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Pollination Efficiency of *Amegilla calens* (Hymenoptera: Apidae) on *Gossypium hirsutum* L. (Malvaceae) Variety L457 Flowers at Meskine (Maroua, Cameroon)

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Abstract: This study was carried out to evaluate the impact of *Amegilla calens* bee on fruit and seed yields of *G. hirsutum* in an experimental field, in September 2018 and 2019. The experiments were carried out on 540 flowers divided in four treatments: 120 flowers accessible to all visitors; 120 flowers bagged to avoid all visits; 200 flowers protected and uncovered when they were opened, to allow *A. calens* visits; 100 flowers bagged then uncovered and rebagged without the visit of insects or any other organism. Bee's daily rhythm of activity, its foraging behaviour on flowers, its pollination efficiency, the fruiting rate, the number of seeds per fruit and the percentage of normal seeds were evaluated. Among the 20 insect species recorded on *G. hirsutum* flowers, *A. calens* was the most frequent insect with 30.72 % of 655 visits. On flowers, individual bee intensely collected pollen and slightly harvested nectar. The mean duration of a visit per flower was 23.56 sec for pollen harvest and 13.68 sec for nectar collection. For the two years, through its pollination efficiency, *A. calens* increased the fruiting rate by 20.30 %, as well as the percentage of normal seeds by 32.39 %.

Keywords: *Amegilla calens*, *Gossypium hirsutum*, Meskine, pollination efficiency, yields.

1. Introduction

The importance of pollinating insects in agricultural production, mainly with regard to that of domestic and wild bees, is no longer to be proven but remains generally unknown (AREM, 2011). In the natural environment and in agro ecosystems, flowering insects in general and Apoidea in particular have great ecological and economical importance because they have a positive influence on food production [2,3]. Insect pollination in many crops is essential for fruit and seed productions [4].

Gossypium hirsutum (cotton), commonly called the white gold, is the second most important oil-seed crop after *Glycine max* (soybean) [5]. This plant species grows up to 1.5 m in height with vegetative and fruiting branches [6]. Leaves are alternate, petiolate, palmate with 3 to 5 lobes, hirsute, blade cordate, 7.5 cm wide and 15 cm long [6]. Seeds are oval in shape, slightly pointed, about 10 mm long and 4 mm wide, and dark brown in colour (black seed) [5]. The capsule can produce 20 to 25 seeds [7]. Commercially, *G. hirsutum* is cultivated as an annual plant for seed and fiber [8,7,5]. Its flowers produce pollen and nectar that attract insects [9].

To our knowledge, the data published after detailed studies on the interactions between insects and *G. hirsutum* are those from Sudan [10], Russia [11], Australia [12,13,14], USA [11,15,16,17] and Cameroon [18,3,19,20].

In all these investigations, the foraging behaviour and pollination activity were carried out in detail only on *Apis mellifera* and *Macronomia vulpina*. The flowering entomofauna and the impact of insects on pollination, fruit and seed yields of a plant species may vary with the species of insect, time and space [21]. Cameroon is classified among the countries with a severe level of hunger with an overall hunger index of 17.6 with 22 % of the undernourished population [22]. In this country, the demand for cottonseed is high (240 000 tons/year) while its production is low (250 000 tons/year) [23]. Currently, the processing of seed cotton produces around 15 million litres of cottonseed oil per year for an estimated population of 25 million inhabitants [23]. Cotton yields can be increase in this country if its flowering insects are well known and exploited [20]. Therefore, it is important to investigate on the possibilities of increasing the production of this valuable plant in the country.

The general objective of this work is to contribute to the understanding of the relationships between *A. calens* and *G. hirsutum*, for their optimal management. Specific objectives are to: (a) determine the place of *A. calens* in the *G. hirsutum* floral entomofauna; (b) study the activity of this Apidae on flowers of the Malvaceae; (c) evaluate the impact of the flowering insects including *A. calens* on pollination, fruit and seed yields of *G. hirsutum*; (d) estimate the pollination efficiency of *A. calens* on this plant species.

2. Material and Methods

2.1. Study site, experimental plot and biological material

The experiment was carried out from 13th September to 12th December 2018 and from 11th September to 1st December 2019 at Meskine within an experimental fields (latitude: 10° 32'.26 N''; longitude: 014° 14'.53'' E; altitude: 424 m above sea level). This Region belongs to the ecological zone with three phytogeographical areas (Sudano-Sahelian, Sahelian and Sudanian altitude) periodically flooded, with unimodal rainfall [24]. The climate is characterized by two seasons: a dry season (November to May) and a rainy season (June to

October); August is the wettest month of the year [25]. Annual rainfall varies from 400 to 1100 mm [25]. The annual average temperature varies between 29°C and 38°C; daily temperature range between 6°C and 7°C [25]. The experimental plot was a field of 437 m^2. The bees, *A. calens* of the experimental station were recruited among the arthropods naturally present in the environment. The vegetation was represented by wild and cultivated species. The plant material was represented by the seeds of *G. hirsutum* variety L457 provided by Institute of Agricultural Research for Development of Maroua.

2.2. Sowing and weeding

From June 15th to 21st 2018 and from July 20th to 24th 2019, the experimental plot was divided into eight subplots of 8*4.5 m^2 each. Four seeds were sown per hole on six lines per subplot. There were 16 holes per subplot. Holes were separated 50 cm from each other, while lines were 70 cm apart [18]. Weeding was performed manually as necessary to maintain plots weeds-free.

2.3. Determination of the reproduction mode of *Gossypium hirsutum*

On September 12th 2018, 240 flowers of *G. hirsutum* at bud stage were labeled and divided in two treatments: 120 unprotected flowers (treatment 1) and 120 flowers bagged using gauze bags net to prevent insect visitors (treatment 2) [26]. Similarly, on September 10th 2019, 240 flowers at the budding stage were labeled of which 120 were left unprotected (treatment 5), while 120 were bagged (treatment 6). In the both years, after harvest, the number of fruits formed in each treatment was assessed.

For each treatment, the fruiting index (*Pi*) was then calculated as described by [26]:

$Pi = F_b / F_a$, where F_a is the number of viable flowers initially set and F_b the number of the formed fruits. For each year, the allogamy rate (*Alr*) from which derives the autogamy rate (*Atr*) was expressed as the difference in fruiting indexes between treatment *X* (unprotected flowers) and treatment *Y* (bagged flowers) [27]: $Atr = \{[(FiX - FiY) / FiX] * 100\}$, where *FiX* and *FiY* are the fruiting indexes in treatments *X* and *Y* respectively; $Alr = 100 - Atr$.

2.4. Estimation of the frequency of *Amegilla calens* visits on *Gossypium hirsutum* flowers

The frequency of *A. calens* visits on *G. hirsutum* flowers was determined based on observations of flowers of treatments 1 and 5, every day, from 13th to 22nd September 2018 and from 11th September to 1st October 2019 according to six daily time frames: 6 - 7 am, 8 - 9 am, 10 - 11 am, 12 - 13 pm, 14 - 15 pm and 16 - 17 pm. In a slow walk along all labelled flowers of treatments 1 and 5, the identity of all insects that visited *G. hirsutum* flowers was recorded [28]. Specimens of all insect taxa were caught using insect net on unlabelled flowers and conserved in 70 % ethanol, excluding butterflies that were preserved dry [29], for subsequent taxonomic identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *A. calens* in the anthophilous entomofauna of *G. hirsutum* [28]. Data obtained were use to determine the frequency of visits (F_i) of each insect species on *G. hirsutum* flowers. For each study period, $F_i = [(Vi / Vt) * 100]$, were *Vi* is 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.

2.5. Study of the activity of *Amegilla calens* on *Gossypium hirsutum* flowers

2.5.1. Floral product harvested

In addition to the determination of the flower visiting insect frequency, direct observation of the foraging activity of *A. calens* on flowers was made in the experimental field. The floral product (nectar or pollen) harvested by *A. calens* during each flower visit was registered based on its foraging behaviour. Nectar foragers were seen extending their proboscis between the base of the corolla and stamens, while pollen gatherers scratched the anthers using their mandibles and legs [29,30]. During the same time the duration of *A. calens* visits on flowers were registered, the type of floral product harvested by this solitary bee was noted [30]. In the morning of each sampling day, the number of opened flowers was counted. Data obtained were used to determine the relationship between the number of visits of *A. calens* and the corresponding flowers [28].

2.5.2. Duration of visits and foraging speed

During the same days as for the registration of the frequency of visits, the duration of individual flower visit was recorded (using stopwatch) according to six daily time frames: 7 - 8 am, 9 - 10 am, 11 - 12 am, 13 - 14 pm, 15 - 16 pm and 17 - 18 pm. Moreover, the number of visits during which the bee came into contact with the stigma [31], was registered. Regarding the foraging speed (F_s) which is the number of flowers visited by an individual bee per minute [31], data were registered during the same dates and according to the same time frames and date as for the 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 *G.*

hirsutum 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 duration (in sec) given by stopwatch and F_s the number of flowers visited during d_v [28]. During the observation, when a forager returned to previously visited flower, counting was performed as on two different flowers [28].

2.5.3. Abundances per flower and per 1000 flowers

The abundance of foragers (highest numbers of individuals foraging simultaneously) [32] per flower and per 1000 flowers (A_{1000}) were recorded on the same dates and daily time frames as that for the registration of the 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 know number of opening flowers and A_{1000} was calculed 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 [32].

2.5.4. Foraging ecology

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *A. calens* was assessed by direct observations. For the second parameter, the number of times that the solitary bee left this Malvaceae flowers to another plant species and vice versa was noted through the investigation period.

During each observation date, temperature and relative humidity in the station were registrated every 30 minutes using a mobile thermo-hygrometer installed in the shade [28], from 6 am to 6 pm.

2.5.5. Evaluation of the impact of flowering insects including *Amegilla calens* on *Gossypium hirsutum* yields

Parallel to the constitution of treatments 1, 2, 5 and 6, 600 flowers at bud stage were laballed in 2018 and in 2019, to form two treatments:

- treatment 3 in 2018 or 7 in 2019: 400 flowers protected using gauze bag nets to prevent insect or any other organism visits and destined to be visited exclusively by *A. calens;* as soon as each flower of these treatments was opened, the gauze bag was removed and this flower was observed for up to 10 minutes; the flower visited once by *A. calens* was marked and then protected once more [33];

- treatment 4 in 2018 or 8 in 2019: 200 flowers protected using gauze bag nets and destined to be uncovered then rebagged without the visit of insects or any other organism; as soon as each flower of these treatments was opened, the gauze bag was removed and this flower was observed for up to 10 minutes, while avoid insect or any other organism visits.

At the maturity, fruits were harvested and counted from each treatment. The fruiting rate, the percentage of seeds per fruit and the percentage of normal seed were then determined for each treatment [34].

The evaluation of the effect of insects including *A. calens* on *G. hirsutum* production was based on the impact of flowering insects on pollination, the impact of pollination on *G. hirsutum* fruiting and the comparison of the fruiting rate, the number of seeds per fruit and the percentage of normal (that is well developed) seed [35] of treatments 1, 2, 4, 5, 6 and 8. For each year, the fruiting rate due to the foraging insects including *A. calens* (*Fri*) was calculated using the following formula [35]: $Fri = \{[(FX - FZ) / (FX + FY - FZ)] * 100\}$, where *FX*, *FY* and *FZ* are the fruiting rates in treatment *X* (flowers left in free pollination), treatment *Y* (flowers protected from all insect visits) and treatment *Z* (flowers bagged then uncovered and rebagged without insect or any other organism visit). The fruiting rate of a treatment (*F*) is $F = [(b / a) * 100]$, where *b* is the number of fruits formed and *a* the number of viable flowers initially set [26]. The impact of flower visiting insects including *A. calens* on the percentage of seeds per fruit and the percentage of normal seeds was evaluated using the same method as mentioned above for the fruiting rate [26].

2.5.6. Assessement of the pollination efficiency of *Amegilla calens* on *Gossypium hirsitum*

The contribution of *A. calens* on the fruiting rate, the number of seeds per fruit and the percentage of normal seeds, was calculared using the data of treatments 3 and 4 for 2018 and those of treatments 7 and 8 for 2019.

For each observation year, the contribution of *A. calens* in the fruiting rate (*FrA)* was calculated using the following formula: $FrA = \{[(FA - FZ) / FA] * 100\}$ [33], where *FA* is the fruiting rate in treatment *A* (flowers visited exclusively by *A. calens*).

The impact of *A. calens* on the fruiting rate, number of seed per fruit and the percentage of normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

2.6. Data analysis

Statistical analyses were performed using R commander, version i386 3.2.0., descriptive statistics, Microsoft Excel 2010, 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,

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pearson correlation coefficient (*r*) for the study of the association between two variables and chi-square (χ^2) for the comparison of percentages.

3. Results

3.1. Reproduction mode of *Gossypium hirsutum*

Podding indexes of *G. hirsutum* were 0.89, 0.53, 0.80 and 0.67 for treatments 1, 2, 5 and 6 respectively. Thus, in 2018, the allogamy rate was 41.12 % and the autogamy rate was 58.53 %. In 2019 the corresponding figures were 16.67 % and 83.33 %. For the two cumulated years, the allogamy rate was 28.89 % and the autogamy rate was 70.93 %. It appears that the variety of *G. hirsutum* used in our experiments has a mixed reproducting regime that is allogamous-autogamous, with the predominance of autogamy over allogamy.

3.2. Place of *Amegilla calens* in *Gossypium hirsutum* floral entomofauna

Among 655 visits of 11 and 19 insect species recorded on *G. hirsutum* flowers in 2018 and 2019 respectivily, *A. calens* ranked third with 32 visits (13.22 %) after *Lasioglossum* sp. 1 (36.36 %) and *Apis mellifera* (14.82 %) in 2018 and first with 172 visits (41.64 %) in 2019 (Table 1). The difference between the percentages of *A. calens* visit for two years is highly significant (χ^2 = 57.48; *df* = 1; P < 0.001). For the two cumulated year *A. calens* ranked first with 204 visits (30.72 %).

Table 1. Diversity of flowering insects on *Gossypium hirsutum* in 2018 and 2019, number and percentage of visits of different insects.

Insects			2018		2019		Total	
Order	**Family**	**Genus and species**	n_1	P_1 (%)	n_2	P_2 (%)	n_t	P_t (%)
Diptera	Muscidae	*Musca domestica* (ne)	3	1.23	4	0.96	7	1.07
	Calliphoridae	*Chrysomia chloropyga* (ne)	7	2.06	-	-	7	2.52
Coleoptera	Scarabaeidae	(sp. 1) (po)	30	12.39	9	2.17	39	5.99
		(sp. 2) (po)	4	1.65	2	0.48	6	0.92
		(sp. 3) (po)	-	-	6	1.45	6	1.45
	Melo idae	*Mylabris* sp. (ne. po)	10	4.13	10	2.42	20	3.07
Hymenoptera	Apidae	*Apis mellifera* (ne. po)	36	14.87	21	5.08	57	8.75
		Amegilla calens (ne. po)	32	13.22	172	41.64	204	30.72
		Amegilla sp. 1 (ne. po)	-	-	3	0.72	3	0.72
		Amegilla sp. 2 (ne. po)	-	-	4	0.96	4	0.96
		Amegilla sp. 3 (ne. po)	-	-	2	4.48	2	4.48
		Amegilla sp. 4 (ne. po)	-	-	19	0.60	19	0.60
		Amegilla sp. 5 (ne. po)	9	3.71	-	-	9	3.78
		Tetralonia sp. (ne. po)	-	-	7	1.69	7	1.07
	Halictidae	*Lasioglossum* sp. 1 (ne. po)	88	36.36	107	25.90	195	29.95
		Lipotriches azarensis (ne. po)	-	-	1	0.24	1	0.24
	Vespidae	(sp. 1) (ne. po)	5	2.06	34	8.23	39	5.99
		(sp. 2) (po)	-	-	6	1.45	6	1.45
Lepidoptera	Acraeidae	*Acraea acerata* (ne)	12	4.95	3	1.72	15	2.30
	Pieridae	*Eurema* sp. (ne)	6	2.47	3	1.72	9	1.38

Total	**242 100** **12**	**413 100** **18**	**655 100** **20 species**

n_1 and ***n_2***: number of visits on 120 flowers in 2018 and in 2019; **ne**: collection of nectar; **po**: collection of pollen; ***p_1*** and ***p_2***: percentages of visits; **sp**: undetermined species.

$p_1 = (n_1 / 242) * 100$;

$p_2 = (n_2 / 413) * 100$;

$p_t = (n_t / 655) * 100$

3.3. Duration of visits per flower

The mean duration of *A. calens* visit per *G. hirsutum* flower varied according to floral product harvested (Table 2). In 2018, the mean duration of a flower visit for nectar harvest was 16.29 sec (n = 34; s = 7.42) and that for pollen collection was 25.80 sec (n = 72; s = 12.55); the difference between these means is highly significant (t = 4.82; df = 104; P < 0.001). In 2019, the corresponding figures were 11.08 sec (n = 37; s = 4.60) for nectar harvest and 21.33 sec (n = 76; s = 10.82) for pollen collection. The difference between these two means is higly significant (t = 6.99; df = 111; P < 0.001). For the two cumulated years, the mean duration of a flower visit was 13.69 sec (n = 71; s = 6.01) for nectar harvest and 23.57 sec (n = 148; s = 11.69) for pollen collection. The difference between these two later means is highly significant (t = 8.22; df = 217; P < 0.001).

Table 2. Duration of *Amegilla calens* visits per flower of *Gossypium hirsutum* in 2018 and 2019 at Meskine.

Years	**Harvested products**	**Duration of visit (sec)**					**Comparison of means**		
		n	***m***	***sd***	***mini***	***maxi***	***t-value***	***df***	***p-value***
2018	Nectar	34	16.29	7.42	8	42			
	Pollen	71	25.80	12.55	7	58	19.42	103	< 0.001 VHS
2019	Nectar	37	11.08	4.60	2	21			
	Pollen	76	21.32	10.81	5	48	27.31	111	< 0.001 VHS
Total	Nectar	71	13.68	6.01	2	42			
	Pollen	147	23.56	9.11	5	48	57.21	216	< 0.001 VHS

n: nomber of durations registred; ***m***: mean; ***sd***: standard deviation; ***maxi***: maximum; ***mini***: minimum; **VHS**: very highly significant difference; ***df***: degre of freedom

3.4. Activity of *Amegilla calens* on *Gossypium hirsutum* flowers

3.4.1. Floral products harvested

Individuals of *A. calens* were seen collecting nectar (Figure 1A) and pollen (Figure 1B) on *G. hirsutum* flowers. Pollen collection was regular and intensive whereas nectar collection was less. For 106 visits recorded in 2018, 72 (67.92 %) were devoted to pollen collection and 34 (32.08 %) to nectar harvest; in 2019, for 113 visits registered, 76 (67.25 %) were devoted to pollen collection and 37 (32.74 %) to nectar harvest. For the two cumulated years on 219 visits recorded, 148 (67.57 %) were devoted to pollen collection and 71 (32.42 %) to nectar harvest. Nectar and pollen were harvested during all scheduled observation daily time frames. For harvesting nectar, the individuals of *A. calens* lands either on the stamens or on the stigma, then moves towards the inside of the corolla and deploys its proboscis to collect nectar. For the pollen collection the bee comes into contact with the flower from above, the thorax being in contact with the stigma; subsequenly, it uses the hind legs, mandibules and abdominal hair to collect pollen wich is sored at the level of their hing legs.

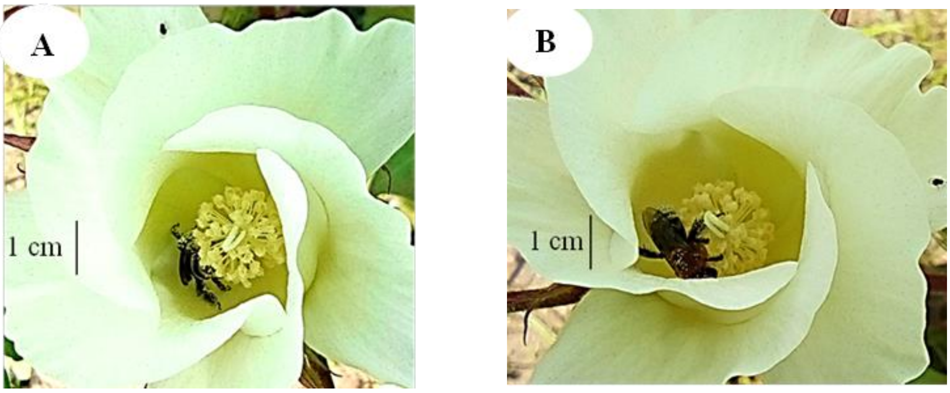
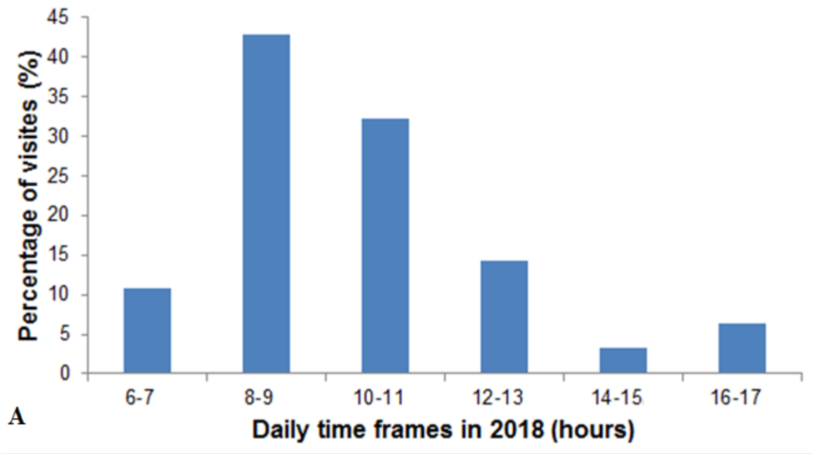


Figure 1. *Amegilla calens* collecting nectar (**A**) and pollen (**B**) in *Gossypium hirsutum* flower at Meskine in 2019.

3.4.2. Daily rhythm of visits

Amegilla calens was active on *G. hirsutum* flowers from 6 am to 5 pm in 2018 and in 2019. The peak of activity was situated between 8 and 9 am in 2018 as well as in 2019 (Figure 2).



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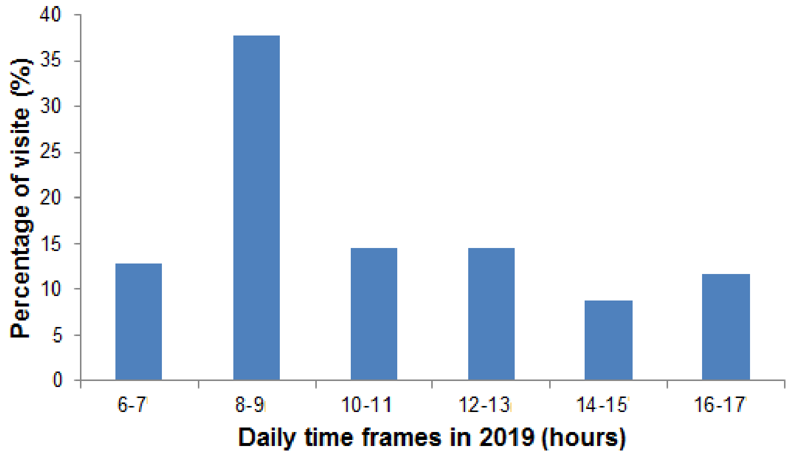
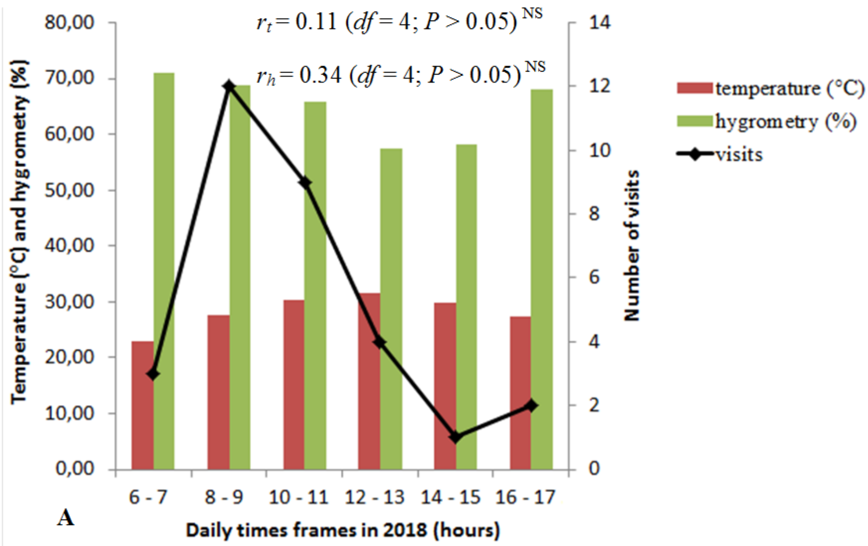


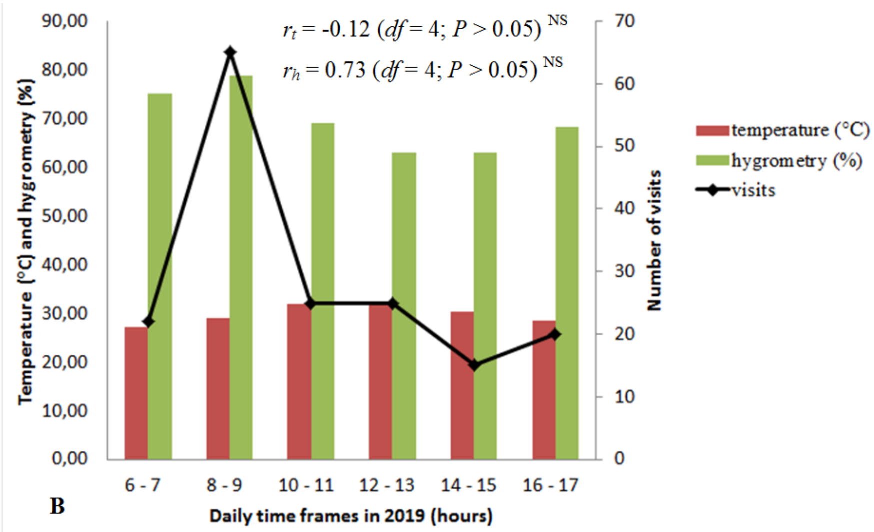
B

Figure 2. Variatons of the number of *Amegilla calens* visits on *Gossypium hirsutum* flowers according to the daily time frames in 2018 (**A**) and 2019 (**B**) at Meskine.

In 2018, the correlation was not significant between the number of *A. calens* visits and the temperature (r = 0.11; df = 4; P > 0.05) and between the number of visits and relative humidity (r = 0.34; df = 4; P > 0.05). In 2019, the correlation was equally not significant between the number of *A. calens* visits and the temperature (r = -0.12; df = 4; P > 0.05) and between the number of these visits and relative humidity (r = 0.73; df = 4; P > 0.05) (Figure 3).



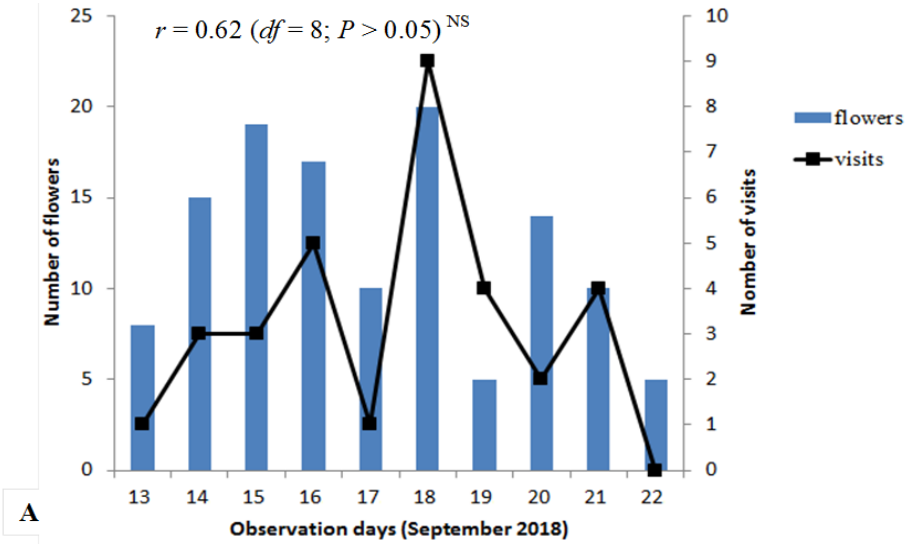
A



r_t*:** correlation between number of *A. calens* visits and temperature; ***r_h*:** correlation between number of *A. calens* visits and hygrometry; ***df: degre of freedom; ***P***: level of significance; ***NS***: not significant difference

Figure 3. Variation of the temperature, the humidity and the number of *Amegilla calens* visits on *Gossypium hirsutum* flowers according to the daily frames time in 2018 (**A**) and 2019 (**B**) at Meskine.

In 2018 as well as in 2019 *A. calens* visits were apparently more numerous on *G. hirsutum* individual plant when their number of opened flowers was highest. But, the correlation between the number of opened flowers and the number of visit was not significant in 2018 (r = 0.62; df = 8; P > 0.05) as well as in 2019 (r = 0.14; df = 7; P > 0.05) (Figure 4).



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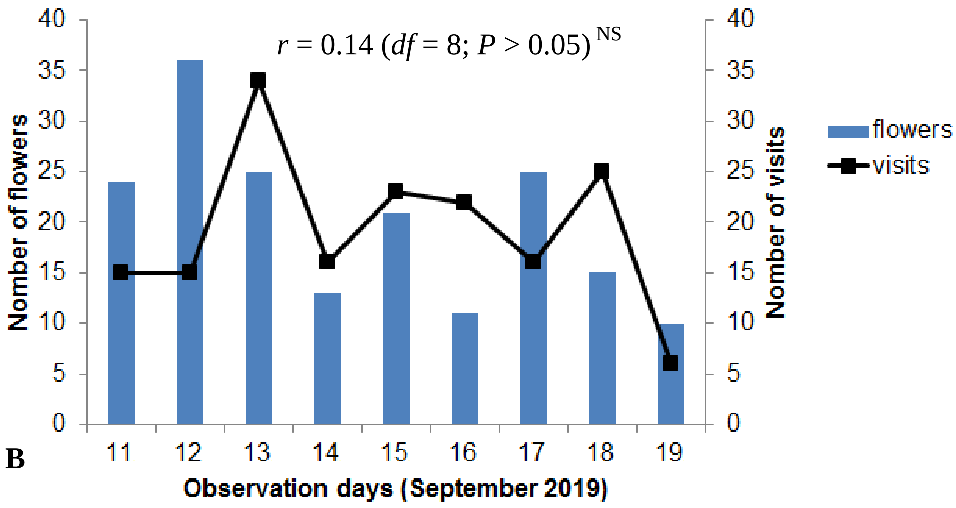

r: correlation; ***df***: degre of freedom; ***P***: level of significance; ***NS***: not significant difference

Figure 4. Seasonal variations of the number *Gossypium hirsutum* opened flowers and the number of *Amegilla calens* visits on these organs in 2018 (A) and 2019 (B) at Meskine.

3.4.3. Foraging speed of *Amegilla calens* on *Gossypium hirsutum* flowers

In the experimental field, *A. calens* visited between 1 and 6 flowers per minute in 2018 and between 2 and 13 flowers per minute in 2019 (Table 3). The mean foraging speed was 3.07 flowers per minute (n = 69; s = 2.74) in 2018 and 2.69 flowers per minute (n = 60; s = 2.52) in 2019. There is not difference between these two means (t = 0.81, df = 127; $P > 0.05$). For the two cumulated years, the mean foraging speed was 2.88 flowers per minute (n = 129; s = 2.64).

Table 3. Foraging speed of *Amegilla calens* on *Gossypium hirsutum* flowers in 2018 and 2019 at Meskine.

Years	**Number of flowers / minute**					**Comparison of means**
	n	***m***	***sd***	***mini***	***maxi***	
2018	69	3.07	2.74	1	6	
2019	60	2.69	2.51	2	13	t = 0.82; df = 127; $P > 0.05)^{NS}$.
Total	129	2.97	2.37	1	13	

n: nomber of speeds registred; ***m***: mean; ***sd***: standard deviation; ***maxi***: maximum; ***mini***: minimum; ***Ns***: not significant difference

3.4.4. Abundance of *Amegilla calens*

In 2018, the highest mean number of *A. calens* individuals simultaneously in activity was 1 per flower (n = 47; s = 0) and 533.80 per 1000 flowers (n = 36; s = 329.42). In 2019, the corresponding figures were 1 per flower (n = 89; s = 0), and 547.67 per 1000 flowers (n = 102; s = 325.06). There is no difference between the mean number of foragers per 1000 flowers in 2018 and 2019 (t = 0.22; df = 136; P > 0.05). For the two cumulated years the mean number of foragers per 1000 flowers was 540.74.

3.4.5. Influence of neighbouring flora

During the observation period, flowers of many other plant species growing in the environment of *G. hirsutum* field were visited by *A. calens*, for their nectar (ne) and/or pollen (po). Amongst these plants were: *Solanum lycopersicum* (Solanaceae: po); *Cosmos sulphureus* (Asteraceae: ne and po), *Hibiscus sabdariffa* (Malvaceae: ne and po), *Abelmoschus esculentus* (Malvaceae: ne and po), and *Ceratotheca sesamoides* (Pedaliaceae: ne and po). During the two years of study, we observed no passage of *A. calens* from *G. hirsutum* flowers to flowers of another plant species and vice versa. Hence during foraging trips on *G. hirsutum*, individuals of *A. calens* were faithful to this Malvaceae.

3.4.6. Influence of fauna

Individuals of *A. calens* were disturbed in their foraging activity by other individuals of the same species or those from other species, that were the competitor for *G. hirsutum* nectar and/or pollen. In 2018, for 106 visits, 4 (3.77 %) were interrupted by *Lasioglossum* sp. 1 and 2 (1.88 %) by individuals of an Vespidae (sp. 1). In 2019, for 113 visits, 3 (2.65 %) were interrupted by an Vespidae (sp. 1) and 1 (0.88 %) by *Mylabris* sp.

3.5. Impact of anthophilous insects including *Amegilla calens* on *Gossypium hirsutum* yields

The fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds in the different treatments of *G. hirsutum* are shown in Table 4.

Table 4. Fruiting rate, percentage of fruits with seed and percentage of normal seeds according to different treatments of *Gossypium hirsutum* in 2018 and 2019 at Meskine.

Years	**Treatments**	**NF**	**NFF**	**FR (%)**	**Seeds / fruit**			**TNS**	**NS**	**%NS**
					mean	***sd***	***n***			
2018	**1 (Uf)**	120	107	89.16	26.68	6.25	95	2535	2408	94.99
	2 (Pf)	120	63	52.5	24.09	6.38	55	1325	1162	87.69
	3 (Fpva)	177	157	88.70	25.54	6.08	48	1226	1131	92.25
	4 (Fpwv)	123	105	85.36	23.82	4.46	34	810	710	87.65
2019	**5 (Uf)**	120	96	80.00	31.25	6.03	78	2438	2242	91.96
	6 (Pf)	120	80	66.66	25.67	6.26	73	1874	1583	84.47
	7 (Fva)	159	137	86.16	25.38	5.68	62	1574	1474	93.64
	8 (Fpwv)	125	70	56.00	23.68	4.94	51	1208	1052	87.08

Uf: unprotected flowers; **Pf**: protected flowers; **Fpva**: flowers visited exclusively by *Amegilla calens*; **Fpwv**: flowers bagged then uncovered and rebagged without visit by insect or any

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other organism; **NF**: number of flowers; **NFF**: number of fruits formed; **FR**: fruiting rate; **TNS**: total number of seeds; **NS**: number of normal seeds; **%NS**: percentage of normal seeds

This table shows that:

a) The fruiting rates were 89.16 %, 52.5 %, 88.70 %, 85.36 %, 80 %, 66.66 %, 86.16 % and 56 % in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant (χ^2 = 114.27; df = 7; P < 0.001). The two to two comparisons showed that the difference observed is highly significant between treatments 1 and 2 (χ^2 = 39.05; df = 1; P < 0.001), 2 and 4 (χ^2 = 30.75; df = 1; P < 0.001), 7 and 8 (χ^2 = 32.22; df = 1; P < 0.001), 4 and 8 (χ^2 = 25.74; df = 1; P < 0.001), significant between treatements 5 and 6 ($\chi 2$ = 5.46; df = 1; P < 0.05), and not signicant between treatments 1 and 5 (χ^2 = 3.87; df = 1; P > 0.05), 3 and 4 (χ^2 = 0.73; df = 1; P > 0.05), 6 and 8 (χ^2 = 2.93; df = 1; P > 0.05), 1 and 3 (χ^2 = 0.016; df = 1; P > 0.05), then 5 and 7 (χ^2 = 1.89; df = 1; P > 0.05). Consequently, in 2018 and 2019, the fruiting rate of unprotected flowers (treatments 1 and 5) was higher than that protected flowers (treatments 2 and 6).

b) The mean numbers of seeds per fruit were 26.68, 24.09, 25.54, 23.82, 31.25, 25.67, 25.38 and 23.68 in treatments 1 to 8 respectively. The differences between these eight means are globally highly significant (F = 11.4; df_1 = 7; df_2 = 488; P < 0.001). The two to two comparison showed that the difference is highly significant between treatments 5 and 7 (t = 5.87; df = 138; P < 0.001), 1 and 5 (t = 4.85; df = 171; P < 0.001), 5 and 6 (t = 5.53; df = 149; P < 0.001), so significant between treatments 1 and 2 (t = 2.40; df = 148; P < 0.05), not significant between treatments 7 and 8 (t = 1.69; df = 111; P > 0.05), 2 and 4 (t = 0.23; df = 87; P > 0.05), 4 and 8 (t = 0.13; df = 83; P > 0.05), 3 and 4 (t = 1.46; df = 80; P > 0.05), 1 and 3 (t = 1.04; df = 141; P > 0.05), then 6 and 8 (t = 1.96; df = 122; P > 0.05). Thus, in 2018 as well as in 2019, the mean number of seeds per fruit of unprotected flowers was higher than that of protected flowers.

c) The percentages of normal seeds were 94.99 %, 87.69 %, 92.25 %, 87.65 %, 91.96 %, 84.47%, 93.64 % and 87.08 % in treatments 1 to 8 respectively. The differences be tween these eight percentages are globally highly significant (χ^2 = 204.18; df = 7; P < 0.001). Pairewise comparisons showed that the difference observed is highly significant between treatments 1 and 2 (χ^2 = 66.59; df = 1; P < 0.001), 5 and 6 (χ^2 = 59.31; df = 1; P < 0.001), 7 and 8 (χ^2 = 35.21; df = 1; P < 0.001), 1 and 5 (χ^2 = 18.79; df = 1; P < 0.001), 3 and 4 (χ^2 = 11.90; df = 1; P < 0.001), 1 and 3 (χ^2 = 11.16; df = 1; P < 0.001), significant between treatments 5 and 7 (χ^2 = 3.98; df = 1; P < 0.05), 6 and 8 (χ^2 = 4.05; df = 1; P < 0.05), and not signicant between treatments 2 and 4 (χ^2 = 0.001; *df* = 1; *P* > 0.05), then 4 and 8 (χ^2 = 0.14; *df* = 1; *P* > 0.05). Hence, in 2018 and 2019, the percentage of normal seeds of unprotected flowers was higher than that of flowers protected during their opening period.

In 2018, the numeric contribution of anthophilous insects in the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *G. hirsutum* were 6.76 %, 10.61 % and 7.72 % respectively. In 2019, the corresponding figures were 26.47 %, 23 % and 5.46 %. For the two cumulated years, the numeric contribution of anthophilous insects including *A. calens* were 16.62 %, 16.81 % and 6.59 % for the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds of *G. hirsutum* respectively.

3.5. Pollination efficiency of *Amegilla calens* on *Gossipium hirsutum*

During a single flower visit of *A. calens* for nectar or pollen harvest on *G. hirsutum* flowers, this bee regularly came into contact with anthers and stigma (100 %), increasing the possibility of the Malvaceae pollination.

The fruiting rates (Table 4) due to *A. calens* were 88.70 % in 2018, 86.16 % in 2019 and 87.43 % for the two cumulated years. Therefore, in 2019, the fruiting rate of flowers protected and visited exclusively by *A. calens* was higher than that of flowers protected and rebagged without insect or any other organism visit. The mean numbers of seeds per fruit due to *A. calens* were 25.54 in 2018, 25.38 in 2019 and 25.46 for the two cumulated years. Thus, in 2018 as well as in 2019 the numbers of seeds per fruit in protected flowers and visited exclusively by *A. calens* was not higher than that of flowers protected and rebagged insect or any other organism visit. The percentage of normal seeds due to *A. calens* were 92.25 % in 2018 93.64 % in 2019 and 92.95 % for the two cumulated years. For each of the two years, results pointed out that flowers visited by *A. calens* have the highest number of normal seeds compare to those protected then uncovered and rebagged without the visit of insect or any other organism.

In 2018, the contribution of *A. calens* on the percentage of normal seeds via on single flower visit was 4.99 %. In 2019, the fruiting rate and the percentage of normal seeds were 35 % and 28.63 % respectively. For the two cumulate years, the numerique contribution of *A. calens* via a single flower visit on the fruiting rate and the percentage of normal seeds were 20.30 %, and 32.39 % respectively.

4. **Discussion**

4.1. Activity of *Amegilla calens* on *Gossypium hirsutum* flowers

Results obtained from these experiments indicated that *A. calens* was the main floral insect visitor of *G. hirsutum* flowers. The high frequency of individual foragers of *A. calens* on the flowers could be explained by the large number of individuals of this bee inside the experimental field and the good attractiveness of the floral products of this Malvaceae towards this bee. The significant difference between the percentages of *A. calens* visit for the two years of study could be attributed to a combination of climatic factors and seasonal variation in floral resources availability. It is well known that the anthophilous insect fauna of a plant varies over time [28,3]. Other researches have revealed *Apis mellifera* [12,11,10,18,3] and *M.* vulpina [19] as the main insect visitors on the flowers of this Malvaceae. This difference could be explained by the absence of the nests of these two bee species within and near the exprimental site, and the presence of other plant species with flowers able to attract *Apis mellifera* and *M. vulpina* foragers.

The significant difference observed between the mean duration of a pollen harvest visit and that of nectar harvest visit could be explained by the importance and accessibility of each of these floral products. Pollen is produced by the anthers, which are on the top of the stamens, whereas nectar is between the base of the style and stamens [32]. *Amegilla calens* do not make honey. It harvests more pollen and less nectar to make bread which is the protein source of young larvs [36].

The high abundance of individual bees per 1000 flowers and the attractiveness of *G. hirsutum* nectar and pollen for *A. calens* could be partially explained by the higher availability of theses substance, their accessibility and the needs of *A. calens* during the flowering period of the Malvaceae.

The absence of the passage of *A. calens* from *G. hirsutum* flowers to flowers of another plant species and vice versa could be explained by the fedelite of this solitary bee to the flowers of this plant durring foraging bouts. This phenomenon is called floral constancy [37]. It is explained by the fact that the solitary bees in general are able to memorize the form, the color and the smell of the flowers visited during previous foraging trips [38]. These same observations have been made for honey bees on the flowers of cotton in Mayel-Ibe [3] (Maroua-Cameroon).

The disruption of visits by other insects in 2018 by *Lasioglossum* sp. 1 (3.77 %) and Vespidae (sp. 1) (1.88 %), then in 2019 by Vespidae (sp. 1) (2.65 %) and *Mylabris* sp. (0.88 %) reduced the duration of some *A. calens* visits. This obliged some individuals of this bee to visit more flowers during a foraging trip to maximize their pollen or nectar loads. Similar observations have been made on *Apis mellifera* workers foraging on the flowers of this Malvaceae in Maroua [3] and in Garoua [20].

4.2. Impact of anthophilous insects including *Amegilla calens* on *Gossypium hirsutum* yields

The numeric contribution of anthophilious insects to the fruiting rate, the number of seeds per fruit and the percentage of normal seeds of *G. hirsutum* was positive. During nectar and/or pollen harvest on *G. hirsutum*, foraging insect in general always shook flowers and regularly contacted anthers and stigma, increasing self-pollination and/or cross-pollination possibilities of this Malvaceae. Our results agreed with those obtained in: Mayel-Ibbe (Maroua) [3], Dang (Ngaoundere) [18] and Djamboutou (Garoua) [20] on this Malvaceae.

4.3. Pollination efficiency of *Amegilla calens* on *Gossypium hirsutum*

During a single flower visit of *A. calens* for the collection of nectar and/or pollen on each flower, individuals of *A. calens* always come into contact with the stigma and anthers (100 %) and thus increasing the possibilities of *G. hirsutum* pollination. They could thus enhance self-pollination by applying pollen of one flower on its own stigma. *Amegilla calens* could provide allogamous pollination through carrying of pollen with their hairs, legs, mouthparts, thorax and abdomen, which is then deposited on flowers belonging to a different plant of the same species (geitogamy) [26]. The intervention of *A. calens* on the pollination of *G. hirsutum* is especially probable since their abundance per 1000 flowers and their foraging speed were high. The positive and significant contribution of *A. calens* in the fruiting rate and the percentage of normal seeds of *G. hirsutum* is justified by the action of this bee on the pollination of visited flowers. This significant contribution of *A. calens* on the fruiting rate and the percentage of normal seeds of *G. hirsutum* is in agreement with similar findings for *Apis mellifera* [18,3,20] and *M.* vulpina [19] on the same Malvaceae.

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