

Pollination And Yield Attributes Of (Cowpea) *Vigna Unguiculata* L. Walp. (Fabaceae) As Influenced By The Foraging Activity Of *Apis Mellifera* (Hymenoptera: Apidae) At Malang (Ngaoundere, Cameroon)

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Abstract—To determine the apicultural value of *Vigna unguiculata* (L.) Walp. (Fabaceae) and evaluate the *Apis mellifera adansonii* Latreille (Hymenoptera: Apidae) activity on its pod and seed yields, the bee foraging and pollinating activities were studied in Malang. The experiment was carried out on 240 flowers differentiated in two lots, based on the protection/or not of plant inflorescences against insect visits. The bee's seasonal rhythm of activity, its foraging behaviour on flowers, the fructification rate, the number of seeds/pod, the percentage of normal seeds/pod, and the pod length were evaluated. Results show that *A. mellifera* foraged on plants throughout the whole blooming period. Worker bees intensively and preferably harvested nectar. These findings allow the classification of *V. unguiculata* as a highly nectariferous bee plant. The number and dry weight of seed/pod, the pod length and the percentage of normal seeds/pod from unprotected flowers were significantly higher than those of flowers protected from insects. The fructification rates were 71.66 and 59.16%, while the percentages of healthy seeds were 88.46 and 78.70%, respectively in unprotected and protected

inflorescences. The installation of *A. mellifera* colonies close to *V. unguiculata* field. *Vigna* could be recommended to improve its pods and seeds production in the region.

Keywords—*Apis mellifera adansonii*, *unguiculata*, bee plant, foraging, pollination, increased yield.

I-INTRODUCTION

Pollination of crops benefit food increase through yield quantity and/or quality [1–4]. Globally, pollinators are responsible for pollinating approximately 30,000 plant species [5]. Among these plant species, 75% are crops that benefit directly or indirectly from the ecosystem service provided by pollinators [1]. Nowadays, the abundance and diversity of insect pollinators have been widely documented [6]. *Apis mellifera* is the most important for the highest quantity of honey production they account for [7]. This insect visits flowers of various plant species where it use to collect nectar or pollen (Louveaux, 1984). Nectar is used to make honey. Pollen and honey are stored in cells built by bee workers [8]. These stored products

are sampled by human for nutritional and therapeutic needs [9]. Several vegetable crop species including cowpea, *Vigna unguiculata* (L.) Walp., have been reported to be attractive to pollinators [19]. Cowpea (blackeye pea), is an important vegetable crop grown widely by small- edium scale growers and also on large commercial scale in the southern USA [20]. The crop is utilized both as a vegetable crop and a dry bean [21]. Increased yield was attributed to benefits derived from intercropping (improve soil fertility, control of some pests and favorable environment for beneficial insects) and not to those associated with floral resources of cowpea. Cowpea floral resources, especially nectar, are attractive to pollinators [26]. Information on the successful use of cowpea as an intercrop for the purpose of enhancing pollinator activity and increase crop production is lacking. To have a better knowledge of the diversity and abundance of pollinators associated with the cowpea we conducted field experiments with the main objective to identify the pollinator diversity associated with the studied variety of Cowpea.

II. MATERIAL AND METHODS

A. MATERIAL

A.1 STUDY SITE

The experiment was carried out from 10th May to 09th August 2018 and 2019 at Malang (latitude 07°39.26'N, longitude 13°43.94'E and altitude 1206 m above sea level), Ngaoundéré III Subdivision, Vina Division, Adamaoua Region in Cameroon.

The site belongs to the high altitude Guinean savannah agroecological zone [15]. The climate is characterized by a rainy season (April to October) and dry or season (November to March), with an annual rainfall of approximately 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70 % [15]. The vegetation is represented by crops, ornamental, hedge and native plants of savannah and gallery forests.

A.2 BIOLOGICAL MATERIALS

The plant material was *V. unguiculata* seeds were provided by the Institute of Research for Agricultural Development (IRAD) at Garoua (Fig 1).

The animal material was mainly represented by insects naturally present in the environment, *X. olivacea* digs its nest in the trunks of the trees and 15 colonies of *A. mellifera* Linnaeus (Hymenoptera: Apidae).

B. Methods

B.1 Sowing and weeding

From April 15th to May 6th 2019, the experimental plot was delimited, ploughed and divided into nine subplots, each measuring 4*3 m². On May 10th 2019, sowing was done on five lines per subplot, each of which had eight holes per line. Five seeds were sown per hole. Holes were separated 50 cm from each

other, while lines were 75 cm apart [9]. From germination to the blooming, the field was regularly weeded with hoe and was performed manually as necessary to keep plots weed-free until the maturation of pods. A week after germination, the plants were thinned and only two were left per hole.

B.2 Determination of the reproduction mode of *Vigna unguiculata*

On July 25th 2013, 240 flowers at bud stage were labeled and divided in two treatments: 120 unprotected flowers (treatment 1) and 120 bagged flowers using gauze bags net to avoid all visits (treatment 2) [16]. Similarly, on July 31th 2014, 240 females flowers at the budding stage were labeled of which 120 were left unprotected (treatment 4), while 120 were bagged (treatment 5). For each cropping year, a week after shedding of the last labeled flower, the number of pods was assessed in each treatment. The podding index (*Ifr*) was then calculated as described by [16] :

$Ifr = (Na / Nf)$, where *Na* is the number of pods formed and *Nf* the number of viable flowers initially borne.

For each study season, the difference between the podding indexes in the treatments for flowers left in free pollination and that in the treatment for flowers protected from insects made it possible to assess the rates of allogamy (*TC*) and autogamy (*TA*) according to the following formulas [17] :

- $TC = \{[(IfrX - IfrY) / IfrX] * 100\}$, where *Ifr X* and *Ifr Y* are the fruiting indices in treatments *X* (flowers in free pollination) and *Y* (protected flowers) ;

- $TA = [100 - TC]$.

B.3 Study of the activities of *Apis mellifera* on *Vigna unguiculata* flowers

Observations were conducted on flowers of treatments 1, every day, from 26th July to 31th September 2018 and 2019. During each observation day, before starting visit counts, the number of open flowers in each treatment was counted. Data were taken according to four daily time frames : 8 - 9 am, 10 - 11 am, 12 - 13 pm and 14 - 15 pm. In a slow walk along all labeled flowers of treatments 1 and 4, the identity of insects that visited *V. unguiculata* flowers was recorded [16].

All insects encountered on flowers were registered [18] and the cumulated results expressed as the number of visits to determine the relative frequency of each insect species in anthophilous entomofauna of *V. unguiculata* [19]. Data obtained were used to determine the frequency of visits (*Fi*) of each insect species on *V. unguiculata* flowers. For study period :

$Fi = [(Vi / Vt) * 100]$, with *Vi* the number of visits of insect *i* on treatment with unprotected flowers and *Vt* the total number of insect visits of all recorded insect species on these flowers [16]. Specimens (3 to 5) for all insect taxa, excluded *A. mellifera* were caught

using insect net on unlabeled flowers and conserved in 70 % ethanol, excluding butterflies that were preserved dry [20] for subsequent taxonomic identification.

B.4. Evaluation of the apicultural value of *Vigna unguiculata*

The apicultural value of *V. unguiculata* was assessed as in other plant species [38], using data on flowering intensity and the attractiveness of *A. mellifera* workers with respect to nectar and pollen.

B.5. Duration of visits and foraging speed

During the same days as for the frequency of visits, the duration of individual flower visits was recorded (using stopwatch) according to six daily time frames: 7 - 8 am, 9 - 10 am, 11 - 12 am, 13 - 14 pm and 15 - 16 pm. Moreover, the number of visits during which the bee came into contact with the stigma [22] was registered. Regarding the foraging speed (F_s) which is the number of flowers visited by an individual bee per minute [22], data were registered during the same dates and according to same time frames and daily period as for duration of visits. The stopwatch, previously set to zero was switched on as soon as an individual landed on a flower and the number of visited, flowers was concomitantly counted. The stopwatch was stopped as soon as the visitor was lost to sight or when it left *V. unguiculata* flower for another plant species. The foraging speed (F_s) was calculated using the following formula :

$F_s = (Nf / d_v) * 60$, where d_v is the time (sec) given by a stopwatch and Nf the number of flowers visited during d_v .

During the observation, when a forager returns to previously visited flower, counting is performed as two different flowers [18].

B.6. Abundances per flower and per 1000 flowers

The abundances of foragers (highest numbers of individuals foraging simultaneously) per flower and per 1000 flowers (A_{1000}) were recorded on the same dates and daily time frames as for the registration of duration of visits. Abundance per flower was recorded as a result of direct counting. For determining the abundance per 1000 flowers, foragers were counted on a known number of opened flowers and A_{1000} was calculated using the following formula :

$A_{1000} = [(Ax / Fx) * 1000]$, where Fx and Ax are respectively the number of flowers and the number of foragers effectively counted on these flowers at time x [16].

B.7. Foraging ecology

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *V. unguiculata* was assessed by direct observations [18]. For the second parameter, the number of times that the Apidae left *V.*

unguiculata flowers to other plant species and vice versa was noted through the investigation period [18].

During each daily period of investigation, ambient temperature and relative humidity in the station were registered every 30 minutes using a mobile thermohygrometer (HT-9227) [18] installed in the shade.

B.8. Evaluation of the impact of the flowering insects including *Apis mellifera* on *Vigna unguiculata* yields

Parallel to the constitution of treatments 1 and 2, 120 flowers at bud stage were protected using gauze bag nets to prevent insect visits and destined to receive one visit of *A. mellifera*. As soon as the flowers were opened, each flower of treatments 3 were inspected. Hence, gauze bag was delicately removed and this flower was observed for up to 10 minutes; the flowers visited by *A. mellifera* were marked and then reprotected. At maturity, pods were harvested and counted from each treatment. The mean number of seeds per pod and percentage of normal (well developed) seeds [23] were then evaluated.

The estimation of the effect of insects including *A. mellifera* on *V. unguiculata* production was based on the impact of flowering insects on pollination, the impact of pollination on *V. unguiculata* podding and the comparison of yields (podding rate, number of seeds per pod and percentage of normal seeds) of treatments 1 and 2. For each observations year, the podding rate due to the flowering insects including *A. mellifera* (Pr) was calculated using the following formula :

$Pr = \{[(PX - PY) / PX] * 100\}$, With PX the podding rate in treatments X (flowers left in free pollination) and PY , the podding rate in treatments Y (flowers protected from all insect visits). The podding rate of a treatment (Pr) is :

$Pr = [(b / a) * 100]$, where a is the number of viable flowers initially set and b the number of formed pods [16]. The impact of flower visiting insects including *A. mellifera* on the number of seeds per pod and the percentage of normal seeds were evaluated using the same method as mentioned above for the podding rate.

B.9. Assessment of the pollination efficiency of *Xylocopa olivacea* on *Vigna unguiculata*

The contribution of *A. mellifera* on the podding rate, the number of seeds per pod and the percentage of normal seeds was calculated using the data of treatments 2 and 3. For each observation year, the contribution of *A. mellifera* on the podding rate (PrX) was calculated using the following formula :

$PrX = \{[(PY - PZ) / PY] * 100\}$, where PY is the podding rate in treatment Y (flowers visited exclusively by *A. mellifera*) and PZ the podding rate in treatment Z (protected flowers to avoid all visits) [23]. The impact of *A. mellifera* on the number of seeds per pod

and the percentage of normal seeds were evaluated using the same method as mentioned above for the podding rate.

B.10. Data analysis

Data were analyzed using descriptive statistics (means, standard deviation and percentages), ANOVA (*F*) for the general comparison of means of more than two samples, student's *t*-test for the comparison of means of two samples, Pearson correlation coefficient (*r*) for the study of the association between two variables and chi-square (χ^2) for the comparison of percentages and using Microsoft Excel 2010 software.

III. RESULTS

A-Reproduction mode of *Vigna unguiculata*

The fruiting indexes of *V. unguiculata* for treatments 1 and 2 were 0.62 and 0.57 respectively. Thus, the autogamy rate was 91.22%; whereas the allogamy rate was 8.77%. It appears that the variety of *V. unguiculata* used in our experiments has a mixed reproduction regime that is autogamous-allogamous, with the predominance of autogamy over.

B-Place of *Apis mellifera* in the Flower Entomofauna of *Vigna unguiculata*

Amongst the 480 and 507 insect visits of six species recorded on *V. unguiculata* *A. mellifera* ranked first accounting for 205 (42.60%) and 216 (42.60%) of all visits in 2018 and 2019 respectively (Table 1).

Table 1: Insects registered on *Vigna unguiculata* flowers in 2018 and 2019, number and percentage of visits of different insects.

Insects			2018		2019	
Order	Family	Genus and species	n	P (%)	n	P (%)
Hymenoptera	Apidae	<i>Apis mellifera</i> (ne)	205	42,70	216	42.60
		<i>Xylocopa olivacea</i> (ne)	74	15,41	86	16.96
		<i>Amegilla</i> sp. 2 (ne)	34	7,08	61	12.03
		Total Hymenoptera	313	65,20	366	72.18
		<i>Eurema eximia</i> (ne)	66	13,75	41	8.08
		<i>Graphilum angolanus</i> (ne)	45	9,375	55	10.84
		<i>Cotopsilia florella</i> (ne)	56	11,66	45	8.87
		Total Pieridae	167	34,79	141	27.81
Total		480	100	507	100	

n : number of visits on 120 flowers in six days ; sp. : undetermined species ; P : percentages of visits : $P_1 = (n_1/480)*100$; $P_2 = (n_2/507)*100$; ne : nectar collection.

C. Activity of *Apis mellifera* on *Vigna unguiculata* flowers

On *V. unguiculata* flowers, individuals of *A. mellifera* were seen intensively collected the nectar. No pollen harvest visit was observed.

Apis mellifera visits were numerous on *V. unguiculata* plants when the number of opened flowers was highest (Figure 2). Furthermore, we found a positive and highly significant correlation between the number of *A. mellifera* visits and the number of *V. unguiculata* opened flowers in 2018 and 2019 ($r = 0.99$; $df = 4$; $P < 0.01$).

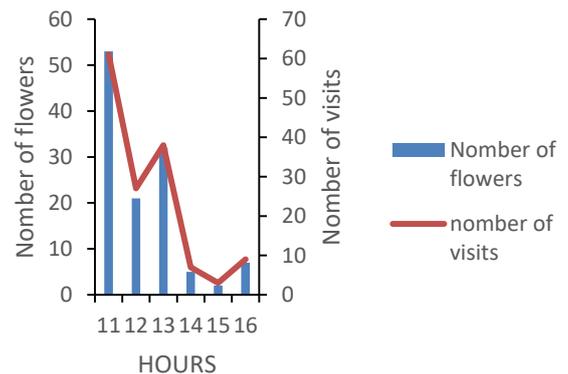


Figure 2. Seasonal variation of the number of *Vigna unguiculata* opened florets and the number of *Apis mellifera* visits on these organs in 2018 and 2019 at Malang

Apis mellifera was active on *V. unguiculata* flowers from 8 h to 15 h in 2019, with a peak of visits between 10 h and 11 h (Figure 3). This daily period probably correspond to that of the highest availability of nectar on flowers of this Fabaceae. Indeed, the period of daily activity of many flowering insects on a given plant species depends on the availability of pollen [24]. or nectar [25]. in its flowers.

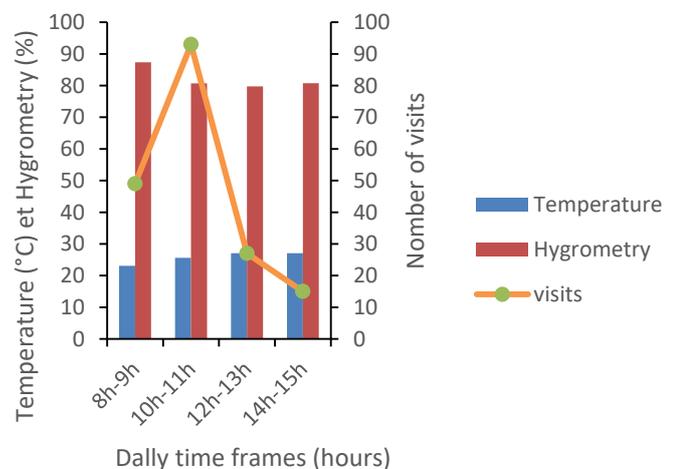


Figure 3: Variations of the temperature, the hygrometry and the number of *Apis mellifera* visits on the flowers of *V. unguiculata* according to the daily time frames in 2018 and 2019 at Malang

This activity could also be conditioned by some climatic factors (Abrol, 1988). In 2019, the correlation was not significant between the number of *A. mellifera* visits and the temperature ($r = 0.4$; $df = 4$; $P > 0.05$) and between the same number of visits and the relative humidity ($r = - 0.60$; $df = 4$; $P > 0.05$) (Figure 3).

In 2019, the highest mean number of *A. mellifera* individuals simultaneous in activity was 1/flowers ($n = 58$; $s = 0$) and 196.44 per 1000 flowers ($n = 58$; $s = 71.79$; $min = 55.56$; $max = 380.28$). The high abundance of *A. mellifera* individuals per 1000 flowers, and the positive and significant correlation between the number of *V. unguiculata* flowers and the number of *A. mellifera* visits, highlight the attractiveness of *V. unguiculata* nectar for *A. mellifera*. This could be explained by the availability and the accessibility of this floral product.

The mean duration of *A. mellifera* visit per *V. unguiculata* flowers was 6.59 sec ($n = 688$; $s = 5.93$) in 2019.

The mean foraging speed was 9.97 flowers per minute ($n = 89$; $s = 4.66$; $min = 2.5$; $max = 30$) in 2019.

D. Floral substances harvested

From our field observations, *A. mellifera* workers were found to harvest exclusively nectar on *V. unguiculata* flowers (Table 1).

E. Impact of flowering insects including *Apis mellifera* on *Vigna unguiculata* Production

During pollen and nectar harvest from *V. unguiculata*, foraging insects were always in contact with the anthers and stigma. Thus these insects increased the pollination possibilities of this plant species. Table 2 presents the results on the podding 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 pod and seed yields from flowers of plants visited by insects (treatment 1) was higher than that of flowers protected from insects (treatment 2);

b) The differences observed between the three podding rates were highly significant ($\chi^2_{global} = 37.99$; $df = 3$; $P < 0.001$). The difference between the podding rates of treatments 1 and 2 ($\chi^2 = 14.52$; $df = 1$; $P < 0,001$) ; and between the podding rates of treatments 2 and 3 ($\chi^2 = 23.44$; $df = 1$; $P < 0.001$) were highly significant and is not significant between treatments 1 and 3 ($\chi^2 = 0.65$; $df = 1$; $P > 0,05$). Consequently, the podding rate of protected flowers was highly from that of flowers bagged during their flowering period. The mean podding rate due to the action of flowering insects was 61.62%.

c) The differences observed between the three mean number of seeds were highly significant ($F = 10.93$; $df1 = 3$; $df2 = 360$; $P < 0.001$). The difference between the mean number of seeds of treatments 1 and 2: ($t = 3.98$; $df = 156$; $P < 0.001$) and between the mean number of seeds of treatments 2 and 3 ($t = 6.25$; $df = 186$; $P < 0.001$) were highly significant and is not significant between treatments 1 and 3 ($t = 1.78$; $df = 214$; $P > 0.05$). Consequently, the number of seeds per pod of exposed flowers was higher than that of protected flowers. The mean number of seeds per pod attributed to the activity of flowering insects was 16.23%.

d) The differences observed between the four percentage of normal seeds indicate were highly significant ($\chi^2_{global} = 66.49$ $df = 3$, $P < 0,001$). The difference between the percentage of normal seeds of treatments 1 and 2: ($\chi^2 = 3.91$; $df = 1$, $P < 0.05$) and between the percentage of normal seeds of treatments 1 and 2 ($\chi^2 = 8.79$; $df = 1$, $P < 0.01$) were not significant and is highly significant between the percentage of normal seeds of treatments 2 and 3 ($\chi^2 = 24.20$; $df = 1$, $P < 0,001$). Thus, the percentage of normal seeds in opened flowers was higher than that of protected flowers. The percentage of the normal seeds due to the action of insects was 14.74%.

The mean numeric contribution of pollinating insects to the fruiting rate, the mean number of seeds per pod and the percentage of normal seeds were respectively 11.70%, 17.07% and 5.37%. The impact of pollinating insects on pod and seed yields was positive and significant.

Table 2: Fruiting rate, number of seed per pod and percentage of normal seeds according to different treatments of *Vigna unguiculata* in 2018 and 2019 at Malang

Traitment	flowers	pod	podding rate	Seeds / pod			Total seeds	Normal seeds	% Normal seeds
				n	m	s			
1(Unprotected flowers)	120	86	71.66	86	3.26	4,30	130	115	88,46
2 (Bagged flowers)	120	71	59.16	71	3.86	4.26	155	112	78,70
3 (<i>Apis mellifera</i>)	120	79	65.83	79	3.7	4.37	148	138	93,24

Comparison of the podding rates: $\chi^2_{global} = 37.99$ ($df = 3$, $P < 0.001$) ; T1 / T2 : $\chi^2 = 14.52$ ($df = 1$, $P < 0.001$); T1 / T3 : $\chi^2 = 0.65$ ($df = 1$, $P > 0.05$); T2 / T3 : $\chi^2 = 23.44$ ($df = 1$, $P < 0.001$).

Comparison of the mean number of the seeds per pod: $F = 10.93$ ($df1 = 3$, $df2 = 360$; $P < 0.001$); T1 / T2 : $t = 3.98$ ($df = 156$, $P < 0.001$) ; T1 / T3 : $t = 1.78$ ($df = 214$, $P > 0.05$) ; 2 / 3 : $t = 6.25$ ($df = 186$, $P < 0.001$).

Comparison of the percentage of normal seeds: $\chi^2_{global} = 66.49$ ($df = 3$, $P < 0.001$); T1 / T2 : $\chi^2 = 3.91$ ($df = 1$, $P < 0.05$); T1 / T3 : $\chi^2 = 8.79$ ($df = 1$, $P < 0.01$) T2 / T3 : $\chi^2 = 24.20$ ($df = 1$, $P < 0.001$).

F. Pollination Efficiency of *Apis mellifera* on *Vigna unguiculata*

During pollen and nectar harvests on the cotton flowers, individuals of *A. mellifera* always came into contact with anthers and stigma, increasing the possibilities of *V. unguiculata* pollination. With this pollen, they flew frequently from flowers to flowers inside the same or different individuals of *V. unguiculata*.



Figure : *Apis mellifera* collecting nectar on a flower of *Vigna unguiculata*

IV-DISCUSSION

Activity of Apis mellifera on Vigna unguiculata flowers Results indicate that in Malang, *Apis mellifera* was the main floral visitor of *V. unguiculata* during the observation period. This bee has been reported as the main floral visitor of this plant in Benin [26]. and in Cameroon [27-29]. *Apis mellifera* was also shown to be the most abundant floral visitors of other Fabaceae members such as *Glycine max* in Douala [30]. *Phaseolus vulgaris* in Ngaoundéré [31]. and in Maroua [32]. The peak of the activity of *A. mellifera* on *V. unguiculata* flowers was located between 9 and 10 am, which correlated with the highest availability period of nectar on *V. unguiculata* flowers.

The weak frequency of the visit of *X. olivacea* on *V. unguiculata* flowers compare to that of *A. mellifera* could be explained by the strategies adopted by the honey bee that consist of recruiting a great number of workers for the exploitation of an interesting food source [33]. Besides, during our investigations, the number of honeybee colonies (35) in the study site was much higher than that of *X. olivacea* (7). Other researches have revealed *A. mellifera* among most frequent insects on *Croton macrostachyus* [34], *Physalis minima* [35], *Vigna unguiculata* [36] and *Helianthus annuus* [37].

The strong attractivity of the nectar of this Fabaceae with respect to *A. mellifera* could be partly explained by the availability and the quality of this food as well as the best time to harvest it at the level of the flowers [33].

Our results are not in line with those obtained with the *X. olivacea* by [38]. on the same plant in Ngaoundere. According to these authors, *X. olivacea* had a peak of activity situated between 8 and 9 am

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