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Diversity of flowering insects and their impact on yields of *Abelmoschus esculentus* (L.) Moench, 1794 (Malvaceae) in Yaoundé (Cameroon)

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Abstract
To evaluate the diversity and impact of insect pollinators on pod and seed yields of *Abelmoschus esculentus*, its foraging and pollinating behavior were studied in Yaoundé, during the mild raining season (March-June) in 2014 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 32 ± 2 flowers per treatment. The insects' activities, its foraging behavior, and its impact on pollination were recorded. The foraging activities of insect pollinators increased the number of seeds/pod by 15.09% in 2014 and 20.57 in 2017 and the normal seeds by 44.49% in 2014 and 5.38% in 2017. Therefore, conservation of nests and colonies of insect pollinators close to *A. esculentus* crop fields should be recommended to improve pod and seed production in the region.

Keywords: Flowering insects, *Abelmoschus esculentus*, foraging, flower, pollination

1. Introduction

Abelmoschus esculentus (L.) Moench (Malvaceae), commonly called okra, lady's finger or gumbo is an important vegetable in the tropics and sub-tropics [1]. This crop plant probably originated in the Ethiopian region of Africa but is now widely grown throughout Africa; especially in Sudan, Egypt and Nigeria [2]. Up to 4 m tall, erect, more or less strongly branched; cylindrical stem, with stiff hairs disseminated, glabrescent, often spotted with red; branches set to curved downwards [2]. The leaves are arranged spirally, their color vary from clear to purplish red. Flowers are solitary, axillary and cream-colored, yellow or golden yellow with a dark red zone at the base of the petals [2]. Okra is self-compatible, and self-pollination can take place in its hermaphrodite flowers [3]. The flowers open in the early hours of dawn. Anthers are dehiscent at anthesis [2] and produces nectar and pollen which attract insects [2, 3, 4, 5]. Previous results of Azo'o *et al*. [5] indicate that the flower of Okra is very deep; this makes it difficult for foragers to reach nectaries, which lie in its bottom. Two wild bees, *Eucara macrognatha* (Gerstaecker) and *Tetralonia fraterna* (Freise) were found as the main pollinators of this vegetable in 2010 cultivating season. Thus, the visits of *E. macrognatha* identified on *A. esculentus* flowers in the locality of Domayo (Maroua) were only devoted to the collection of pollen. No previous research has been reported on the relationships between *A. esculentus* and its anthophilous insects in Yaoundé, although, the activity and diversity of flowering insects of a plant species vary with Agro-ecological region [4-6]. The main objective of this research was to gather more data on the relationships between *A. esculentus* and flower visiting insects in Cameroon. Specific objectives were to determinate the reproduction system of this plant, to study the activities of flowering insects on *A. esculentus* flowers in Yaoundé (Cameroon) and to evaluate the impact of those insects on pollination and production of pods and seeds yields of this Malvaceae in this region.

2. Materials and methods

2.1 Study site, experimental plot and biological material

The studies were conducted from March to June in 2014 and 2017 (mild rainy season) in the fields located 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 [7]. 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) [8]. The experimental plot was an area of 500 m^2. The animal material was represented by insects naturally present in the environment and one colony of *Apis mellifera* Latreille (Hymenoptera: Apidae) located in the roof of a building at 10 m from the experimental field. Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of *A. esculentus* were bought at the Mokolo market in Yaoundé (Cameroon).

2.2 Sowing and weeding

On March 19 and 22 respectively in 2014 and 2017 the experimental plot was divided into 15 logs (15*1.5*0.4 m^2). Seeds were sown on one line per log; the line had 15 holes and each hole received three seeds. Each hole was 4 cm depth. Two weeks after germination, the plants were thinned and only two were left per hole. Weeding was performed manually as necessary to maintain subplots weeds-free.

2.3 Determination of the reproduction system of *Abelmoschus esculentus*

In May 08 and 22 respectively in 2014 and 2017, 65 flowers of *A. esculentus* at the bud stage were labeled and 34 of the total flowers carry by 25 plants distributed on 15 logs were allowed for treatment *1* (open pollinated) and 31 other flowers carry by 20 plants distributed on the same number of logs belong to treatment *2* (bagged with gauze bag to prevent visitors or external pollinating agents) (figure 1) [6, 9]. 10 days after shading of the last flower, the numbers of pods were assessed in each treatment. The podding index (*Pi*) was then calculated as described by Tchuenguem [6]: $Pi = F2/F1$. Where *F2* is the number of pods formed and *F1* 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 [10], as follows: $Alr = [(Pi1 - Pi2) / Pi1]*100$. Where *Pi1* and *Pi2* are the podding average indices of treatments *1* and *2* respectively; $Atr = 100 – Alr$.

2.4 Foraging activity of flowering insects on *Abelmoschus esculentus* flowers

Observations were conducted on 34 individually opened pollinated flowers of treatment *1* each day from May 14 to June 5 in 2014 and from May 18 to June 8 in 2017 at interval from 8 to 17 h (8-9h, 10-11h, 12-13h, 14-15h et 16-17 h). In a slow walk along all labeled open flowers of treatment *1*, the identity of all insects that visited *A. esculentus* 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 [11].

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 9am-16pm (9-10h, 11-12h, 13-14h, 15-16h). 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 [12]. The temperature and relative humidity in the station were also registered everyone hour using a mobile thermo-hygrometer during all sampling periods.

2.5 Evaluation of the effect of flowering insects on *Abelmoschus esculentus* yields

The impact of visiting insects on pollination of *A. esculentus*, 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 calculate. The fruiting rate due to the influence of activity of insects (*Fri*) was calculated using the formula: $Fri = \{[(Fr1–Fr2)/Fr1]*100\}$; *Fr1* and *Fr2* are the fruiting rate in treatments *1* and *2*. The fruiting rate (*Fr*) was calculated as follows: $Fr = [(F2/F1)*100]$; *F2* is the number of pods formed and *F1* the number of opened flowers initially set [6]. 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 *Abelmoschus esculentus*

Podding index of *A. esculentus* was 0.91 (n = 34) and 0.77 (n = 31) respectively for treatment *1* and *2* in 2014 and 0.85 (n = 34) and 0.67 (n = 31) in 2017. In 2014, the allogamy and autogamy rates were 15.38% and 84.62%. In 2017 the same result was 21.17% and 78%. For the two cumulated years; for autogamy and allogamy rate were 81.31% and 18.27% respectively. It appears that the variety of *A. esculentus* used in our experiments has a mixed production regime that is autogamous-allogamous, with the predominance of autogamy over allogamy.

3.2 Activities of insects

3.2.1 Frequency of flowering insects of *Abelmoschus esculentus*

Amongst the 101 and 1008 visits of 10 and 9 insect species were recorded in two years (May 14 to June 05, 2014) and (May 18 to June 8, 2017) on *A. esculentus*, *Xylocopa olivacea*, *Synagris cornuta* and the ants were the most frequently insect species observed, with 52 (4.69%), 195 (17.58%) and 653 (58.88%) visits respectively (Table 1). The difference between these three percentages of visits is very high significant (χ^2 = 900.81, df = 2, p<0.001). The distribution of flower-visiting insect species according to their daily regularity is reported in table 2. Three groups of anthophilous insects were found on *A. esculentus* flowers: constant species that include Hynemoptera and Hemiptera, accessory species (Hynemoptera and Lepidoptera) and accidental species (Lepidoptera, Orthoptera and Nevroptera).

3.2.2 Floral rewards harvested

In 2014 (from May 14 to June 5), 41 visits of *X. olivacea* recorded, 32 (78.04%) were devoted to pollen collection and 9 (21.96%) at the nectar harvest. Whereas in 2017 (from May 18 to June 8), on 53, 43 and 16 visits of ants, *S. cornuta, Dysdercus voelkeri* respectively. 11 (20.75%), 26 (60.43%) and 7 (26.92%) were devoted to pollen collection and 42 (79, 24%), 17 (39.53%) and 09 (73.07%) were belong to the nectar harvest from *A. esculentus* flowers (Table 3). The difference between the collection of nectar and pollen; in 2017 the percentage of pollen collection and nectar harvest for the three insects is very high significant ($\chi^2 = 15.85$, $df = 2$, $p<0.001$).

3.2.3 Relationship between visits and flowering stages

We found a positive and highly significant correlation between the number of opened flowers and the number of insect visits (*X. olivacea*) in 2014 ($r = 0.86$; $ddl = 9$; $P<0.05$). Furthermore, a positive and significant correlation was found ($r = 0.56$, $ddl = 9$, $P<0.05$) and a positive highly significant correlation ($r = 0.72$, $ddl = 9$, $P<0.05$) between the number of opened flowers and the number of insect visits (hymenoptera and others group of flowering insects) in 2017.

3.2.4 Abundance of flowering insects

In 2014, the highest mean number of insect simultaneously in activity on a flower of *A. esculentus* vary from 0 for ants to 1 for *X. olivacea, S. cornuta* and *D. voelkeri*; in 2017 the corresponding figures vary from 1 for *X. olivacea, S. cornuta* and *D. voelkeri* to 11 for ants. In 2014 the mean abundance per 1000 flowers vary from 0 for ants to 525 ± 61, 71 for *X. olivacea*; In 2017 the result vary from 32 ± 0.59 for *Synagris cornuta* to 1358 ± 102.05 for ants (Table 4).

3.3 Impact of insect pollinators on seed yields of *Abelmoschus esculentus*

During pollen and nectar harvest, flowering insects of *A. esculentus* were in regular contact with the anthers and stigma. Thus these insects increased the pollination possibilities of this plant species. Table 5 presents the results on the fruiting rate, the number of seeds per pod and the percentage of normal seeds in different treatments. From this table, we documented the following:

a) The comparison of the fruiting rates were not significant between opened flowers (treatment *1*) and bagged flowers (treatment *2*) in the first year ($X^2 = 0.20$; $ddl = 1$; $P>0.05$) and in the second year ($\chi^2 = 0.37$, $df = 1$, $p>0.05$).
b) The comparison of the mean number of seeds per pod was highly significant between treatments *1* and *2* ($t = 3.21$, $df = 53$, $p<0.001$) in the first year and in the second year ($t = 4, 20$, $dd1 = 1$, $P<0.001$). Thus, the number of seed per pod was higher in opened flowers (treatment *1*) than bagged flowers (treatments *2*).
c) The comparison of the percentage of normal seeds were highly significant between opened flowers (treatment *1*) and bagged flowers (treatment *2*) in the first year ($\chi^2 = 1183.14$; $ddl = 1$; $P<0,001$) and in the second year ($\chi^2 = 44.19$; $ddl = 1$; $P<0,001$). The percentage of normal seeds in opened flowers was higher than that of protected flowers in 2014 and 2017.

The numeric contribution of pollinating insects to the mean number of seeds per pod and the percentage of normal seeds were respectively 15.09% and 44.49% in 2014. The corresponding figures were 20.57% and 5.38% in 2017. For the two cumulate years, the numeric contributions were 17.83% and 24.93% for the mean number of seeds per pods and the percentage of normal seeds respectively. The impact of pollinating insects on pod and seed yields was positive and significant.

Table 1: Diversity of floral insects on *Abelmoschus esculentus* flowers in 2014 and 2017, number and percentage of visits of different insects.

Insects			2014		2017		Total	
Orders	family	Genus, species	n_1	p_1(%)	n_2	p_2(%)	$n_{1,2}$	$p_{1,2}$(%)
Diptera	Muscidae	*Musca domestica* (nt, po)	0	0	17	1,68	17	1,53
Coleoptera		(sp.) (nt, po, df)	0	0	93	9,22	93	8,39
Hymenoptera	Apidae	*Xylocopa olivacea* (nt, po)	41	40,59	11	1,09	52	4,69
		Apis mellifera (nt, po)	14	13,86	0	0	14	1,26
	Formicidae	(sp.) (nt, po)	0	0	653	64,78	653	58,88
	Vespidae	*Synagris cornuta* (nt, po)	9	8,91	186	18,45	195	17,58
Hemiptera	Pyrrhocoridae	*Dysdercus voelkeri* (nt, po)	12	11,88	26	2,57	38	3,43
Lepidoptera	Acraeidae	*Acrea acerata* (nt)	0	0	13	1,28	13	1,17
	Pieridae	*Catopsila florella* (nt)	7	6,93	0	0	7	0,63
	Pieridae	sp.1 (nt)	11	10,89	0	0	11	0,99
	Pieridae	sp.2 (nt)	2	1,98	0	0	2	0,18
Orthoptera		sp.1 (df)	2	1,98	0	0	2	0,18
		sp.2 (df)	3	2,97	0	0	3	0,27
		sp.3 (df)	0	0	5	0,49	5	0,45
Nevroptera		(sp.) (pr)	0	0	4	0,39	4	0,36
Total		15 species	101	100	1008	100	1109	100

n1: number of visits on 40 flowers in 10 days. n2: number of visits on 40 flowers in 10 days. p1 et p2: percentages of visits. p1 = (n1 / 101) x 100. p2= (n2 / 1008) x 100. n1, 2= (n1 + n2)/2. p1, 2 = (p1 + p2)/2. nt : visitor collected nectar. po: visitor collected pollen. df: defoliator. rt: rest. pr: predator. sp.: undetermined species.

Table 2: Distribution of flowering insects according to the seasonal frequency of visits in 2014 and 2017

Insects	2014		2017		Total		1. **Category of insects**
	n_1	f_1(%)	n_2	f_2(%)	$n_{1,2}$	$f_{1,2}$(%)	
Dysdercus voelkeri	10	100	4	40	14	63.33	2. Constant species (f ≥ 50%)
Xylocopa olivacea	10	100	1	8.33	11	50.00	
Synagris cortuna	8	80	5	50	13	59.09	
Ants	0	0	12	100	12	54.54	
Apis mellifera	7	70	0	0	7	31.88	3. Accessory species (25% ≤ *f* < 50%)
Acrea acerata	0	0	6	60	6	27.27	
Coleoptera (sp.)	2	20	3	30	5	22.72	4. Accidental species (f < 25%)
Pieridae (sp.1)	5	50	0	0	5	22.72	
Catopsilia florella	3	30	0	0	3	13.63	
Pieridae (sp.2)	3	30	0	0	3	13.63	
Orthoptera (sp.1)	1	10	0	0	1	4.54	
Orthoptera (sp.2)	1	10	0	0	1	4.54	
Nevroptera (sp.)	0	0	1	10	1	4.54	
Total	10		12		22	100	

Table 4: Abundances of Ants, Dysdercus voelkeri, Synagris cornuta and Xylocopa olivacea on flowers of Abelmoschus esculentus in 2014 and 2017.

Insects	Years	Abundance of insect					Comparison of percentage
		per flower			per 1000 flowers		
		n	*m*	*s*	*m*	*s*	
Xylocopa olivacea	2014	30	1	0	525	61.71	$t = 22.83$, $df = 38$, $p<0.001$
	2017	10	1	0	65	12.01	
	Total	40	1	0	32	78.04	
Synagris cornuta	2014	5	1	0	32	0.59	$t = -8.59$, $df = 33$, $p<0.001$
	2017	30	1	0	81	12.37	
	Total	35	1	0	26	60.43	
Ants	2014	0	0	0	0	0	
	2017	30	11	0.23	1385	102.05	
	Total	30	11	0.23	11	20.75	
Dysdercus voelkeri	2014	10	1	0	29	8.62	$t = -5.29$, $df = 28$, $p<0.001$
	2017	20	1	0	54	13.07	
	Total	30	1	0	7	26.92	

Table 5: Fruiting rate, number of seed per pod and percentage of normal seeds according to different treatments of *Abelmoschus esculentus* in 2014 and 2017 at Yaoundé

Treatment	Year	Flowers	Pods	Fruiting rate (%)	Seed / Pods		Total Seeds	Normal Seeds	% normal Seeds
					m	*s*			
Unlimited visits	2014	34	31	91.17	92.24	15.53	3269	2966	91.40
Bagged flowers		31	24	77.41	78.93	14.17	2651	1345	50.73
Unlimited visits	2017	34	29	85.29	81.60	19.06	2463	2374	96.38
Bagged flowers		31	21	67.74	61.70	11.18	1261	1150	91.19

Fig 1: Plants of *Abelmoschus esculentus* showing flowers isolated from insects

4. Discussion

Ants, *S. cornuta*, *D. voelkeri* and *X. olivacea* were the main floral visitor of *A. esculentus* during the observation period. The significant difference observed between the percentages of visits of those flowering insects studied could be attributed to the variation of environmental factors in experimental site and the needs of those insects. The temperature and hygrometry positively influenced the insect activity flowers; foraging insects preferred sunny days for good floral activity [11]. The abundance of Ants, *S. cornuta, D. voelkeri* and *X. olivacea* on 1000 flowers and the positive and highly significant correlation between the numbers of *A. esculentus* flowers indicated the availability and the good attractiveness of floral products of *A. esculentus*. During each of the two flowering periods of *A. esculentus*, those insects' intensely and regularly harvested nectar or pollen. This could be attributed to the needs of individual insect species [6]. From this research, we observed that insect's pollinators can provide benefits to pollination management of *A. esculentus*. 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 *A. esculentus* was found and in Cameroon [5] which showed that *A. esculentus* 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 [4]. 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 *A. esculentus* 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 *A. esculentus* fields should be recommended for the increase of pods and seed yields of this valuable crop.

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