Foraging and pollination effectiveness of *Apis mellifera* (Hymenoptera: Apidae) on *Vigna unguiculata* (L.) Walp. 1843 (Fabaceae) in Yaounde-Cameroon

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Abstract
Experiments were made in Yaoundé to determine the effects of *Apis mellifera* visit on the pollination and the productivity of cowpea, *Vigna unguiculata*. Two treatments for each experiment were used on 40 randomly-selected plants each. These included Autonomous Self-Pollination (ASP) with flowers from which insects visit, with airborne pollen flow excluded (treatments 1 and 3), flowers that received a single bee visit (SBV) of *A. mellifera* (treatments 2 and 4). This honey bee mainly foraged for nectar and pollen resources. The mean foraging speed was 11.55 flowers/min and the duration of visits was 10.94 sec to collect nectar and pollen. All flowers produced pod with or without insect visits. *Apis melliferai* was effective pollinator, and of course their visits increased pod production (fruiting rate), number of seeds (number of seeds/pod) and normal seed (percentage of normal seed). *Apis mellifera* foraging resulted in a significant increment of the fruiting rate by 31.18 %, as well as the number of seeds/pod by 29.97 % and the percentage of normal seeds by 15.46 % in the two years in Yaounde. Conservation of *A. mellifera* colonies close to *V. unguiculata* fields could be recommended to increase pod and seed production in the region.

Keywords: *Vigna unguiculata*, flowers, *Apis mellifera*, pollination, yields

Introduction

Cowpea (*Vigna unguiculata*) is of vital importance to the livelihood of millions of people in West and Central Africa (Adeyanju *et al*., 2012) [1]. From its production, rural families derive food, animal feed and cash income. It provides nutritious grain and an inexpensive source of protein for both rural poor and urban consumers. Cowpea grain contains about 25% proteins and 64% carbohydrate (Ntoukam *et al*., 1993) [14] and therefore has a tremendous potential to contribute to the alleviation of malnutrition among resource-poor farmers (Adeyanju *et al.*, 2012) [1]. Cowpea has several advantages. The crop is cultivated in the semi-arid regions of lowland tropics and sub-tropics where the soil is poor in fertility and rainfall is scanty (Mortimore *et al*., 1997) [14]. Cowpea is shade provides ground cover, conserves moisture, suppresses weeds, provides protection against soil erosion (Quin, 1997) [22]. Tender leaves and green pods are used as vegetables while, haulms are used as fodder for cattle. Seeds are boiled and eaten with rice or alone, and can be processed and eaten as moi-moi (steamed paste) and bean cake (fried paste: Mohammed *et al*., 2010) [13]. The showy papillionaceous flowers vary in colour from white, cream, yellow or light to dark purple and in different combinations. The pistil and stamens are enclosed within the keel and this enhances self-pollination. Usually pollination occurs in cowpea flowers before they open (Blackhurst and Miller, 1980) [4]. The style, which is bent and hairy towards the stigma, is surrounded by the filaments with attached anthers located close to the stigma. Generally, flowers of cultivated cowpea open as early as 6.30 a.m. and are closed by 12.00 noon same day (Ige *et al.*, 2011) [10]. When days are hot and dry the flowers close earlier than during the humid rainy season (Egho, 2011) [8]. The stigmas are receptive over a short period of time (Tchuenguem *et al*., 2009) [25]. There are extrafloral nectaries at the base of the corolla (Gopinathan and Babu, 1987) [9] and intrafloral nectaries at the base of ovaries (Ojehomon, 1968) [16], which attract insects to cowpea flowers. Some of the insects such as the bumble and honey bees are possibly attracted to the flowers by the nectaries (Ige *et al.*, 2011) [10]. Pollen grains of cowpea are heavy and sticky and could not be readily carried by the wind (Ige *et al.*, 2011) [10]. Insects are therefore mostly responsible for cross-pollination in cowpea. In some parts of Brazil cowpea grain yield had been reduced due to the widespread use of insecticides, which kept away the pollinating insects (Vaz *et al*., 1998; Djonwangwé *et al*., 2017) [27, 6]. Only a heavy insect such as the bumble bee is capable of depressing the wings of cowpea flowers thereby allowing the stamens and stigma to be pushed out of the corolla, a process that facilitates pollination (Asiwe, 2011; Pando *et al.*, 2014) [2, 18].

In Cameroon, the quantity of *V. unguiculata* available to consumers is very low (130101 tones/years for 115374 hectares: MINADER, 2010) [12], the demand for cowpea seeds is high (184744 tones/years: DSCE, 2009) [7], and its pod and seed yields are low (MINADER, 2010) [12]. It is therefore important to investigate how the production of this plant could be increased in the country. The studies by Tchuenguem *et al.* (2009) [25] have shown that at Ngaoundéré, *A. mellifera* visits *V. unguiculata* flowers to harvest both nectar and pollen and increases its pollination possibilities. There has been no previous research reported on the pollination efficiency of *A. mellifera* on *V. unguiculata* flowers. This study was carried out to assess the foraging activity of *A. mellifera* foragers on *V. unguiculata* flowers during a longer rainy season and the effects of pollination efficiency of *A. mellifera* on yields of the Fabaceae. During preliminary investigations on flower–insect relationships in Yaoundé before 2008 (unpubl. data), *A. mellifera* have been seen intensively visiting flowers of *V. unguiculata*. This bee can be managed for pollination (Tchuenguem *et al*., 2009) [25].

Materials and methods

The experiment was carried out twice, first from August to November 2008 and then September to December 2009 at Nkolbisson (3°51.800′N, 11°27.450′E, 726 m above sea level), Yaoundé, central Cameroon. This region belongs to the forest agroecological zone, with bimodal rainfall pattern. The climate is of the Guinea type, characterized by four seasons: a brief rainy season (March to June), a short dry season (July to August), a longer rainy season (September to November) and a more extended dry season (November to March). The annual rainfall varies from 1500 to 2000 mm. The average annual temperature is 25°C, while the mean annual relative humidity is 75%. The experimental plot was 26 x 16 m where in seeds of *V. unguiculata* purchased from the local Bafia seed outlets were planted. Two Kenya Top Bar Hives (KTBH) with *Apis mellifera* Latreille (Hymenoptera: Apidae) colonies were installed close to the experimental plot (Figure 1).

Sowing and weeding

On the 21 August 2008 and 20 September 2009, the experimental plot was divided into four subplots (10 x 5 m each). The sowing was done on ten lines per subplot, each line with ten holes and in each hole, three seeds were placed. The space was 75 cm between holes and 75 cm between lines. Weeding was performed manually as necessary to maintain weed-free plots. Direct observations on flowers were made daily during 20 days of the blooming period and between 7:00 and 11:00 am (local time) since preliminary observations indicated that cowpea flowers were fully visited by *A. mellifera* between 6.00 and 15.00 am (unpubl. data). At least, ten specimens of *A. mellifera* were captured with the pliers and were conserved in a box containing 70% of ethanol for future taxonomy.

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

Floral products harvested

The floral products (nectar or pollen) harvested by *A. mellifera* during each floral visit were registered based on its foraging behavior. Nectar foragers were seen extending their proboscises to the base of the corolla while pollen gatherers scratched anthers with the mandibles or the legs. During the same time that *A. mellifera* encounters on flowers were registered, we noted the type of floral products collected by this bee. This parameter was measured to determine if *A. mellifera* is strictly a pollenivore, nectarivore or pollenivore and netarivore. This could give an idea of its implication as a cross-pollinator of *V. unguiculata*.

Duration of visits and foraging speed

During the same days as for the registration of visits, the duration of the individual flower visits was recorded (using a stopwatch) at least three times: 7.00–8.00 hours, 9.00–10.00 hours, 11.00-12.00 hours and 13.00–14.00 hours. Moreover, the foraging speed, according to Jacob-Remacle (1989) [11], is the number of flowers visited by a bee per min. According to Tchuenguem *et al.* (2004) [24], the foraging speed could be calculated by this formula: $Vb = (Fi / di) \times 60$ where di is the time (s) given by a stopwatch and Fi is the number of flowers visited during di.

Assessment of the pollination efficiency of *Apis mellifera* on *Vigna unguiculata*

To assess of the pollination efficiency of *A. mellifera* for each experiment, two treatments were done: (1) Autonomous self-pollination (ASP) in which flower buds were isolated with hydrophilic bags (Figure 2) (12 × 16 cm; Osmolux®, Pantek France, Montesson) a day before anthesis to prevent anthophilous insect visitation and airborne pollen flow the following day (2008: treatments 1 and 2009: treatment 3). These bags were removed on the day following anthesis. (2) Single bee visit (SBV) in which flower buds were isolated (2008: treatment 2 and 2009: treatment 4). Between 7.00 hours and 11.00 hours of each observation date, the hydrophilic bags were delicately removed from each inflorescence carrying new opened flowers and this inflorescence observed for up to 20 min by four-man observer team was positioned in the study field. The flowers visited by *A. mellifera* were marked and the new opened flowers that were not visited were eliminated. Each flower was monitored until it received a single visit by *A. mellifera*.

After *A. mellifera* visit, the flower was bagged with a hydrophilic plastic bag (12 × 16 cm) until the next day to avoid any additional insect visitation (Vaissière *et al*., 1996) [26], after which the flower and the equivalent plant were also tagged. At maturity, pods were harvested from each treatment and the number of seeds per pod counted. The mean number of seeds per pod and the percentage of normal seeds (well-developed seeds) were then calculated for each treatment. The fruiting rate due to the influence of foraging *A. mellifera* (*Fri*) was calculated by the formula:

$$Fri = \{[(Fr_Y - Fr_X) / Fr_Y] \times 100\},$$

where Fr_Y and Fr_X were the fruiting rate in treatment Y (protected flowers and visited exclusively by *A. mellifera*) and treatment X (protected inflorescences).

$$Fr = [(F_2 / F_1) \times 100]$$

where F_2 is the number of pods formed and F_1 the number of viable flowers initially set. The impact of flowering *A. mellifera* on seed yields was evaluated using the same method as mentioned above for fruiting rate (Pando *et al.*, 2011) [17].

During the study period, flowers of several other plant species including *Aspilia africana* (Pers.) C.D. Adams, 1956; *Mimosa pudica* (Linneaus, 1753), *Cajanus cajan* (Linnaeus) Millsp., 1900; *Senna javanica* (Linnaeus, 1753); *Phaseolus coccineus*

(Linnaeus, 1753); *Ipomoea involucrata* (P. Beauv., 1817) and *Psidium guajava* (Linnaeus, 1753) in bloom in the vicinity of the experimental plot were observed to attract *A. mellifera*.

Data were analysed using descriptive statistics, Student's *t*-test for the comparison of means of the two samples, chi-square (χ^2) for the comparison of two percentages using SPSS statistical software (SPSS Inc., Chicago, IL, US) and Microsoft Excel.

Results

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

From this study's field observations, *A. mellifera* foragers were found to collect nectar and pollen on *V. unguiculata* flowers. Nectar collection was intensive and regular (more than 81.45% of visits each year), whereas pollen collection was very low (02.61%: Table 1). Other individuals collect, during the same floral visit, both nectar and pollen (15.94%: Table 1). The activity of the honey bee observed foraging on cowpea flowers was mainly for pollen and nectar gathering base to their foraging behavior.

In 2008 and 2009, the mean duration of *A. mellifera* visits was 7.53 sec (n = 95; s = 1.07; max = 15 sec) and 7.11 sec (n = 160; s = 2.22; max = 14) for nectar harvests respectively. The difference between the duration of the visit to harvest nectar in 2008 and 2009 was not significant (t = 1.56 [df = 253; P = 0.12]). For pollen, the corresponding numbers were 4.08 sec (n = 95; s = 1.08; max = 7 sec) and 3.14 sec (n = 160; s = 1.32; max = 7 sec) in 2008 and 2009, respectively. The difference between duration of visit for pollen in 2008 and 2009 was significant (t = 5.86 [df = 253; P < 0.05]), for pollen and nectar, the corresponding values were 11.61 sec (n = 95; s = 2.09; max = 14 sec) and 10.26 sec (n = 160; s = 2.60; max = 18 sec) in 2008 and 2009, respectively. The difference between the duration of visit for pollen and nectar in 2008 and 2009 was higher significant (t = 4.31 [df = 253; P < 0.01]), The mean duration of *A. mellifera* visits per *V. unguiculata* flower varied significantly according to the type of food harvested (t_{2008} = -16.66 [df = 253, P < 0.001]; t_{2009} = -19.41 [df = 253, P < 0.001]).

During the observation period, flowers of many other plant species growing near *V. unguiculata* were visited by *A. mellifera* for nectar (N) and/or pollen (P). Among these plants were *Aspilia africana* (Asteraceae; N and P), *Mimosa pudica* (Mimosaceae, N and P), *Cajanus cajan*, *Senna javanica* and *Phaseolus coccineus* (Fabaceae; N and P) *Ipomoea involucrata* (Convovulaceae, N) and *Psidium guajava* (Myrtaceae; P). During one foraging trip, an individual bees foraging on *V. unguicuilata* was not observed moving from *V. unguiculata* to the neighbouring plant and vice versa. On the experimental plot of *V. unguiculata*, *A. mellifera* visited between two and 24 flowers/min in 2008 and between two and 23 flowers/min in 2009. The mean foraging speed was 10.78 flowers/min (n = 96; s = 4.71) in 2008 and 12.31 flowers/min (n = 112; s = 3.99) in 2009, which was significantly different (t = -2.57 [df = 206; P < 0.001]).

Pollination efficiency of *Apis mellifera* on *Vigna unguiculata*

During the collection of pollen or nectar on flowers (Figure 3), foragers regularly contacted anthers, stigma and carried pollen. With this pollen, they flew frequently from flower to flower. The percentage of the total number of visits during which forager bees came into contact with the stigma of the visited flowers was 100.00 % for pollen collect and 57.80 % for nectar harvest (Table 2). Thus *A. mellifera* greatly increased the pollination possibilities of *V. unguiculata* flowers. Table 3 shows the fruiting rate, the mean number of seeds per pod and the percentage of normal seed in treatments 1 and 3 (ASP) and treatments 2 and 4 (SBV). It appeared from this table that each flower turned into a pod, regardless of the treatment it received.

- The fruiting rate ranged from 69.00 % in treatment 1 to 92.00 % in treatment 2 in 2008 and from 57.00 % in treatment 3 to 91.00 % in treatment 4 in 2009. The comparison of the fruiting rate showed that the differences observed were highly significant between treatments 1 and 2 (χ^2 = 16.85 [df = 1; P < 0.001]), treatments 3 and 4 (χ^2 = 30.04 [df = 1; P < 0.001]) and not significant between treatments 2 and 4 (χ^2 = 0.06 [df = 1; P > 0.05]). Consequently, the fruiting rate of flowers bagged and visited exclusively by *A. mellifera* (treatments 2 and 4) was higher than that of flowers bagged during their flowering period (treatments 1 and 3). The percentage of the fruiting rate due to *A. mellifera* activity was 31.18 %.
- The mean number of seeds per pod ranged between 13.35, 19.64, 14.47 and 20.07, and was affected by the pollination treatment. The difference was significant between treatments 1 and 2 (t = 15.15 [df = 161; P < 0.001]), 4 and 4 (t = 26.37 [df = 148; P < 0.001]). Consequently, the number of seed yields per pod of flowers bagged and visited exclusively by *A. mellifera* (treatments 2 and 4) was higher than that of flowers bagged during their flowering period (treatments 1 and 3). The contribution of *A. mellifera* to the increment of the number of seeds per pod was 29.97 %.
- The percentage of normal seed was 81.54%, 98.28 %, 84.97 % and 98.69 % in treatments 1, 2, 3 and 4 respectively. The difference between treatments 1 and 2 (χ^2 = 250.56 [df = 1; P < 0.001]), and treatments 3 and 4 (χ^2 = 202.81 [df = 1; P < 0.001]) was highly significant and was not significant between treatments 2 and 4 (χ^2 = 0.08 [df = 1; P > 0.05]). Consequently, the percentage of normal seeds of flowers bagged and visited exclusively by *A. mellifera* (treatments 2 and 4) was higher than that of flowers bagged during their opening period (treatments 1 and 3). This may show high pollination deficit on the crop, indicating need for *A. mellifera* management to increase developed seeds. The contribution of *A. mellifera* to the increment of the percentage of normal seed attributable to the influence of *A. mellifera* was 15.46%.

Table 1: Products harvested by *Apis mellifera adansonii* on the inflorescences of *Vigna unguiculata* in 2008 and 2009

Year	Number of visits studies	Visits for nectar harvest		Visits for pollen harvest		Visits for nectar and pollen harvest	
		Number	%	Nombre	%	Nombre	%
2008	278	234	84.17	7	02.52	37	13.31
2009	525	420	80.00	14	02.67	91	17.33
Total	803	654	81.45	21	02.61	128	15.94

Table 2: Number and frequency of contacts between *Apis mellifera adansonii* and the stigma during floral visits to *Vigna unguiculata*

	November 2008			**December 2009**			**Total**		
Products harvested	Number of studied visits	Visits with stigmatic contacts		Number of studied visits	Visits with stigmatic contacts		Number of studied visits	Visits with stigmatic contacts	
		Number	%		Number	%		Number	%
Nectar	271	140	51.66	511	312	61.06	782	452	57.80
Pollen	44	44	100.00	105	105	100.00	149	149	100

Table 3: Fruiting rate, mean number of seeds yield per pod and percentage of normal seeds according to the treatments of *Vigna unguiculata*

Year	**Treatment**	**Flowers**	**Pods**	**Fruiting rate**	**Seeds/pod**		**Total seeds**	**Normal seeds**	**% normal seeds**
					Mean	**SD**			
2008	1 (Bagged flowers)	100	69	69.00	13.35	3.16	921	751	81.54
	2 *(A. m. adansonii* flowers)	100	92	92.00	19.64	1.29	1807	1776	98.28
2009	3 (Bagged flowers)	100	57	57.00	14.47	2.71	825	701	84.97
	4 (*A. m. adansonii* flowers)	100	91	91.00	20.07	1.38	1826	1802	98.69

Fig 1: *Apis mellifera* colonies were installed close to the experimental plot

Fig 2: Bagged flowers of *Vigna unguiculata*

Fig 3: *Apis mellifera* collecting on *Vigna unguiculata.*

Discussion

Our study indicates that *A. mellifera* was visited cowpea flowers to collected nectar or/and pollen. The attractiveness of *V. unguiculata* nectar could be partially explained by its reported high production and total sugar concentration (34.13–54.27 %: Tchuenguem *et al.*, 2009) [25], compared to range 15–75 % in which most of the plant species fall (Proctor *et al.*, 1996) [21]. According to Pauly *et al.* (2009) [19] in Benin, Tchuenguem *et al.* (2009) [25] in Cameroon and Ige *et al.* (2011) [10] in Nigeria, nectar produced by *V. unguiculata* attracts *A. melllifera* in natural conditions. The significant difference observed between the duration of pollen harvest visits and that of nectar collection visits could be explained by the accessibility of each of these floral products. Pollen is produced by the anthers, which are situated on the top of the stamen and are thus easily accessible to *A. melliferai*, whereas nectar is between the base of the style and stamens and is thus less accessible (Rodolphe- Edouard *et al.*, 2002) [23]. Under these conditions an individual bee must spend much more time on flowers to obtain its nectar load, compared to the time she needs for pollen load. This result confirms other findings reported by Tchuenguem *et al.* (2009) [25] in Ngaoundéré and Djonwangwé *et al.* (2017) [6] at Maroua. The present study shows that during one foraging trip, an individual bee foraging on a given plant species scarcely visited another plant species. This result indicates that *A. mellifera* shows flower constancy (Basualdo *et al.*, 2000) [3] for the flowers of each of the plant studied. The bee foragers had a high affinity with respect to *V. unguiculata* compared with the neighbouring plant species, indicating their faithfulness to this Fabaceae, a phenomenon known as “floral constancy” (Basualdo *et al*., 2000) [3]. This flower constancy could be partially due to the high sugar content of the nectar (45.98 %: Pando *et al*., 2014) [18]. Indeed, it is known that the workers of *A. mellifera* are generally constant on a plant species when the concentration in sugars of its nectar is more than 15% (Philippe, 1991) [20]. *Apis mellifera* has been previously reported as good constancy visitor as such; effective pollinators (Pauly *et al.*, 2009; Tchuenguem *et al.,* 2009; Egho, 2011) [19, 25, 8]. Flower constancy is an important aspect in management of pollination and this shows *A. mellifera* can provide the advantages of pollination management for *V. unguiculata.* Investment in *A. mellifera* management may provide high returns to investment on this crop. During the collection of nectar and pollen on each flower, *A. mellifera* regularly comes into contact with the stigma. It could enhance auto-pollination, which has been demonstrated in the past by Tchuenguem *et al.* (2009) [25] and Ige *et al.* (2011) [10] in Cameroon and Nigeria respectively. *Apis mellifera* would provide allogamous pollination through carrying of pollen within their furs, legs and mouth accessories, which is consequently deposited on another flower belonging to different plant of same species. This has also been observed by other studies (Pauly *et al*., 2009; Asiwe, 2011; Egho, 2011; Djonwangwe *et al.,* 2017) [18, 2, 8, 6]. The positive and significant contribution of *A. mellifera* in the pod and seeds yields of *V. unguiculata* is justified by the action of this forager bee on self-pollination and cross-pollination. During foraging behaviour on flowers of *V. unguiculata*, *A. mellifera* played a positive role: when collecting nectar and/or pollen, *A. mellifera* shook flowers and this movement could facilitate the liberation of pollen by anthers, for the optimal occupation of the stigma. Similar phenomenon was also reported by Pauly *et al.* (2009) [19] in Benin, Tchuenguem *et al.* (2009) [25] in Cameroon and Ige *et al.* (2011) [10] in Nigeria for this bee on cowpea. Nevertheless, the morphology of *V. unguiculata* flowers seems to avoid auto-pollination and seems to favour cross-pollination (Asiwe, 2011; Ige *et al*., 2011) [2, 10]. Pollen grains of cowpea are heavy and sticky and could not be readily transferred by wind, therefore insects are responsible for transfer of pollen grains and consequently cross pollination in cowpea plant. *Apis mellifera* has been previously reported as good pollen collectors and as such; effective pollinators (Tchuenguem *et al.,* 2004) [24]. During our investigations, the falling of pollen carried by the foragers and the deposition of this pollen on the stigma and stamens of the flowers to be visited by the action of gravity and that of wind have been observed. Such pollen losses by bees are frequent at the end of single flower or inflorescence visits, especially during the hovering flight of foragers above these organs. The most important yield (pods, seeds, percentage of normal seeds in pods) recorded in unlimited and visited flowers exclusively by *A. mellifera* can be attributed to the important role the pollinating insect. Similar result have been reported in Bénin by Pauly *et al.* (2009) [19], Cameroon by Tchuenguem *et al.* (2009) [25] and Nigeria by Ige *et al*. (2011) [10]. The flowers that were exposed exclusively to *A. mellifera* provided more pods, more seeds per pod with the heavier seeds and of better shape than the bagged flowers, in agreement to previous results reported by Tchuenguem *et al.* (2009) [25] and Ige *et al.* (2011) [10].

Conclusion

In Yaoundé-Cameroon, *V. unguiculata* benefits highly from pollination by *A. mellifera*. The comparison of pods and seeds set of protected inflorescences with that of inflorescences visited exclusively by *A. mellifera* underscores the value of this bee in increasing pod and seed set as well as seed quality. The study thus shows investment in management of *A. mellifera* in terms of Kenya Top Bar Hives (KTBH) provision at the proximity of *V. unguiculata* field is worthy while for growers. The conservation and/or the kept of KTBH with *A. mellifera* (Hymenoptera: Apidae) colonies were installed close to the *V. unguiculata* plots should be recommended for Cameroonian farmers and horticulturists to increase pods and seeds yields. Cowpea should to be cultured more in Cameroon to contribute to the food supply and to favor populations of *A. mellifera.*

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