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Full Length Research Paper

Pollination of the broad bean (*Vicia faba* L.var. *major*) (Fabaceae) by wild bees and honey bees (Hymenoptera: Apoidea) and its impact on the seed production in the Tizi-Ouzou area (Algeria)

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An experiment conducted with caged plots confirmed the large seed yield of *Vicia faba* L. in the presence of pollinating insects during the flowering season of the year 2003. The plants that were accessible to pollinators provided more pods per plant, more seeds per pods; the pods were longer and the seeds were heavier than the encaged plants. Among the pollinating insects of *Vicia faba*, in the Tizi Ouzou area (Algeria), the wild bee *Eucera pulveracea* Dours was the most abundant and seemed to be the most effective pollinator. His visits were all able to fertilize the flowers. On the contrary, several floral visits of the honey bee *Apis mellifera* L. and all visits of *Xylocopa violacea* were "nectar robbery" through the holes made by the bumblebees at the base of the corolla. The floral visits of *Eucera pulveracea* L. were also faster (10 visits per minute).

Key words: Wild bees, *Vicia faba,* pollination, yield, foraging behaviour, *Eucera pulveracea*, *Apis mellifera*.

INTRODUCTION

The broad bean is one of the most food crop cultivated in the world. It is a vegetable whose origin has long been discussed. Today, there are Mediterranean regions which are considered as the origin of this vegetable, its culture represents almost 25% of the total cultivated area (Saxena, 1991). The broad bean is nutritional, economic and environmental interesting vegetable.

North Africa is one of the top-producing regions of the broad bean in the world. The average area reserved for this crop is between 23000 and 73000 ha, with an annual production of 137000 to 410000 quintals (Maatougui, 1996). Broad bean occupes the first place among the pulses in Algeria because it has high nutritional value and divers' uses. It is mainly cultivated in the plains and sublittoral regions and has an important role in the national economy and agricultural production. In the wilaya of Tizi-Ouzou, this culture extends over large areas. The broad bean has a very important place in the culinary arts of this region.

In our region, the bean is sown in autumn and blooming between February and April. The flowering lasts 30 days and runs from bottom to the top of the stem. A broad bean's stalk produces 50 to 80 flowers grouped in clusters of 2 to 9 flowers. The broad bean is an annual plant with a cycle effected in 24 to 28 weeks. The maturation phase of pods and the flowering overlapped with each other. We can find pods at widely varying from bottom to top of the plant: pods were being filled and pods in terminal stage, with a few flowers at the vertices of the stems. The formation of pods occurred in March-April, the maturity period was noted in May.

The autopollens guarantee less fertilization of all ova (Barbier et al., 1986 cited in Philippe, 1991). The more number of pollen grains is important on the stigma, the more rate of fructification is higher.

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Vicia faba is a partially allogamous species. The Apoides play a decisive role in the pollination of allo-games lines. The importance of bees in cross-pollination of this plant and the improvement of its production has been demonstrated and recognized by several authors (Pritsch, 1971; Poulsen, 1975; Svendsen and Brodsgaard, 1997).

In flowering, this species attracts almost exclusively Hymenoptera, particularly Apoides having sufficient strength and weight to lower the hull and wings, to reveal the stamens and pistil, come into contact with them to forage for pollen and / or nectar. According to the observations of several authors (Tasei, 1976; Pesson and Louveaux, 1984; Stoddard and Bond, 1987; Philippe, 1991; Varis, 1996; Pierre et al., 1997, 1999) in particular in Europe, Apoides pollinators of this plant are composed of honeybees *Apis mellifera*, bumblebees, and diverse solitary bees. Among the latter, the most cited are *Anthophora spp*., *Eucera spp*., *Xylocopa spp*. (El Berry et al., 1974; Knott et al., 1994), which are indeed large size bees. The same is true for bumblebees which are more-over regarded as the best pollinators of the bean because of their weight, speed of their flights; their body is more suitable to transport pollen because of their hairy system (Pierre et al., 1997). In south Spain, there is *Eucera numida* Lepeletier which is the main pollinator of the broad bean (Cartujo et al., 1998; Pierre et al., 1999). This result is confirmed by Benachour et al. (2007) in Algeria. On the other hand, *Anthophora plumipes* Pallas which is the main pollinator in Cambridge (England).

In Algeria, the remaining works on wild bees for the first authors go back to those of Schulthess (1924). The only recent studies about this fauna are those of Louadi and Doumandji (1998a, 1998b), Louadi (1999) in the region of Constantine (Eastern Algeria) and those of Tazerouti (2002) in the region of Algiers. In the region of Tizi-Ouzou, there are not any recent data related to the Apoides' fauna. As for the study of pollination cultivated plants by bees, the only work was made by Benachour and al. (2007) in the region of Constantine. It seemed worthwhile to consider this issue under the terms of cultures of our region. The main aim of our study was to evaluate the role of pollinators and their impact on seed yield of the broad bean by comparing seed yield of plants left free access to pollinators with caged plants.Others objectives of the study were the inventory of pollinating Apoidea, the estimation of foraging Apoidea density, the estimation of floral density, the observation of foraging behaviour, and foraging speed for the principal pollinators.

MATERIALS AND METHODS

Characterization of the study station

The study was conducted in spring 2003 in the region of Tizi-Ouzou, located 100 km East far of Algiers. The chosen site is located in an agricultural fallow at Boukhalfa 8 km away from the city of Tizi-Ouzou (36° 43'N 4° 00'E, 180 m). The observations were made in a plot (area: 30 square-meters, 10 m long and 3 m wide). The main melliferous species growing at the edges of the plot were *Oxalis pes-caprae* L. (Oxalidaceae), *Borago officinalis* L. (Boraginaceae) and *Sinapis arvensis* L. (Bracaceae). The variety of the broad bean used in our experimentation is *Vicia faba* L. var. *major* Hartz (local variety). The seedlings were realized on December 10th 2002 at the rate of 36 plants per square-meter. For the monitoring of flowering, according with Delbrassine and Rasmont (1988), we consider that the beginning of flowering is reached during when half of the plants have begun to bloom, and the end of flowering is the time when half of the plants have no flowers.

Inventory and density of the pollinating entomofauna

A first work was to count the number of bees on the broab bean flowers. The counting method applied was the "quadrate-method" (Lecomte, 1962b; Banaszak, 1980; Sonnet and Jacob-Remacle, 1987; Abrol, 1988). This method allows also the study of the floral density. In order to study these two criteria, we have delineated 6 quadrates 1 m² each, using wire and piles. The quadrates were arranged in rows of 1 m apart from each other and oriented north-south so that they are well exposed to the sun. The observations were made regularly every two days during the whole flowering period. So as to study the insects density in broad bean crop, the bees which were in gathering activity, were counted; the observer also travelled slowly for at least 5 min each of the plots from 08 a.m to 4p.m up, every hour. The captured bees were determined byProf. P. Rasmont for species of genera *Bombus* and *Anthophora* and by Mr. Terzo for species of *Eucera*, *Lasioglossum* and *Xylocopa*. In addition, in order to prove if there is a correlation between floral display (number of available flowers/m²) and pollinators' density (number of pollinators/m²), in parallel counting of pollinators, flowers counts were made in the mid-afternoon, a time that corresponds to the optimal opening of the flower. This count allows estimate mean number of "opened" flowers likely to be visited by the insects. In considering this argument, we based on the most common method currently used to compare pollinators' densities in crops (Pierre et al., 1997; 1999). This method is to bring out the number of insects counted on a defined number of 100 flowers.

Apoides foraging behavior

It is noted that the pollinating efficiency of gatherers is dependent on their position on the flowers. Nectar-gatherers standing on petals or both on petals and staminal filaments and inserting the proboscis or the head through the androecium generally made non-pollinating visits (lateral visits). However, nectar-atherersstanding on anthers and/or stigmas came into contact with stigmas of blossoms (frontal visits). The Xylocopes and Bourdon *Bombus terrestris* auct. limit to foraging nectar after drilling a hole at the base of the corolla, a fraction of honeybees pass through these holes to collect nectar. Such visits are called negative visits, not allowing the fertilization of the flower. Many other species forage for nectar and pollen by the natural opening of the flowers corolla, triggering staminal column witch bring it into contact with stigmas (Stoddard and Bond, 1987). In such a case, the movement of bees among flowers can ensure the outcrossing (Carré et al., 1994). This is the case of solitary bees whose foraging are often all positive (Stoddard and Bond, 1987). This is also true for several species of bumblebees such as *Bombus hortorum* L., *Bombus agrorum* L. and *Bombus ruderatus* Scopoli (Newton and Hill, 1983; Mesquida et al., 1990). In this study frequency of contacts with stigmas of main species was observed. Their foraging speed was also taken into account to assess their pollinator effectiveness. The pace at which the flowers were

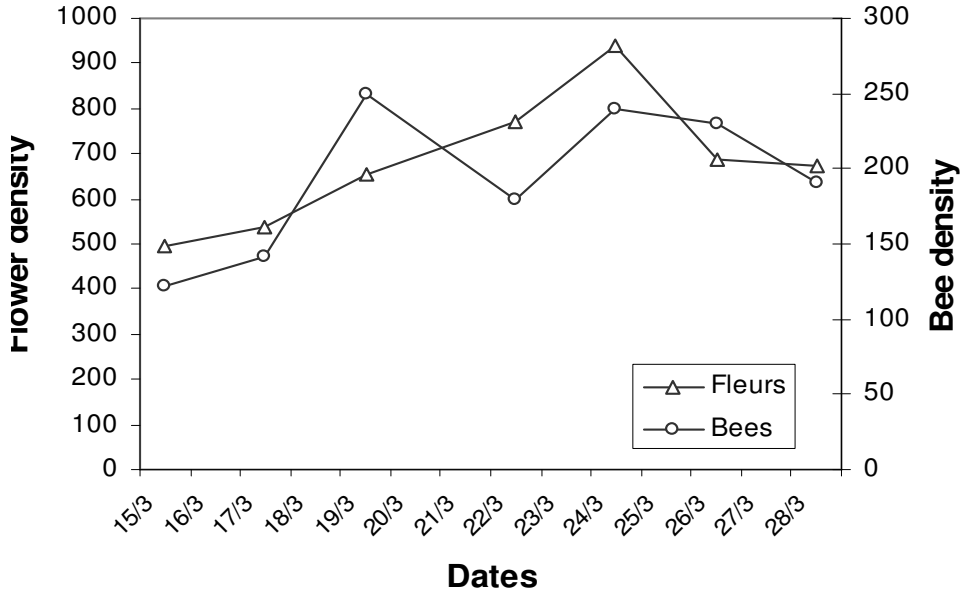

Figure 1. Vicia faba flowering cycle and bee density.

Table 1. Temporal evolution of number of pollinators observed during the flowering period (from 15 / 03 to 28 / 03 / 2003).

Species \ Dates		15 /3	17 /3	19 /3	22 /3	24 /3	26 /3	28 /3	Total
E.pulveracea Dours,1873	♂	29	29	57	48	68	44	39	314
	♀	39	27	68	56	71	75	40	376
Apis mellifera L.,1758		74	78	89	98	74	97	70	580
A. dispar Lepeletier, 1841		4	0	0	15	0	43	28	90
Xylocopa violacea L.,1758		4	6	9	1	3	0	0	23
Total		150	140	223	218	216	259	177	1383

foraged along with pollinating species. It consisted of counting the number of flowers visited per minute. The results are subject to simple statistical treatment through the calculation of the averages.

Evaluation of the effect of entomophilous pollination on seed yield

To highlight the impact of Apoides pollination on seed yield in the bean, we have proceeded to encagement plots before flowering. Three quadrates of 1 square-meter were left free access to pollinator insects. Three others were caged with tulle (2 m height) in order to prevent access to pollinator insects. Several parameters were measured to compare yields of free plots and caged ones. The components of returns that were taken into account are: the average length of the green pods at low level and ready for consumption, the average weight of the crop per plot, the average weight of seeds per plant, the average weight of seeds per pod, the mean number of pods per plant, the mean number of seeds per plant and the percentage of abortion. The counting and weighing of the seeds were carried after harvest. Seeds were collected in maturation phase when the pods were dry and black.

A one-way analysis of variance by a mean comparison test (Newman-Keuls at a 5 % risk level) was performed to value the beneficial effects of bees in the broad bean pollinisation.

RESULTS

Vicia faba flowering and apoides pollinators phenology

During flowering, the flowers were first observed at the base of the stems, the development continued progressively to the high level during the following week. The first inflorescences appeared on the 08th of March. The beginning of flowering was achieved in mid-march. The period of full bloom lasted from 17th to 24th of March when more than 50% of plants were in bloom. The mean number of flowers per stem decreased progressively, the end of flowering is recorded on 28th of March (Figure 1). The main species of bees attending broad bean's flowers were: honeybee *A. mellifera* L., the wild bees *E. pulveracea* Dours, *Anthophora dispar* Lepeletier and *X. violacea* L. Generaly, bees foraged broad bean during the full flowering period but visits were more numerous during the period of intense flowering. The first individuals appeared on the 15th of March (Table 1). It was observed that increasing the number of flowers increases the polli-

Table 2. Number of specimens (N) and related frequencies (% N) of identified Apoidea.

Family	Species		N	% N
Apidae	*Apis mellifera* L.,1758		580	42
	Bombus terrestris auct.			
Anthophoridae	*Eucera pulveracea* Dours, 1873	♂	314	
		♀	376	
	Eucera numida Lepeletier, 1841			
	Anthophora dispar Lepeletier, 1841		90	6.5
	Xylocopa violacea L., 1758		23	1.6
	Xylocopa valga Gerstaecker, 1872			
Halictidae	*Lasioglossum clavipes* Dours, 1872			
	Lasioglossum villosullum Kirby,1802			
Total			1383	100

Table 3. Mean numbers of pollinators per 100 available flowers of *Vicia faba* and per square-meter.

Species		Number /100 fls	Number / m²
Apis mellifera		2	15.42
Eucera pulveracea	♂	<1	6.71
	♀	1	10.14
Anthophora dispar		<1	2
Xylocopa violacea		<1	0.6

pollinator density. Indeed, the number of bees reached a maximal value during the intense flowering of this plant. Two peaks of affluence marked on 19th of March and 24th of March. A decline was noted on 22th of March which could be due to the effect of wind which would have disturbed the foraging activity. Wind speed corresponding to this date had reached an average of 2.6 m/s, a value that exceeded the monthly average recorded in March (1.2 m/s).

Commonly, the broad bean floral display and pollinator density took place in a synchrone way. This positive nutritional relationship has been observed in our cultivar. It has also been shown that there was a clear influence of the flowers availability on foraging activity.

Pollinator diversity and density

Based on our observations, insects which foraged on broad bean flowers were all *Hymenoptera Apoides* belonging to 10 species spread over 03 families: *Apidae*, *Anthophoridae*, *Halictidae*. Encountered species are listed in Table 2; as for the relating results to the pollinator density, they are provided in Table 3.

The most recorded species on the broad bean flowers was *E. pulveracea* which alone accounted for half (49.9%) of all visits, and a population density of 16.8 bees per square meter. The honeybee contributed from 42% of Apoidea visitors. *A. dispar* frequency was relatively low: 6.5%, as for *X. violacea*, it was just occasional with a tiny proportion of 1.6% of the floral visits observed on *Vicia faba*.

Foraging behaviour and pollinating efficiency

In order to determine the pollinating efficiency of some Apoides species on broad bean flowers, we conducted observations of foraging behaviour to understand the nature of visits (positive or negative) and measure the speed of pollination. As to the first aspect, we noted that among the observed species of bees, some always made pollinating visits because they entered the flower and induced tripping; it was case of *E. pulveracea.* Others foragers which made non pollinating visits behaved as “robbers” by collecting nectar through a hole made by the bumblebees at the base of the corolla. It was the *X. violacea*, of the visits made to flowers by honey bees *A. mellifera*, 94% of them were pollinating visits. We can then infer that the most powerful species was *E. pulveracea*, followed by *A. mellifera*. As for *X. violacea*, its role was deemed null as to pollination because its foraging did not fertilize the flower. It can be seen that in species, the broad bean was pollinated by Apoides in 91% cases (Table 4). The second factor of the pollination efficiency that has been taken into consideration is the

Table 4. Number of floral visits, number of the pollinating visits of main bees (only one visit per individual).

Species		Number of observed spécimens	Number of pollinating visits	Proportion (%) of pollinating visits
Apis mellifera		437	412	94
Eucera pulveracea	♂	416	416	100
	♀	514	514	100
Xylocopa violacea		147	0	0
Total		1912	1740	91

Table 5. Speed foraging of main bee species on *Vicia faba*.

Species	Number of observed specimens	Mean number of flowers visited per minute
Eucera pulveracea ♂	45	11.4 ± 5.1
Apis mellifera	45	9.7 ± 4.3
Eucera pulveracea ♀	45	9.3 ±,5.8
Anthophora dispar	45	8.9 ± 6.8
Xylocopa violacea	45	3.3 ± 6.4

Table 6. Mean length of pods during the fructification period at low level of *Vicia faba* and the percentage of abortion.

Plots	Length in cm	Percentage of abortion
Free plots	24.80	19
Caged plots	21.16	37

Table 7. Mean seed yield of *Vicia faba* in free plots and caged plots.

Plots / Weight (grammes)	Free plots	Caged plots
Mean weight of the crop	664.47	575.5
Mean weight of seeds per plant	36.24	30.29
Mean weight of seeds (5 seeds)	19.63	17.63

speed of foraging. If we consider the number of flowers visited per minute, the most frequent visits were those of male of *E. pulveracea* which foraged flowers at an average rate of 11.4 flowers per minute (fls/min). *A. mellifera* and female of *E. pulveracea* came in the second place. *A. dispar* was a little slower than previous species; visit speed of *X. violacea* was relatively low, its average rate was only 3.3 flowers/min (Table 5).

Consequences of pollination entomophilious on seed yield

Bees studied had a positive impact on the production, their presence improved the yield of broad bean culture. The mean length (24.80 cm) of the green pods from the free plots exceeded the one in green pods from the caged plots (21.16 cm) (Table 6). The difference between these two average lengths (calculated from 85 pods) was significant at the 5% threshold. The average weights of the crop seeds as well as the average weight of seeds per plant were higher in the case of plants subject to cross-pollination and in the presence of bees compared to plants subject to the self alone. The average weight of seeds (calculated from 30 samples of 5 seeds randomly from each of the harvest) was significantly greater at 5% threshold in the open plots compared to caged plots (Table 7). The plants that were accessible to pollinators provided more pods per plant, more seeds per pods; by contrast, plants presented a mean number of pods per plant superior in the absence of pollinating insects (Table 8). As to the percentage of abortion, it was much higher in the case of plants placed in a cage and in the absence of bees (37%) than in the case of plants foraged (19%) (Table 6).

DISCUSSION

The study of *Vicia faba* L. during flowering 2003 revealed that this vegetable attracts pollinator insects composed almost exclusively of Hymenoptera apoïdes of the Apidae family. These observations were comparable to those by various authors (Philippe, 1991; Duc, 1997; Pierre et al. 1997) who reported that this vegetable is usually visited by 80% honey bee, 50% of solitary bees and 15% of bumblebees. In our study area, the most abundant species on broad bean flowers was the solitary bee *E. pulveracea* which alone accounts for 49.9% of observed visits. The flowers of *V. faba* seem to be appreciated by

Table 8. Mean index of fertility of *Vicia faba*.

Plots / Calculated index	Free plots	Caged plots
Mean number of seeds per pod	5.52	3.27
Mean number of seeds per plant	19.87	17.27
Mean number of pods per plant	3.60	5.28

solitary bees genera *Eucera*. These Apoidea were well attracted by flowers of this plant. In fact, entomological studies, using similar experimental (Benachour et al., 2007 in Constantine and Pierre et al., 1997 in south Spain) reported relative frequencies on visits of *Eucera numida* on bean flowers that are respectively in the order of 70 and 89.4%. The domestic bee came in the second place in terms of abundance in the region of Tizi-Ouzou and the region of Constantine, as well as *X. violacea* and *Bombus terrestris* who were poorly represented.

The foraging behaviour of the main species showed that visits of honey bees could be fertilizing in 94% cases; this species seems to be less effective in Constantine where the proportion of positive foraging was an average of 59% (Benachour et al., 2007). The visits of *E. pulveracea* were very fast (10.3 flowers per minute) and always result in pollination. The same observations were made by Benachour et al. (2007) for *E. numida* which always made a positive foraging at an average rate (10.4 flowers per minute) very close to that recorded for *E. pulveracea*. Conversely, *X. violacea* always made a negative foraging on broad bean flowers taking advantage of holes drilled at the base of the corolla by *B. terrestris* (Newton and Hill, 1983). Its visits were three times slower than those of the preceding species.

According to our observations, pollinators may play a prominent role in improving *Vicia faba* production. The calculation of the components of yield of plots showed that the numbers of pods as well as the seed yield obtained by cross-pollination are higher than those obtained by self. Our results corroborate those of Pritsch (1971), Pinzauti and Frediani (1979), Varis and Brax (1990) and Benachour et al. (2007).

Pollination by bees made an impact on the number of developed seeds. In a similar test, Free (1966) demonstrates that the absence of pollinating insects during flowering of the bean has hurt the average number of seeds per plant at 15.1 instead of 23.9. Similar findings have been made by Pinzauti and Frediani (1979), the dry seed yield obtained after pollination by bees was 1.2 to 8 times higher than that obtained without bees. According Free (1966), lack of bees, the weight of seeds per plant at the *V. faba* var. *equina* Steudel.falled to 10 g instead of 21.4 g in the presence of bees.

Moreover, it is also clear from our findings that the length of pods and the number of seeds they contain were more important in the free plots. Our results were in agreement to those of Barbier et al. (1986 cited in Philippe, 1991). According to this author, the pods from cross-pollination (entomophilous) were those having an important length and contained more seeds than those obtained after the self (without insects). About this, Vaissière (2002) confirmed that the size of a fruit was well correlated with the number of seeds it contained.

Regarding the number of pods per plant, our results were different from those of Benachour et al. (2007), who observed a higher number of pods per plant in the free access to bees quadrates. However, the comparable findings were obtained by Tamas et al. (1979) and Stephenson (1980) who thought that the observed drop in young pods on the free plants could be explained by the fact that the plants of free plots about the flowers are fully pollinated, could not ensure the maturity of all seeds, as a result pods from recent pollinated flowers aborted at the stage of young pods. This parameter has also been studied by Free (1966), who pointed out that in absence of bees, only 10 to 20% of the flowers managed to give harvestable pods. Moreover, Picard (1960) showed that the weather intervened heavily on the transformation of flowers to pods and therefore on the number of formed pods. In the case of our experiment, the tulle used for cage plots have slowed the speed of the wind. This modified parameter climate would have decreased the fall of flowers and young pods.

In conclusion, the technique of caged parcel has been confirmed that the production of seeds in *V. faba* benefited greatly from the activity of bees. Their visits increased seed yield either by facilitating self-fertilization by mechanical trigger and the release of staminal column, either by allowing the crossings in intervening as vehicle pollen between flowers or between plants.

In the region of Tizi-Ouzou, the solitary bee *E. pulveracea* was the principal pollinator of *V. faba*. The visits of this species were both very frequent and very effective with a foraging that could be fertilizing in all cases. It is a bee endowed with a speed visit relatively high. The honey bee *A. mellifera* was also a species that visit actively culture, but seemed less adapted to the floral morphology of the Vicia faba comparing with *E. pulveracea.* It is suggested to conserve the nesting sites in surrounding areas of crops and to ovoid unjustified us of pesticides during the flowering period. These Apoides may play a non-negligible pollination role in blooming culture where their density is hight enough. Consequently

quently, it is important to maintain wild bee populations at sufficiently high levels. It is therefore necessary to preserve these populations of wild and honey bees and promote their development by protecting their habitats.

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