

Diversity of Flowering Insects and Their Impact on Yields of *Phaseolus Vulgaris* L. (Fabaceae) in Yaoundé (Cameroon)

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Abstract

To evaluate the diversity and impact of insect pollinators on pod and seed yields of *Phaseolus vulgaris* (red bean with small seeds), its foraging and pollinating behavior were studied in Yaoundé, during the mild raining season (March-June) in 2016 and 2017. Treatments included unlimited floral access by all visitors and bagged flowers to avoid all insect pollinators. For each year of study, observations were made on 55 ± 38 flowers per treatment. The seasonal rhythm of insects activities, its foraging behavior, and its impact on pollination (fruiting rate, number of seeds/pod and percentage of normal seeds) were recorded. Fourteen insect species visited *P. vulgaris* flowers. Out of 667 visits, *Xylocopa olivacea*, *Halictus* sp., *Chalicodoma* sp. and *Apis mellifera adansonii* were the most frequent visitors with 21.43 %, 19.49 %, 12.44 % and 10.04 % visits respectively. These insects collected nectar and pollen intensely and regulatedly. The foraging activities of insect pollinators increased the fruiting rate by 23.56 %, the number of seeds/pod by 46.31 % and the normal seeds by 21.49 %. Therefore, conservation of nests and colonies of insect pollinators close to *P. vulgaris* crop fields should be recommended to improve pod and seed production in the region.

Keywords: Flowering insects; *Phaseolus vulgaris*; Foraging; Nectar; Pollen; Pollination.



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1. Introduction

Phaseolus vulgaris is an annual plant that originated from America [1]. Bean plants grow vertically to an average height of upright to 65 cm; climbing stems can reach two to three meters high. The leaves are generally trifoliolate, their color vary from green to purple. Flowering starts 35 days after sowing, the flower is pink, but can vary from white to red [2] and produces nectar and pollen which attract insects [3-5]. Cross-pollination by insects is generally observed [3, 5-7]. *Phaseolus vulgaris* flowers were reported to produce fewer seeds per pod in the absence of insect pollinators in the United States of America [3] and in Cameroon [4, 5, 8]. The research conducted in Kenya [9] and in Cameroon [4, 5, 8, 10] has revealed *Apis mellifera* and *Xylocopa olivacea* visiting *P. coccineus* and *P. vulgaris* flowers. No previous research has been reported on the relationships between *P. vulgaris* and its anthophilous insects in Yaoundé, although, the activity and diversity of flowering insects of a plant species vary with Agro-ecological region [11, 12]. The main objective of this research was to gather more data on the relationships between *P. vulgaris* and flower visiting insects. Specific objectives were to study the activities of flowering insects on *P. vulgaris* flowers, to evaluate the impact of visiting insects on pollination, pods and seeds yields of this Fabaceae.

2. Materials and Methods

2.1. Study Site, Experimental Plot and Biological Material

The studies were conducted from March to June in 2016 and 2017 (mild rainy season) in the fields located

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at the campus of Higher Teacher's Training College of University of Yaoundé I (Latitude 10° 62'N, Longitude 14° 33'E) in the Center Region Cameroon. This region belongs to the tropical rainforest agro-ecological zone [13]. The climate is equatorial, guinean-type with four seasons : the peak rainy season (August to November), the peak dry season (November-March), the mild rainy season (March-July) and the mild dry season (July-August) [14]. The experimental plot was an area of 500 m². The animal material was represented by insects naturally present in the environment and a colony of *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) housed in the building entrance located 5 m from the experimental logs. Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of *Phaseolus vulgaris* (red and small seed) provided by the Institute of Agricultural Research for Development in Nkolbisson (Yaoundé-Cameroon) (IRAD).

2.2. Sowing and Weeding

On March 10 and 15 respectively in 2016 and 2017, the experimental field was prepared and divided into 8 logs (1 x 6 x 0.3 m). Twelve (12) holes were made in one line per log and two seeds were sown each. Holes were separated by a distance of 50 cm. Weeding was performed manually as necessary to maintain weed-free logs.

2.3. Determination of the Reproduction System of *Phaseolus Vulgaris*

In April 15 and 20 respectively in 2016 and 2017, 10 flowers of *P. vulgaris* at the bud stage were labeled on each log for a total of 80 flowers. 40 of the total flowers were allowed for treatment 1 (open pollinated) and 40 others flowers belong to treatment 2 (bagged with gauze bag to prevent visitors or external pollinating agents) (figure 1). 25 days after shading of the last flower, the numbers of pods were assessed in each treatment. The podding index (P_i) was then calculated as described by Delaplane, *et al.* [15]: $P_i = F_2/F_1$. Where F_2 is the number of pods formed and F_1 is the number of viable flowers initially set. The allogamy rate (Alr) from which autogamy rate (Atr) was derived was expressed as the difference in podding indexes between unprotected flowers (treatment 1) and protected flowers (treatment 2). This was done using the formula of Demarly [16], as follows: $Alr = [(P_{i1} - P_{i2}) / P_{i1}] \times 100$. Where P_{i1} and P_{i2} are the podding average indices of treatments 1 and 2 respectively; $Atr = 100 - Alr$.

2.4. Foraging Activity of Flowering Insects on *Phaseolus Vulgaris* Flowers

Observations were conducted on 40 individually opened pollinated flowers of treatment 1 each day from April 20 to May 2nd of 2016 and from April 25, to May 07 of 2017 at 2 h interval from 8 to 18 h (8-9 h, 11-12 h, 14-15 h, 17-18 h). In a slow walk along all labeled flowers of treatment 1, the identity of all insects that visited *P. vulgaris* flowers was recorded. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits to determine the relative frequency of flowering insects.

Direct observations of the foraging activity of insects on flowers were made. The floral rewards (nectar or pollen) harvested by flowering insects during each floral visit were registered based on its foraging behavior. Nectar foragers were expected to extend their proboscis to the base of the corolla and the stigma, while pollen gatherers were expected to scratch the anthers with their mandibles or legs [17].

In the morning of each observation day, the number of opened flowers was counted. In the same day (as for the frequency of visits), the duration of individual flower visits was recorded (using a stopwatch) at least four times at two hourly intervals from 8am -18p.m. The abundance of foragers was defined as the highest number of individuals simultaneously foraging on a flower and on 1000 flowers (A_{1000}) was recorded [11]. The temperature and relative humidity in the station were also registered every one hour using a mobile thermo-hygrometer during all sampling periods.

2.5. Evaluation of the Effect of Flowering Insects on *Phaseolus Vulgaris* Yields

The impact of visiting insects on pollination of *P. vulgaris*, and the comparison of yields (fruiting rate, mean number of seed per pod and percentage of normal or well developed seeds) of treatments 1 and 2 (open and bagged flowers) were done. The fruiting rate due to the influence of activity of insects (Fri) was calculated using the formula: $Fri = \{[(Fr_1 - Fr_2) / Fr_1] \times 100\}$ [Pando, 2011 #10]; Fr_1 and Fr_2 are the fruiting rate in treatments 1 and 2. The fruiting rate (Fr) was calculated as follows: $Fr = [(F_2/F_1) \times 100]$; F_2 is the number of pods formed and F_1 the number of opened flowers initially set. At maturity, pods were harvested from each treatment and the mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

2.6. Data Analysis

Data was analyzed using descriptive statistics with Microsoft Excel 2007, Student's (t) test for the comparison of means of two samples, Anova (F) test for comparison of several averages, Correlation coefficient (r) for the study of the association between two variables, Chi - Square (χ^2) for the comparison of percentages.

3. Results

3.1. Pod Production of *Phaseolus Vulgaris*

Podding index of *P. vulgaris* was 0.92 and 0.76 respectively for treatment 1 and 2 in 2016 and 0.95 and 0.78 in 2017. In 2016 the allogamy rate was 17.40 % and the autogamy rate was 82.60 %. In 2017, the allogamy and autogamy rates were 15.71 % and 81.30 % respectively. It appears that the variety of *P. vulgaris* used in our experiments (small red seed) has a mixed production regime that is autogamous-allogamous, with the predominance of autogamy over allogamy.

3.2. Activity of Insects

3.2.1. Frequency of Flowering Insects of *Phaseolus Vulgaris*

Amongst the 667 visits of 14 insects species recorded in two years (2016 and 2017) on *P. vulgaris* flowers, *Xylocopa olivacea*, *Halictus* sp., *Chalicodoma* sp. and *Apis mellifera adansonii* were the 1st, 2nd, 3rd and 4th most represented insects with 21.43 %, 19.49 %, 12.44 % and 10.04 % of 667 visits respectively (Table 1). The difference between these four percentages of visits is very high significant ($\chi^2 = 29.30$, $df = 3$, $p < 0.001$).

3.2.2. Floral Rewards Harvested

During each of the two flowering periods, *A. m. adansonii*, *Chalicodoma* sp., *Halictus* sp. and *X. olivacea* collected nectar and pollen from *P. vulgaris* but these insects collected mostly nectar than pollen (Table 2) (Figure 2). The difference between the collection of nectar and pollen is very high significant ($\chi^2 = 13.59$, $df = 3$, $p < 0.001$).

3.2.3. Relationship between Visits and Flowering Stages

A positive and significant correlation was found between the number of *P. vulgaris* opened flowers and the number of *A. m. adansonii* visits ($r = 0.73$, $df = 8$, $p < 0.05$) and *Halictus* sp. ($r = 0.66$, $df = 8$, $p < 0.05$) in 2016; in 2017 the results were ($r = 0.65$, $df = 8$, $p < 0.05$), ($r = 0.86$, $df = 8$, $p < 0.05$) respectively from *Chalicodoma* sp. and *X. olivacea*.

3.2.4. Abundance of Flowering Insects

In 2016 and 2017, the highest mean number of *A. m. adansonii*, *Chalicodoma* sp., *Halictus* sp. and *X. olivacea* simultaneously in activity was 1 per flower ($n = 50$, $s = 0$). The abundance per 1000 flowers varied from 153 ($n = 45$, $s = 28.18$) for *A. m. adansonii* to 256 ($n = 45$, $s = 23.29$) for *Halictus* sp. in 2016. In 2017, the corresponding figures were 53 ($n = 25$, $s = 9.80$) for *Chalicodoma* sp. and 203 ($n = 45$, $s = 27.93$) for *Xylocopa olivacea* (Table 3).

3.2.5. Duration of Visits per Flower

In 2016, the mean duration of a flower visit varied from 1.37 s ($s = 0.48$) for *A. m. adansonii* to 1.79 s ($s = 0.93$) for *Halictus* sp. in 2017, the corresponding data were 4.59 s ($s = 2.09$) for *X. olivacea* to 5.96 s ($s = 4.94$) for *Chalicodoma* sp. The difference between the mean duration visit of four insects was highly significant ($F = 2.05$, $df = 3$, $p < 0.001$) for the two cumulated years (Table 4).

3.3. Impact of Insect Pollinators on Seed Yields of *Phaseolus Vulgaris*

During nectar and pollen harvest on *P. vulgaris* flowers, foraging insects always shake flowers and contact which anthers and stigma increases the cross and self pollination possibility of *P. vulgaris* (Table 5). The results obtained indicated that:

a. The comparison of the fructing rates were significant between free opened flowers (treatment 1) and bagged flowers (treatment 2) in the first year ($\chi^2 = 3.49$, $df = 1$, $p < 0.05$) and highly significant in the second year ($\chi^2 = 25.67$, $df = 1$, $p < 0.001$). The difference between the two years as far as treatment 1 is concerned was highly significant ($\chi^2 = 5.79$, $df = 1$, $p < 0.01$) but for treatment 2 there was no significant difference ($\chi^2 = 0.24$, $df = 1$, $p > 0.05$). Consequently, the fruiting rate of the unprotected flowers was higher than that of protected flowers in 2016 and in 2017. The fruiting rate due to the action of flowering insects was 17.09 % in 2016 and 30.04 % in 2017. For all of the flowers studied, the fructing rate attributed to the influence of insects was 23.56 %.

b. The comparison of the mean number of seeds per pod was highly significant between treatments 1 and 2 ($t = 6.78$, $df = 98$, $p < 0.001$) in the first year and in the second year ($t = 7.07$, $df = 98$, $p < 0.001$). For treatment 1, the difference between the two studied years was significant ($t = 1.51$, $df = 165$, $p < 0.1$), for treatment 2 it was not significant ($t = -1.00$, $df = 31$, $p > 0.05$). Consequently, a higher mean number of seeds per pod in opened flowers (treatment 1) than the bagged flowers (treatments 2). The number of seeds per pod attributed to the activity of flowering insects was 55.29 % in 2016 and 37.34 % in 2017, giving an overall mean of 46.31 %.

c. The comparison of the percentage of normal seeds were very significant between free opened flowers (treatment 1) and bagged flowers (treatment 2) in the first year ($\chi^2 = 13.14$, $df = 1$, $p < 0.001$) and in the second year ($\chi^2 = 22.54$, $df = 1$, $p < 0.001$). For treatment 1, the difference between the two studied years was significant ($\chi^2 = 5.25$, $df = 1$, $p < 0.025$), for treatment 2, there was significance also ($\chi^2 = 3.55$, $df = 1$, $p < 0.05$). The percentage of normal seeds in opened flowers was higher than that of protected flowers in 2016 and 2017. The percentage of the normal seeds attributed to the action of insects was 17.60 % in 2016 and 25.39 % in 2017. For all the flowers studied, the percentage of the normal seeds attributed to flowering insects was 21.49 %.

4. Discussion

Apis mellifera adansonii, *Chalicodoma* sp., *Halictus* sp. and *X. olivacea* were the main floral visitor of *P. vulgaris* during the observation period. They same bee has been reported as the main floral visitor of this crop in Kenya [9] and in Cameroon [4, 5]. The significant difference between the percentages of visiting insects within studied years could be attributed to the experimental site variation. The temperature and hygrometry positively influenced the insect activity on foraged flowers. Foraging insects preferred sunny days for good floral activity

[18]. Similarly, the nearby lake in the study site is an environmental factor that can influence the floral insect activity [19]. The abundance of *A. m. adansonii*, *Chalicodoma* sp., *Halictus* sp. and *X. olivacea* on 1000 flowers and the positive and highly significant correlation between the numbers of *P. vulgaris* flowers indicated the good attractiveness of floral products of *P. vulgaris*. The significant difference between the duration of visits of *A. m. adansonii*, *Chalicodoma* sp., *Halictus* sp. and *X. olivacea* in 2016 and 2017 could be attributed to the availability of floral products or the variation of diversity of flowering insects from one year to another. During each of the two flowering periods of *P. vulgaris*, those insects intensely and regularly harvested nectar or pollen. This could be attributed to the needs of individual insect species. From this research, we observed that insects pollinators' can provide benefits to pollination management of *P. vulgaris*. During the collection of nectar or pollen on each flower, those insects regularly came into contact with the stigma. They were also able to carry pollen with their hairs, legs and mouth accessories from a flower of one plant to stigma of another flower of the same plant (geitonogamy), to the same flower (autogamy) or to the flower of another plant (xenogamy). The significant contribution of pollinating insects in pods and seed yield of *P. vulgaris* was found in United State of America [3] and in Cameroon [4, 5] which showed that *P. vulgaris* flowers produce fewer seeds per pod in the absence of pollinating insects. The weight of insect pollinators played a positive role during nectar or pollen collection, those insects shook flowers, facilitating the liberation of pollen by anthers for the optimal occupation of the stigma [8]. This similar observation was also reported by Pando, *et al.* [10] on *P. coccineus*. This Higher productivity of pods and seeds in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross-pollination.

5. Conclusion

The floral products of *P. vulgaris* red seed attract pollinator insects. This attractiveness is of benefit for the pollination process. The comparison of pods and seeds set of unprotected flowers with that of protected flowers indicated the value of these insect pollinators in increasing pods and seed yields. The installation of nests or hives of insect pollinators at the proximity of *P. vulgaris* small red seed fields should be recommended for the increase of pods and seed yields of this valuable crop.

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Table-1. Diversity of floral insects on *Phaseolus vulgaris* flowers in 2016 and 2017, number and percentage of visits of different insects

Insects			2016		2017		Total	
Order	Family	Genus, species, Sub - sp. ^{po, nt, df}	n_1	$p_1\%$	n_2	$p_2\%$	$n_{1,2}$	$p_{1,2}$
Coleoptera			21	7.92	35	8.70	56	8.39
Diptera	Muscidae	<i>Musca domestica</i> ^{nt}	47	17.7	17	4.22	64	9.59
	Calliphoridae	sp. ^{po}	16	6.02	9	2.38	25	3.74
Hymenopt	Apidae	<i>Apis mellifera adansonii</i> ^{po} ,	59	22.2	8	1.99	67	10.0
		<i>Xylocopa olivacea</i> ^{nt}	0	0	14	3.55	14	2.14
	Eumenidae	<i>Delta</i> sp. ^{nt}	0	0	14	3.48	14	2.09
	Halictidae	<i>Halictus</i> sp. ^{po, nt}	83	31.3	47	11.6	130	19.4
	Megachilid	<i>Chalicodoma</i> sp. ^{nt}	0	0	83	20.6	83	12.4
	Vespidae	<i>Synagris cornuta</i> ^{po, nt}	0	0	12	2.98	12	1.79
Lepidopter	Acraeidae	<i>Acraea acerata</i> ^{nt, rt}	7	2.63	0	0	7	1.04
	Pieridae	<i>Catopsilia flerella</i> ^{nt, rt}	0	0	5	1.24	5	0.74
	Nymphalida	sp. ^{nt, rt}	13	4.8	3	0.74	16	2.39
Orthopter		sp. ^{df}	19	7.15	9	2.23	28	4.18
Nevropter		sp. ^{pr}	0	0	17	4.22	17	2.54
Total		14 species	26	100	40	100	66	100

n_1 : number of visits on 40 flowers in 10 days. n_2 : number of visits on 40 flowers in 10 days. p_1 et p_2 : percentages of visits. $p_{1,2} = (n_1 / 265) \times 100$. $p_{2,2} = (n_2 / 402) \times 100$. $n_{1,2} = (n_1 + n_2) / 2$. $p_{1,2} = (p_1 + p_2) / 2$. nt: visitor collected nectar. po: visitor collected pollen. df: defoliator. rt: rest. pr: predator. sp.: undetermined species

Table-2. Products harvested by *Apis mellifera adansonii*, *Chalicodoma* sp., *Halictus* sp. and *Xylocopa olivacea* on flowers of *Phaseolus vulgaris* in 2016 and 2017

Insect	year	Products Harvested				Comparison of Percentages
		Nectar		Pollen		
		n	$p\%$	n	$p\%$	
<i>Apis mellifera adansonii</i>	2016	45	78.94	12	21.05	$\chi^2 = 2.41, df = 1, p < 0.1$
	2017	8	100	0	0	
	Total	53	81.53	12	18.46	
<i>Chalicodoma</i> sp.	2016	0	0	0	0	
	2017	50	100	0	0	
	Total	50	100	0	0	
<i>Halictus</i> sp.	2016	38	82.60	8	17.39	$\chi^2 = 0.04, df = 1, p > 0.05$
	2017	27	84.37	5	15.62	
	Total	65	83.33	13	16.66	
<i>Xylocopa olivacea</i>	2016	0	0	0	0	
	2017	48	100	0	0	
	Total	48	100	0	0	

Table-3. Abundance per 1000 flowers of *Apis mellifera adansonii*, *Chalicodoma* sp., *Halictus* sp. and *Xylocopa olivacea* on *Phaseolus vulgaris* flowers in 2016 and 2017 in Yaoundé

Insect	Year	n	Abundance per 1000 Flowers				Comparison of Average
			m	s	min	max	
<i>Apis mellifera adansonii</i>	2016	45	153	28.18	38	269	$t = 211.80, df = 78, p < 0.001$
	2017	0	0	0	0	0	
	Total	45	153	28.18	38	269	
<i>Chalicodoma</i> sp.	2013	0	0	0	0	0	
	2017	45	203	27.93	21	296	
	total	45	203	27.93	21	296	
<i>Halictus</i> sp.	2016	45	256	23.29	69	302	
	2017	35	53	9.80	14	174	
	Total	80	159.5	16.54	14	302	
<i>Xylocopa olivacea</i>	2016	0	0	0	0	0	
	2017	45	191	52.81	81	248	
	Total	45	191	52.81	81	248	

Table-4. Duration of visit of *Apis mellifera adansonii*, *Chalicodoma* sp., *Halictus* sp. and *Xylocopa olivacea* on flowers of *Phaseolus vulgaris* in 2016 and 2017

Insect	Year	Products Harvested	n	m	s	Comparison of Means
<i>Apis mellifera adansonii</i>	2016	Nectar	40	1.88	0.23	$m_{(2016)} = 1.37 s \pm 0.48.$ $t_{(nectar/pollen)2016} = -3.78 ;$ $ddl=48 ; p < 0.001.$ $m_{(2016/2017)} = 1.38 s \pm 0.42.$
		Pollen	10	1.67	0.85	
	2017	Nectar	8	1.39	0.19	
		Pollen	0	0	0	
<i>Chalicodoma</i> sp.	2016	Nectar	0	0	0	$m_{(2016/2017)} = 5.96 s \pm 4.94.$
		Pollen	0	0	0	
	2017	Nectar	50	5.96	4.94	
		Pollen	0	0	0	
<i>Halictus</i> sp.	2016	Nectar	38	1.25	0.96	$m_{(2016)} = 1.79 s \pm 0.93.$ $t_{(nectar/pollen)2016} = -2.85 ;$ $ddl=44 ; p < 0.001.$ $m_{(2017)} = 2.17 s \pm 1.02.$ $t_{(nectar/pollen)2017} = -2.69 ; ddl= 30 ; p < 0.001.$ $m_{(2016/2017)} = 1.68 s \pm 0.98.$
		Pollen	8	2.33	0.91	
	2017	Nectar	27	1.66	0.47	
		Pollen	5	2.69	1.58	
<i>Xylocopa olivacea</i>	2016	Nectar	0	0	0	$m_{(2016/2017)} = 4.59 s \pm 2.09.$
		Pollen	0	0	0	
	2017	Nectar	48	4.59	2.09	
		Pollen	0	0	0	

$F = 2.05 ; p < 0.001$

Table-5. *Phaseolus vulgaris* yields under pollination treatments

Treatment	Year	Flowers	Pods	Fruiting Rate (%)	Seed / Pods			Normal Seeds	% Normal Seeds
					m	s			
Unlimited visits	2016	97	90	92.78	4.25	1.03	413	385	86.68
Bagged flowers		13	10	76.92	1.90	1.01	21	15	71.42
Unlimited visits	2017	77	77	100	4.07	0.09	289	255	88.23
Bagged flowers		33	23	69.96	2.55	1.86	79	52	65.82

Figure-1. Plant of *Phaseolus vulgaris* showing flowers under treatment (A: open pollinated flower ; B : bagged flower)



Figure-2. Pollinating insects in foraging activities on *Phaseolus vulgaris* flowers (A: *Xylocopa olivacea*; B: *Chalicodoma* sp.)

