons moyens de miel récolté à partir des ruches avant (29 juin 2006) et après (26 juillet 2006) la floraison des tournesols, dans les sites exposés aux cultures de tournesol (S1 t S2) ou placées dan un environnement forestier (site F).

		Date of sampling	**29th June 2006**			**26th July 2006**		
Family	***Latin name***	**Vernacular name**	**S1**	**S2**	**F**	**S1**	**S2**	**F**
Abietaceae	*Abietaceae*		-	0.2	0.2	-	-	-
Aceracea	*Acer*	Maple	-	1.4	-	-	-	-
Apiaceae or **Umbelliferae**	Type *Daucus*	Like carot	-	-	-	-	1.0	-
Asteraceae or Compositeae	*Centaurea sp.*	Corn flower	-	-	-	-	-	0.4
	Helianthus annuus	Sunflower	-	0.1	-	-	4.5	1.6
	Solidago	Golden rod	-	0.1	-	-	-	-
	Type *Taraxacum*	Like Dandelion	0.1	-	-	-	-	-
Balsaminaceae	*Impatiens sp.*	Jewel weed						
Brassicaceae	*Brassica napus*	Oilseed rape	55.2	59.4	86.2	0.8	-	0.1
Caprifoliaceae	*Lonicera implexa*	Honeysuckle	-	-	-	-	-	0.1
Cistaceae	*Cistus sp.*	Rockrose	-	-	-	-	0.5	1.0
Cornaceae	*Cornus sanguineum*	Common Dogwood	-	-	-	-	-	0.1
Ericaceae	*Erica arborea*	Tree Heather	-	-	-	-	-	4.5
Fagaceae	*Castanea sativa*	Chestnut	30.4	14.3	-	70.6	1.0	1.0
	Quercus sp.	Oak	-	0.3	-	-	11.5	22.8
Leguminosae Papilionoideae or **Fabaceae**	*Lotus corniculatus*	Trefoil	-	-	0.2	-	-	0.6
	Melilotus sp.	Sweet clover	-	3.6	-	-	-	-
	Robinia pseudoacacia	Locust tree		0.2				
	Trifolium pratense	Red clover	2.6	3.1	0.3	0.3	1.0	-
	Trifolium sp.	Clover	-	-	-	-	-	1.2
Malvaceae	*Malva sp.*	Common mallow	-	-	-	0.1	-	-
Myrtaceae	*Myrtus sp.*	Myrtle	-	-	-	-	-	0.7
Oleaceae	*Ligustrum vulgare*	Prime print	-	-	-	-	-	0.3
Poaceae	*Zea mays*	Maize	-	0.1	-	-	1.0	0.2
		Other	0.2	-	0.5	-	-	-
Polygonaceae	*Polygonum fagopyrum*	Buckwheat	-	-	-	-	70.0	1.8
Rhamnaceae	*Rhamnus sp.*	Buckthorn	0.6	2.4	5.8	4.0	3.0	10.9
Rosaceae		Fruit-trees	4.4	11.7	4.1	1.2	5.0	7.7
	Rubus sp.	Bramble	0.3	0.9	1.5	23.0	-	44.4
Salicaceae	*Salix sp.*	Willow	6.2	1.7	1.2	-	-	-
Scrophula-	*Linaria vulgaris*	Yellow	-	0.5	-	-	1.5	-

riaceae		Toadflax						
Tiliaceae	*Tilia sp.*	Linden	-	-	-	-	-	0.5
Vitaceae	*Parthenocissus sp.*	Virginia ceeper	-	-	-	-	-	0.1
	Total number of taxa		**9**	**16**	**9**	**7**	**11**	**20**

Table 4: Composition of pollen loads collected during the sunflower flowering at sites exposed to sunflower cultures (S1 and S2) and in a forest environment (site F). Proportions are given in %.

Tableau 4: Composition des pelotes de pollen collectées pendant la floraison des tournesols, dans les sites exposés aux cultures de tournesol (S1 t S2) ou placées dan un environnement forestier (site F). Les proportions sont données en %.

Family	***Latin name***	***Vernacular name***	**S1**	**S2**	**F**
Asteraceae or **Compositeae**	*Carduus sp.*	Thistle	1.7	0.1	-
	Helianthus annuus	Sunflower	80.7	37.3	-
	Taraxacum	Dandelion	3.7	-	-
	Type *Taraxacum*	Dandelion like	-	0.8	-
Balsaminaceae	*Impatiens sp.*	Jewel weed	-	-	0.8
Cucurbitaceae	*Bryonia dioica*	Wild nep	-	-	0.5
Leguminosae Papilionoideae or **Fabaceae**	*Lotus corniculatus*	Trefoil	-	-	0.8
	Trifolium sp.	Clover	-	-	1.8
Nymphéaceae	*Nymphaea sp.*	Water-lily	-	-	26.0
Plantaginaceae	*Plantago sp.*	Plantain	-	-	6.5
Poaceae	*Zea mays*	Maize	5.8	5.8	9.7
Polygonaceae	*Polygonum fagopyrum*	Buckweat	1.8	-	-
	Rumex	Sorrel	-	-	0.4
Rosaceae	*Rubus sp.*	Bramble	1.8	7.6	8.2
Scrophulariaceae	*Linaria vulgaris*	Yellow toadflax	4.5	48.4	0.2
Vitaceae	*Parthenocissus sp.*	Virginia creeper	-	-	45.1
		Total number of taxa	**7**	**6**	**11**

Table 5: Composition et proportion of pollens (%) in bee bread collected before (29th June 2006) and after (26th July 2006) sunflower blooming at sites exposed to sunflower cultures (S1 and S2) and in a forest environment (site F).

Tableau 5: Composition et proportion des grains de pollens (%) dans le pain d'abeille récoltés avant (29 juin 2006) et après (26 juillet 2006) la floraison des tournesols, dans les sites exposés aux cultures de tournesol (S1 t S2) ou placées dan un environnement forestier (site F).

		Date of sampling	**29 June 2006**			**26 July 2006**		
Family	**Latin name**	**Vernacular name**	**S1**	**S2**	**F**	**S1**	**S2**	**F**
Abietacea			-	-	0.5	-	-	-
Anarcardiacea			-	-	-	-	2.8	-
Apiaceae or **Umbelliferae**	Type *Daucus*	Like carot	-	-	-	-	0.2	-
Asteraceae or **Compositeae**	*Brassica*	Oilseed rape	-	-	2.5	-	-	-
	Carduus	Thistle	-	-	-	0.2	-	-
	Centaurea sp.	Corn flower	1.0	-	-	-	-	1.8
	Helianthus annuus	Sunflower	1.6	-	-	18.5	26.4	0.8
	like *Anthemis*	like Camomile	-	-	-	0.2	-	-
Caprifoliaceae	*Lonicera implexa*	Honeysuckle	-	-	-	-	-	0.1
Chenopodiacea			-	-	-	-	-	0.8
Convolvulaceae	*Convolvulus*	Bindweed	-	-	0.2	-	-	-
Ericaceae	*Erica arborea*	Heather	-	-	-	0.3	0.2	-
Fagacae	*Castanea sativa*	Chestnut	-	15.6	7.2	5.2	-	47.8
	Quercus sp	Oak	-	-	-	-	2.2	-
Hippocastanaceae	*Aesculus hippocastanum*	Horse-chestnuts	-	-	3.5	-	-	-
Leguminosae Papilionoideae or **Fabaceae**	*Trifolium repens*	White clover	5.0	-	-	-	-	2.0
Myrtaceae	*Myrtus sp.*	Myrtle	-	-	-	-	60.5	-
Onagraceae	*Scabiosa*	Scabiosa	-	-	-	-	0.1	-
Poaceae	*Zea mays*	Maize	-	-	-	4.3	0.4	-
		Other	0.2	-	-	-	-	-
Polygonaceae	*Polygonum fagopyrum*	Buckwheat	-	-	-	1.0	-	-
Rhamnaceae	*Rhamnus sp.*	Buckthorn	0.8	-	-	-	-	-
Rosaceae		Fruit-trees	3.2	2.4	25.4	-	-	-
		Other	-	-	-	-	6.0	-
	Rubus sp.	Bramble	87.6	81.2	58.5	68.1	-	43.4
Scrophula-riaceae	*Linaria vulgaris*	Troadflax	-	-	-	2.2	-	0.6
Tiliaceae	*Tilia sp.*	Linden	0.6	-	-	-	-	-
Vitaceae	*Parthenocissus sp*	Virginia creeper	-	-	-	-	1.2	2.7
	Undetermined pollen	-	-	0.8	2.2	-	-	-
	Total number of taxa		**8**	**4**	**7**	**9**	**10**	**9**

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References

Bailey L. (1983). *Melissococcus pluton*, the cause of European foulbrood of honey bees (*Apis* spp.). *Journal of Applied Bacteriology* **55,** p. 65-69.

Banaszak J. (1992). Strategy for conservation of wild bees in an agricultural landscape. *Agriculture, Ecosystems and Environment* **40,** p. 179-192.

Borchert A. (1970). Les maladies et parasites des abeilles. Paris, Vigot Frères Editeurs, 486 p.

Bruderer C. & Hermieu Y. (2008). Les abeilles vont-elles disparaître? *L'Oiseau magazine* **90,** p. 24-27.

Charrière J.-D., Imdorf A., Koenig C., Gallmann D. & Kuhn R. (2006). Cultures de tournesol et développement des colonies d'abeilles mellifères. *Revue Suisse d'Apiculture* **38**, p. 269-274.

Cirnu I., Dumitrache V., Hociota E. & Avramescu P. (1976). Variabilité de la production de nectar et du degré d'attraction des variétés et des hybrides de tournesol *(Helianthus Annuus L)* pour les abeilles. *Revue française d'Apiculture* **344,** p. 332-333.

Delaplane K.S. (1997). Practical science - research helping beekeepers. 2. Colony manipulations for honey production. *Bee World* **78,** p. 5-11.

Detzel A. & Wink W. (1993). Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology* **4,** p. 8-18.

Etievant P.X., Azar M., Pham-Delègue M.H. & Masson C. (1984). Isolation and identification of volatile constituents of sunflowers (*Helianthus annuus* L.). *Journal of Agricultural and Food Chemistry* **32,** p. 503-509.

Fassbinder C., Grodnitzky J. & Coats J. (2002). Monoterpenoids as possible control agents for *Varroa destructor*. *Journal of Apicultural Research* **41,** p. 83-88.

Faucon J.P., Aurières C., Drajnudel P., Mathieu L., Ribière M., Martel A.C., Zeggane S., Chauzat M.P. & Aubert M. (2005). Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Management Science* **61,** p. 111-125.

Fonta C., Pham-Delègue M.H., Marilleau R. & Masson C. (1985). Rôle des nectars de tournesol dans le comportement des insectes pollinisateurs et analyse qualitative et quantitative des éléments glucidiques de ces sécrétions. *Acta Oecologica* **6,** p. 175-186.

Gallai N., Salles J.M., Settele J. & Vaissière B. (2008). Economic valuation of the vulnerability of word agriculture confronted with pollinator decline. *Ecological Economics* **68,** p. 810-821.

Gershenzon J., Ohno N. & Mabry T.J. (1981). The terpenoid chemistry of sunflowers (*Helianthus*). *Revista Latinoamericana de Quimica* **12,** p. 53-61.

Greenleaf S.S. & Kremen C. (2006). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the Natinal Academy of Sciences* USA **103,** 13890-13895.

Imdorf A., Buehlmann G., Gerig L., Kilchenmann V. & Wille H. (1987). Überprüfung der schätzmethode und der anzahl arbeiterinnen in freifliegenden bienenvölkern. *Apidologie* **18,** p. 137-146.

Janssens X., Bruneau E. & Lebrun P. (2006). Prediction of the potential honey production at the apiary scale using a Geographical Information System (GIS). *Apidologie* **37,** p. 351-365.

Kadar A. & Faucon J.P. (2006). Determination of traces of fipronil and its metabolites in pollen by liquid chromatography with electrospray ionization - tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* **54,** p. 9741-9746.

Kevan P.G. (1999). Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agriculture, Ecosystems & Environment* **74,** p. 373-393.

Laurent F.M. & Rathahao E. (2003a). Distribution of [(14)C] imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. *Journal of Agricultural and Food Chemistry* **51,** p. 8005-8010.

Liu F.L., Chen J., Chai J.P., Zhang X.W., Bai X., He D. & Roubik D.W. (2007). Adaptative functions of defensive plant phenolics and a non-linear bee response to nectar components. *Functional Ecology* **21,** p. 96-100.

Louveaux J., Maurizio A. & Vorvohl G. (1978). Methods of melissopalynology. *Bee World* **59,** p. 139-157.

Madelon J. (1998). Le tournesol, le Gaucho et les abeilles. *Oléoscope* **45,** p. 30-31.

Mary M. & Mary P. (2002). Miellées de tournesol 2002: actions et constats de terrain en Champagne berrichonne. *Abeilles et Fleurs* **631,** p. 9-10.

Maxim L. & van der Sluijs J.P. (2007). Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho. *Science of the Total Environment* **376,** p. 1-17.

Odoux J.F., Lamy H. & Aupinel P. (2004). L'abeille récolte-t-elle du pollen de maïs et de tournesol? *La santé de l'abeille* **201,** p. 187-193.

OIE (2000a). *European foulbrood*. Manual of standards for diagnostic tests and vaccines. Fourth Edition, 789-792. Paris.

- (2000b). *Nosemosis of bees*. Manual of standards for diagnostic tests and vaccines. Fourth edition, 793-795. Paris.

Pernal S.F. & Currie R.W. (2000). Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidologie* **31,** 387-409.

Pham-Delègue M.H., Etievant P.X., Guichard E., Marilleau R., Douault P., Chauffaille J. & Masson C. (1990). Chemicals involved in honeybee-sunflower relationship. *Journal of Chemical Ecology* **16,** p. 3053-3065.

Pham-Delègue M. (2001). Le Gaucho est-il l'ennemi des abeilles? *La Recherche* **347,** p. 70-74.

Pinheiro J.C. & Bates D.M. (2000). *Mixed-effect models in S and S-PLUS*. New-York, Springer-Verlag, 528 p.

Pywell R.F., Warman E.A., Carvell C., Sparks T.H., Dicks L.V., Bennett D., Wright A., Critchley C.N.R. & Sherwood A. (2005). Providing foraging resources for bumblebees in intensively farmed landscapes. *Biological Conservation* **121,** p. 479-494.

R Development Core Team (2007). R: A language and environment for statistical computing. http://www.R-project.org. Vienna, Austria, R Foundation for Statistical Computing. Accessed in June 2007.

Richards A.J. (2001). Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Annals of Botany* **88,** p. 165-172.

Ritter W. (1980). Varroa disease of the honeybee Apis mellifera. *Bee World* **62,** p. 141-153.

Sammataro D., Erickson E.H., Garment Jr. & Garment M.B. (1985). Ultrastructure of the sunflower nectary. *Journal of Apicultural Research* **24,** p. 150-160.

Schmid-Hempel P. (1993). Foraging of individual workers in relation to colony state in the social hymenoptera. *The Canadian Entomologist* **125,** p. 129-160.

Schmidt L.S., Schmidt J.O., Rao H., Wang W. & Ligen X. (1995). Feeding preference and survival of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame and sunflower pollen. Journal of Economic Entomology **88,** p. 1591-1595.

Taber S. (1996). Pollen and bee nutrition. American *Bee Journal* November issue, p. 787-791.

Tepedino V.J. & Parker F.D. (1982). Interspecific differences in the relative importance of pollen and nectar to bee species foraging on sunflowers. *Environmental Entomology* **11,** p. 246-250.

Vear F., Pham-Delègue M.H., Tourvieille de Labrouhe D., Marilleau R., Loublier Y., Le Metayer M., Douault P. & Philippon J.P. (1990). Genetical studies of nectar and pollen production in sunflower. *Agronomie* **10,** p. 219-231.

Webster T.C., Briggs T.D., Skinner J. & Parisian T. (1985). Effects of pollen traps on honey bee (Hymenoptera: Apidae) foraging and brood rearing during almond and prune pollination. *Environmental Entomology* **14,** p. 683-686.

Weinberg K.P. & Madel G. (1985). The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and the haemolymph volume of the brood of worker bees and drones of the honey bee *Apis mellifera* L. *Apidologie* **16,** p. 421-436.

Zoltowska K., Lipinski Z. & Dmitryjuk M. (2007). Effects of *Varroa destructor* on sugar levels and their respective carbohydrate hydrolase activities in honey bee drone prepupae. *Journal of Apicultural Research* **46,** p. 110-113.

(45 ref.)