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Foraging behaviour of *Apis mellifera adansonii* and its impact on pollination, fruit and seed yields of *Citrullus lanatus* at Nkolbisson (Yaoundé, Cameroon)

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ABSTRACT
Honeybee (*Apis mellifera adansonii* Latreille (Hymenoptera: Apidae)) foraging activity was observed to evaluate its impact on pollination, fruit and seed yields of *Citrullus lanatus* (Thunb) Mansf (Cucurbitaceae) in the area of Yaoundé (Cameroon). Two lots of 775 female flowers were marked according to the presence or the absence of a protection for insect visits. The honey bees' activity, the fruit set, the number of seeds per fruit were recorded. *A. m. adansonii* primarily foraged for nectar on *C. lanatus* throughout the whole blooming period of each plant and pollen collection was low. The mean maximum number of workers foraging at the same time was 331 ± 173 (n= 41) per 1000 flowers. The mean foraging speed was 10.20 ± 2.75 (n = 154) flowers per minute. The fruit set and the number of seeds per fruit of unprotected female flowers were significantly higher than those of female flowers protected from insects. *A. m. adansonii* pollination contributed 70.14% to the fruit set and seed yields. The conservation of *A. m. adansonii* colonies near *C. lanatus* population must be encouraged.
Key words: *Citrullus lanatus, Apis mellifera adansonii,* pollination, yields, Cameroon.

RESUME
L'activité de butinage d'*Apis mellifera adansonii* Latreille (Hymenoptera : Apidae) a été étudiée afin d'évaluer son impact sur la pollinisation, les rendements fruitiers et grainiers de *Citrullus lanatus* (Thunb) Mansf (Cucurbitaceae) à Yaoundé (Cameroun). Deux lots ont été déterminés par le marquage de 775 fleurs femelles différant selon la présence ou l'absence de protection vis-à-vis des visites d'insectes. Le rythme saisonnier d'activité des abeilles domestiques, le taux de fructification des fleurs femelles et le nombre de graines par fruit ont été évalués. *A. m. adansonii* butinait les fleurs de *C. lanatus* tout au long de la période de floraison de chaque plante. Les butineuses prélevaient intensément le nectar pendant que la collecte du pollen était réduite. Le plus grand nombre d'ouvrières butinant simultanément était de 331 ± 173 (n = 41) par 1000 fleurs. La vitesse moyenne de butinage était de 10.20 ± 2.75 (n = 154) fleurs par minute. Le taux de fructification et le nombre moyen de graines matures par fruit des fleurs non protégées de l'activité des insectes étaient significativement élevés que ceux des fleurs femelles protégées. La contribution d'*A. m. adansonii* sur les rendements fruitiers et grainiers de *C. lanatus* était de 70.14% grâce à l'action pollinisatrice des ouvrières. La conservation des colonies d'*A. m. adansonii* à côté des populations de *C. lanatus* en fleurs doit être encouragée.
Mots clés: *Citrullus lanatus*, *Apis mellifera adansonii*, pollinisation, rendements, Cameroun.

INTRODUCTION
Many insects visit flowers from which they obtain carbohydrate and protein food [1]. During their visits, they usually pollinated the host plant flowers [2, 3, 4, 5, 6, 7, 8]. The role of pollinators of many plants is well known throughout the world and their activity is essential to ecosystem functioning and agriculture [9]. In many regions, the honey bee (*Apis mellifera*) seems to be the main pollinator insect of many cultivated crops [10, 11, 12, 13, 14, 15, 16, 17, 18].

Besides their pollinating activity, honey bees provide a suite of products of a great nutritional, therapeutic and economic importance for mankind [19].
Thus, apicultural research aims to develop management programs to improve both pollination and honeybee products. At the same time others develop techniques on habitat conservation to maintain the population of natural bees [20]. Today, many bee keepers rent their bee colonies for the pollination services to crop farmers at the

same time trying to maximize honey production [21, 22].

The watermelon (*Citrullus lanatus*) originated from Africa and is widely grown throughout the tropics, sub-tropics and arid regions of the world [23]. Its fruits are consumed as dessert and are appreciated for their sweet flesh full of juice. In some parts of Africa, the seeds of watermelon are roasted and eaten [24] but are also used to produce oil [25]. Today, China is the largest world producer of watermelon and Greece is the first producer of this fruit per inhabitant with about 55kg [15]. In Cameroon, the market price varies from Fcfa 500 to Fcfa 3000 per fruit (about 0.8 to 4.6 €).

In several regions of the world, watermelon flowers are exclusively pollinated by insects with *A. mellifera* as the most efficient [15, 23]. In Cameroon, there is no information concerning the role of it endemic honeybees, *A. m. adansonii,* for *C. lanatus* pollination. In fact, the research concerning the activity of anthophilous insects on cultivated crops in this country is almost completely lacking [5]. In general, Cameroonian farmers don't know the importance of insects on the pollination of cultivated crops. They ignore that in the absence of insects during the flowering period of their crops, the fruit and seed yields can be decreased or nulls [1, 26].

The main objective of this research undertaken in Yaoundé is to contribute to the knowledge of the relationships between *C. lanatus* and its anthophilous insects, for an efficient management of this crop in Cameroon. Specific objectives were (1) the registration of the activity of *A. m. adansonii* on *C. lanatus* flowers and (2) the evaluation of its impact on the pollination and on the fruit and seed yields of this crop.

MATERIAL AND METHODS

Study site

The study was carried out in Nkolbisson, a Western suburb of Yaoundé (Cameroon) from December 2004 to February 2005 (dry season). The natural vegetation of Yaoundé is very disturbed at some sites because of urbanisation. It belongs to the semi deciduous forest domain. The relief is characterised by the presence of many hills, which modify considerably the climate. The climate here is Guinean type including four seasons with two differently dry and rainy seasons: the brief rainy season (March to June) is followed by the short dry season (July to August) and the longer rainy season (September to November) is followed by the more extended dry season (November to March). Rainfall varies from 1400 to 2000 mm per year and average temperature is about 25°C.

Experimental design

We delimited 2500 m^2 experimental plots in a more than two years old fallow in the year 2004. After clearing and ploughing of the experimental area before November 15, sugar baby variety of watermelon was planted with 2 seeds per hole on November 21. The space between two holes was 2 m on rows and 1.5 m between rows. Watering was done daily, from 18th November 2004 to 13th January 2005, with one litre of water measured per hole, at the dawn and at the twilight.

Two weeks after germination, one plant per hole was removed and chemical manure 20-10-10 was spread around each plant. On December 19, 80 watermelon plants with about 90cm each were labelled and split into two lots (A and B). In lot A (60 plants), female flowers were left exposed to insect pollinators. In lot B (20 plants), female flowers were covered with gauze bags (5x10 cm) to exclude insect pollination. This operation was daily repeated, as soon as female flowers appeared and just before their opening. In the end a total of 517 and 258 female flowers were available in lot A and lot B respectively.

During the study period, flowers of several other plant species including *Mimosa pudica, Mimosa invisa, Oxalis barrelieri, and Dacryodes edulis* growing in the vicinity of the experimental plots were observed to attract *A. m. Adansonii*.

The abundance of African honeybees on the floral entomofauna of *C. lanatus*

The abundance of *A. m. adansonii* in the entomofauna of *C. lanatus* was daily determined during four time intervals (7-8h, 9-10h, 11-12h, 13-14h) based on observations of the flowers of each labelled plant from 24th December 2004 to 10th February 2005. Flowers typically were completely opened at 7 and closed before 14 h. In transect walks along all labelled plant of lot A, we recorded the identity of all insects that visited *C. lanatus* flowers. Specimens of all insect taxa were caught with an insect net and conserved in 70% ethanol for subsequent taxonomy determination. All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative abundance of *A. m.*

adansonii in the anthophilous entomofauna of *C. lanatus*.

Study of *A. m. ada*nsonii activity on *C. lanatus* flowers

In addition to the determination of the pollinator abundance, direct observations of the foraging activity on both male and female flowers were made on insect pollinator fauna in the experiment field. The observation periods and daily time intervals were the same as those indicated for *A. m. mellifera* abundance. The floral product harvested (nectar or pollen) by worker bees during a floral visit were registered based on the foraging behavior of each. Nectar foragers were seen introducing the head between the stigma or the anther and the corolla while pollen gatherers directly scratched the anthers with the mandibles or the legs. In addition, the number of pollination effective visits (the worker came into contact with the stigma or the anther), as well as the duration of the flower visits were also recorded. Moreover, the maximum daily number of individuals foraging simultaneously on a flower and on 1000 flowers, the duration of individual flower visits (using a stopwatch), the number of flowers visited per minute [13] were measured. Finally, the disruptions of pollination by competing pollinators, as well as the attractiveness of other plants on foragers were assessed.

Evaluation of *A. m. adanson*ii activity on *C. lanatus* yields

The fruit set rate [(number of fruits formed/number of female flowers labelled per a plant) x 100] was determined based on the number of female flowers and the number of actual fruits formed.

The percentage of the fructification rate (*%Tfr*) due to the influence of foraging insects was calculated based on the relative difference in fruit set between bagged (B) and open female flowers (A) (*%Tfr*) = {[(*Tx-Ty*)/*Tx*] x *100*} where *Tx* and *Ty* are the fructification rate in lot A (exposed female flowers) and in lot B (bagged female flowers) respectively [27]. The contribution of honeybees on the overall fruit set was then quantified by {(*%Tfr*) *x* [*Va/100*]} with *Va* = the percentage of the honeybee visits on the flowers of lot A [27].

The impact of anthophilous insects on seed yield was evaluated using the following formula (*%Gr*) = {[(*Mx-My*)/*Mx*] x *100*} where *Mx* and *My* are respectively the mean number of matured seeds per fruit in lot A and lot B respectively. The contribution of honeybees on seed yield is {(*%Gr*) x [*Va/100*]} [27].

Insect identification

The collected insect were identified in the laboratory of Zoology of the University of Yaoundé I, the laboratory of entomology, Institute of Agricultural Research for the Development (Yaoundé) and the contribution of A. Pauly, Department of Entomology, Royal Institute of Natural Sciences of Belgium, who identified some bees of our collection.

Data analysis

Data were analysed using descriptive statistics, correlation coefficient (r) for the evaluation of the association between two variables, the Student *t*-test for the comparison of means of two samples and Microsoft Excel.

RESULTS

A .m. adansonii activity on *C. lanatus*

Frequency of visits

7964 visits of 36 insect species were counted on 12766 flowers of 60 *C. lanatus* plot A plants. *Apis mellifera adansonii* contributed with 5586 visits (= 70.14%) and was the most frequently observed insect species.

Floral products harvested

On *C. lanatus* flowers*, A. m. adansonii* workers collected both nectar and pollen on male flowers and nectar on female flowers. Pollen collection was low and occurred in the morning. Nectar collection was regular, very intensive, and occurred during the flowers opening. For 813 visits counted on male flowers in this effect, 774 (95.21%) were for nectar collection and only 39 (4.79%) for pollen collection.

Density of foragers

The greatest number of *A. m. adansonii* workers foraging simultaneously was 1 per flower. The greatest mean number of foragers per 1000 flowers was 331 ± 173 (n = 41).

Duration of visits per flower

The duration of a bee visit on female flowers varied from 1 to 12 sec, with a mean of 4.93 ± 2.65 (n = 186), that on a male flower from 1 to 13 sec with a mean of 5.91 ± 2.51 (n = 186). The difference between these means was not significant (t = 0.34, df = 370, p < 5%). Only the visits for nectar harvesting were considered because pollen harvesting was very scarce.

Foraging speed

On *C. lanatus* flowers, workers visited between 6 and 14 flowers per minute. The mean foraging speed was 10.20 ± 2.75 flowers per minute (n = 154).

Rhythm of visits according to the flowering stage

Visits were most numerous on a plant when the number of opened flowers was at maximum (Table 1). Furthermore, a positive and significant correlation was found between the number of *C. lanatus* open flowers and the number of *A. m. adansonii* visits ($r = + 0.92$, $n = 17$, $df = 15$, $p < 5\%$).

Daily rhythm of visits

From table 2, it appears that *A. m adansonii* foraged on *C. lanatus* flowers since the dawn with the flowers opening until 14 h (flowers closing). The activity was highest between 9-10 h.

Foraging ecology

During the observation period, flowers of many other plant species growing near *C. lanatus* were also visited by *A. m. adansonii* workers for nectar and / or pollen. During one foraging trip, an individual bee foraging on *C. lanatus* was not observed moving from *C. lanatus* to the neighbouring plant and vice versa.

Workers of honey bee were regularly interrupted by other workers or by other insects collecting *C. lanatus* floral products such as *Xylocopa calens* (nectar), *Meliponula nebulata* (nectar and pollen), *Dactylurina staudingeri* and halictid bees (nectar and pollen) or *A. m. adansonii* predators such as *Philanthus triangulum*, *Polistes* sp. and spiders.

Impact of *A. m. adansonii* activity on pollination, fruit and seed yields of *C. lanatus*

The relative fruit set was 73.11% in lot A and 0 % in lot B (Table 3). The mean number of seeds per fruit was 421.43 ± 92.92 (n = 92 fruits) in lot A and since there were no fruits 0 in lot B. Thus the percentage to both fruit set and seed production was 100% with the honeybee contributing 70.14%.

Consequently the influence of *A. m. adansonii* on fruit and seed yields of *C. lanatus* was positive and significant and over 70% of this yield increase was due to the activity of honey bee workers on the pollination of watermelon.

DISCUSSION

Apis mellifera adansonii activity on *C. lanatus* flowers

Our study confirms other reports [28, 29, 30, 31] that *A. mellifera* is the most frequent and abundant anthophilous insect of *C. lanatus*. Also in India, the genus *Apis* with *Apis cerana* comprised 87% of the pollinating insects founded on *C. lanatus* [32].

The small number of foragers observed per a *C. lanatus* flower is due to its small diameter (diameter of male flowers = 1.87 ± 0.49 cm, for 35 male flowers and diameter of female flowers = 2.53 ± 0.61 cm, for 60 female flowers). The relative high abundance, the observed high density of foragers per 1000 flowers and the positive correlation between the number of flowers and the number of visits are due to the ability of honey bees to recruit a great number of workers for the exploitation of high yield food resources [12]. This factor on *C. lanatus* could be explained by the good accessibility of both nectar and pollen for honey bees.

The disruptions of visits reduced the duration of certain honey bee visits. This could explain the relative high foraging speed obtained. The same observations were made on *Annona senegalensis*, *Croton macrostachyus, Psorospermum febrifugum* and *Syzygium guineense* var. *guineense* flowers at the Adamaoua region of Cameroon [7].

The fact that an individual bee foraging on *C. lanatus* was not observed visiting another plant species indicated that honey bee shows flower constancy [12] for *C. lanatus*. In fact, honey bee foragers are known to be generally constant to a plant species during one or several foraging trips [12].

Table 1: Distribution of the number of *C. lanatus* opened flowers (F) and the total number of *A. m .adansonii* visits (V) (Nkolbisson, 24th December 2004 -10 February 2005).

D	24d	27d	30d	2j	5j	8j	11j	14j	17j	20j	23j	26j	29j	1f	4f	7f	10f
F	23	168	413	578	962	1314	1441	1487	1127	996	936	894	754	603	586	322	162
V	7	53	178	214	485	741	762	954	667	512	274	203	166	124	97	108	42

D= day; d= December; j= January; f= February

Table 2: Daily distribution of *A. m. adansonii* visits on the flowers of sixty *C. lanatus* plants over seventeen days (Nkolbisson, 24th December 2004 -10 February 2005).

	Daily periods (hours)				
	7-8 h	9-10 h	11-12 h	13-14 h	Total
Number of visits	1812	2543	1123	108	5586
% Visits	32.45	45.52	20.10	1.93	100

Table 3: Distribution of the female flowers produced (Fs) by *C. lanatus,* number of fruits formed (Ff) and matured (Fm), fructification rate (Fr) and abortion rate (Ar) in lot A and lot B.

Lots	Fs	Ff	Fr (%)	Fm	Ar (%)
A(n=60)	517	378	73.11	92	82.20
B(n=20)	258	0	0	0	100.00

n = number of *C. lanatus* plants per lot; Fr = (Ff/Fs) x 100; Ar = {[(Fs – Fm)/Fs] x 100}

Impact of *A. m. adansonii* activity on the pollination and yield of *C. lanatus*

During their visits on *C. lanatus* male flowers, foragers contacted anthers and carried pollen with their body hairs. On female flowers, workers came into contact with the stigma and then induced watermelon pollination. In fact, in entomophilous plants, pollination mostly involves pollen transferred on the body of insects [33]. *A. m. adansonii* could carry the pollen from a male flower of one plant to the stigma of a female flower of the same *C. lanatus* plant (geitonogamy) or that of another plant (xenogamy). That is, *C. lanatus* is self and cross-compatible [15, 23]. Consequently, *A. m. adansonii* played a direct and important role on the pollination of *C. lanatus* in which the pollen is not windblown [31]. Moreover, their foraging speed was high, which is a condition that determines the efficiency of *A. m adansonii* as a *C. lanatus* pollinator [13]. In addition, their daily morning periods (9-10 h) of intense activity on *C. lanatus* flowers coincided with the maturation periods of anthers and the optimal receptivity of stigmas of this plant species [15].

The highest fructification rate and mean of mature seeds obtained from unprotected female flowers were the consequences of the direct pollination action of anthophilous insects in general and *A. m. adansonii* in particular on *C. lanatus* flowers. It is known that the more a flower receives pollen grains, the higher the chance of fructification and consequently the higher the amount seeds produced [21]. Moreover, results showed a positive and higher significant correlation between the fruit weight and the number of matured seeds ($r = 0.91$; $df = 59$; $p < 0.05$) and between the number of matured seeds and the diameter of the fruit ($r = 0.96$; $df = 29$; $p<0.05$). These correlations confirm other reports [30] and demonstrate the value of *A. m. adansonii* on *C. lanatus* production.

Of the 387 fruits formed in lot A, only 92 became matured. The abortion rate (82.20%) in lot A could be the effect of the competition for assimilates that affects fruit set in many species [34]. This phenomenon is observed mainly in Cucurbits [35], in which the low fruit set and the dominance of the first set fruits appear to be specific traits of reproductive development [36]. This abortion rate could also be due to the activity of *Dacus bivittatus* (Tephritidae) whose females laid the eggs in young *C. lanatus* fruits and caused important crop loses. Finally, the lack of sufficient honey bees to pollinate every watermelon female flower justified the important crop lose. For this third reason, it is reported that watermelon is dependent on multiple bee visits for pollination requires deposition of 500-1000 pollen grains on the stigmas for production of marketable fruits [30]. Female flowers without bee visitation abort and do not produce fruits [37].

C. lanatus is then strictly an entomophilous crop. It is also neither parthenocarpic nor apomictic under natural conditions as most cultivars of squashes and melons [3].

CONCLUSION

At Nkolbisson, *C. lanatus* is a highly nectariferous bee plant which is exclusively pollinated by insects among which *A. m. adansonii* is by far the most important. Comparison of the fruit and seed sets of unprotect female flowers with those of female flowers protected from insects underscores an increase of 70.14% of the fruit set and the number of matured seeds per fruit due to *A. m. adansonii*. Therefore, the installation of *A. m. adansonii* colonies near populations of *C. lanatus* in bloom appeared as a necessity to improve fruit and seed yields of this plant species.

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