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Repercussions of *Eucara macrognatha* (Hymenoptera: Apidae) duration of visit on the pollination rate and yields of *Abelmoschus esculentus*

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1 SUMMARY

Field experiments were conducted in Domayo (Maroua-Cameroon) to determine whether the duration of a visit of a wild bee *E. macrognatha* affected the pollination rate and the productivity of *A. esculentus* (Okra). Random samples of 30 experimental plants were used. The experiment included flowers having benefited from a single bee visit. Linear regressions were established between the duration of *E. macrognatha* visit and parameters related to Okra production such as pollination rate and number of normal seeds. Another correlation was established between the pollination rate and the fruit length. Results showed that the pollination rate and the number of normal seeds per fruit increased with increasing duration of the wild bee visit. Thus, the pollination rate (R^2 = 0.55; n = 30; *df* = (1; 28); $P < 0.001$) and the number of normal seeds (R^2 = 0.44; n = 30; *df* = (1; 28); $P < 0{,}001$) were positively correlated with the duration of a wild bee visit. In addition, the fruit length of Okra was correlated with the pollination rate (R^2 = 0.53; n = 30; *df* = (1; 28); $P < 0{,}001$). Therefore, the duration of *E. macrognatha* visit is important to the pollination and influences both the seed yield and the fruit length of *A. esculentus.*

2 INTRODUCTION

Several studies have already been done on plant-insect relationships regarding the proportion of floral products harvested by foragers (Tchuenguem *et al.*, 2007; 2008a, b; 2010a, b; Benachour and Louadi, 2011). It appears that the interest aroused by an insect for gathering nectar or pollen depends mostly on the quality, the quantity, the accessibility and the availability of each of these floral products (Tepedino, 1981; Schneider and Hall 1987; Roubik *et al.*, 1995; Segeren *et al.*, 1996; Pierre *et al.*, 1996). The above-listed factors associated with interruption of visits, predation and some abiotic parameters as wind strength and rainfall govern an important pollination parameter namely, the duration of visit (Mc Gregor, 1976; Jacob-Remacle, 1989; Tchuenguem *et al.*, 2008a, b). The duration of a floral visit varies, for an insect, with the plant species, and for a given plant species, with the floral product harvested; in addition, the duration of visit may vary for a given plant species and for the same coveted floral product with insect species (Collison and Martin, 1979; Tchuenguem *et al.*, 2007; 2008a, b). *Abelmoschus esculentus* flowers are solitary, axillary and cream-colored, yellow or golden yellow with a dark red zone at the base of the petals; they exhibit each five free petals. The longitudinal section of the flower of

A. esculentus shows a super ovary containing several eggs. The ovary is topped by a style of variable length according to variety. The style is capped at the anterior end by a flat stigma, rounded and dark purple. The stigma is into direct contact with the anthers in variable numbers enclosed by the style (George, 1989). Okra is self-compatible and self-pollination can take place in its hermaphrodite flowers (Al Ghzawi *et al*., 2003). The flowers open in the early hours of dawn. Anthers are dehiscent at anthesis (George, 1989). After fecundation, the flowers are pink colour, the petals close and fall with the calyx and the staminal column to which they are attached at the base. Okra pollen grains are large and echinate; their diameter is 156 µm and the average length of the spines which cover it is 20µm (Vaissière and Vinson, 1994). Flowers of Okra are insect pollinated (Free, 1993; Al Ghzawi *et al*., 2003; Azo'o *et al*., 2011; 2012). Two wild bees, *Eucara macrognatha* Gerstaecker and *Tetralonia fraterna* Freise were found as the main pollinators of this vegetable in 2010 cultivating season (Azo'o *et al*., 2012). *Eucara macrognatha* Gerstaecker 1870 belongs to the tribe Eucerini often called long-horned bees. This tribe is the most diverse in the family Apidae, with over 32 genera worldwide (Michener, 2007). Species are solitary, though many nest in large aggregations, and it is occasionally possible to find large sleeping aggregations of males (Michener, 2007; Eardley *et al*., 2010). Previous results of Azo'o *et al*. (2011) indicate that the flower of Okra is very deep; this makes it difficult for foragers to reach nectaries, which lie in its bottom. Moreover, the mean value of the sugar contains of the nectar of *A. esculentus* is lower than 30% (Azo'o *et al*., 2012); this proportion is suggested to be unable to allow a net energy gain for bee foragers (Proctor *et al*. 1996). Thus, the visits of *E. macrognatha* identified on *A. esculentus* flowers in the locality of Domayo (Maroua) in 2010 were only devoted to the collection of pollen (Azo'o *et al*., 2011); Furthermore, the average value of the duration of a floral visit registered was 892 seconds for this wild bee species (Azo'o *et al*., 2012). This value appears time consuming and seems to play a significant role in the pollination of Okra whose flowers are endowed with a faculty of passive self-pollination (Hamon and Koechlin, 1991). The main objective of this study is to highlight the importance of the duration of a bee visit as a pollination factor on *A. esculentus*. The specific objectives were to establish the effect of the duration of *E. macrognatha* visit on the pollination rate of *A. esculentus* as well as on the number of mature seeds and the length of fruits produced.

3 METHODOLOGY

The study site was located at the Technical School of Agriculture at Domayo in the neighbourhood of Maroua (10°35' North and 14°20' East) in the Far North Region of Cameroon. The climate is of the Soudano-Sahelian type with two seasons; the dry and rainy seasons. The former runs from November to May followed by the latter that runs from June to October. The rainfall recorded in 2010 stood at 1002.9 mm and temperatures ranged from 27 to 36°C during the Okra growing season, which are good conditions for *A. esculentus* cultivation (George, 1989). Experiments were done on an area of 2500 m^2 during the rainy season. On 20th July 2010, three Okra seeds of "Clemson spineless" variety were sown distanced by 50 cm within and apart on rows. Plantlets were trimmed to one per hole, two weeks after sowing. Direct observations on flowers were made daily for 8 days (from 27th August to 13th September 2010) of the blooming period and between 6:00 am and 8:00 am. To assess the effect of the duration of a visit of *E. macrognatha* in the pollination rate and the productivity of *A. esculentus*, six-man observer team was positioned in the study field. Each observer was placed 2 m away from a newly opened flower of a given plant from 6:00 am (local time) early in the morning, before the arrival of wild bee foragers. Each flower was monitored until it received a single visit by *E. macrognatha*. The duration of each floral visit by the bee concerned was recorded by the observer

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using a stopwatch. In the particular case of the present study, the duration of visit is defined as the time spent by *E. macrognatha* to collect pollen from *A. esculentus* flowers. The stopwatch was brought down to zero before the arrival of the forager on a flower of *A. esculentus*. It was activated at the time when an individual of *E. macrognatha* landed on the flower and was stopped when the wild bee was leaving the flower visited. The duration of a visit corresponded to the value read on the stopwatch when *E. macrognatha* left the flower. During the floral activity of an individual, its foraging behaviour was registered. After a bee visit, the flower was bagged with a hydrophilic plastic bag (12 x 16 cm) until the next day to avoid any additional insect visitation, after which the flower and the equivalent plant were also tagged. Overall, random samples of 30 experimental plants were used. For each plant, only the first flower at the base was considered. The bee visit on each flower was register between 6:10 and 6:25 am. Two weeks after anthesis, each experimental fruit was harvested and tagged for future analysis. The total length was measured using a soft tape to assess the fruit size; then it was cut longitudinally for seed counts. The developed seeds and aborted ovules were counted and the pollination rate of a flower was defined as the proportion of ovules that developed into seeds in the fruit (Gingrass *et al.*, 1999). Data were analyzed using descriptive Statistics. The means were given with their standard deviation. Linear regressions were established between the duration of visit, the pollination rate and the number of normal seeds, and then between the pollination rate and fruit length of *A. esculentus*. The values of the duration of visits failing to verify normality were converted under the form log10 (x + 1) (Gingrass *et al.*, 1999). Statistical analyzes were facilitated through the SPSS software.

4 RESULTS

Data contained in Table 1 showed the duration of a visit of *E. macrognatha* on each flower observed, the length of each fruit from the labelled flower, the number of normal seeds and aborted seeds per fruit from the experienced flower and pollination rate that is deducted from the above-mentioned number of seeds. It is thus clear from the table that for a flower of a given plant, correspond the duration of a visit of *E. macrognatha* and therefore the length of the fruit from of the flower, the number of normal seeds, the number of aborted seeds and the pollination rate. From this table, it also appears that the longest floral duration of *E. macrognatha* visit was to 3420 seconds (57 minutes).

Table 1: Data related to the single visit of *Eucara macrognatha* on thirty Okra flowers

N° Plant	Normal seeds/fruit	Abortive seeds/fruit	Pollination rate (%)	Fruit length (Centimetre)	Duration of visit log10 (Seconds)
1	77	41	65.25	13.7	1.79
2	83	32	72.17	14.6	2.08
3	84	29	74.33	14.6	2.25
4	88	26	77.20	14.8	2.25
5	98	19	83.76	16.2	2.25
6	89	21	80.90	15.2	2.38
7	88	23	79.28	15.1	2.38
8	89	27	76.72	15.8	2.48
9	94	23	80.34	16.4	2.55
10	92	19	82.88	16.2	2.62
11	97	21	82.20	16.4	2.62
12	96	17	84.95	16.7	2.68
13	97	31	75.78	16.8	2.68
14	105	9	92.11	16.1	2.73
15	90	29	75.63	16.4	2.73
16	98	18	84.48	16.2	2.73
17	94	21	81.74	15.4	2.73

18	92	19	82.88	14.8	2.73
19	95	23	80.51	15.6	2.78
20	102	17	85.71	17.6	2.86
21	94	24	79.66	16.3	2.92
22	95	22	81.20	15.6	2.95
23	97	19	83.62	17.1	2.98
24	101	16	86.32	17.2	3.10
25	103	17	85.83	17.6	3.19
26	92	16	85.18	17.3	3.36
27	105	11	90.51	17.2	3.37
28	87	19	82.07	16.9	3.41
29	98	16	85.96	17.7	3.47
30	108	9	92.30	18.2	3.53
M ± SD	**94.26 ± 7.00**	**21.13 ± 6.87**	**81.72 ± 5.85**	**16.20 ± 1.20**	**2.95 ± 2.82**

Legend: M ± SD = Mean ± Standard Deviation; N° = Order; % = percentage.

To this value, corresponds a fruit of 18.2 cm, the longer got into the experiment; this fruit contains 108 normal seeds and 09 aborted seeds for a pollination rate of 92.30%. Similarly to the shorter recorded floral duration of visit which is 62 seconds, is derived a fruit of 13.7 cm containing 77 normal seeds and 41 aborted seeds for a pollination rate of 65.25%. Thus, the longer the duration of a wild bee visit on Okra flower, the lower the number of abortive seeds, the higher the number of normal seeds per fruit and important one is the pollination rate that results; also, the shorter the duration of a visit, the higher the number of abortive seeds, the lower the number of mature seeds per fruit and reduce is the pollination rate that results.

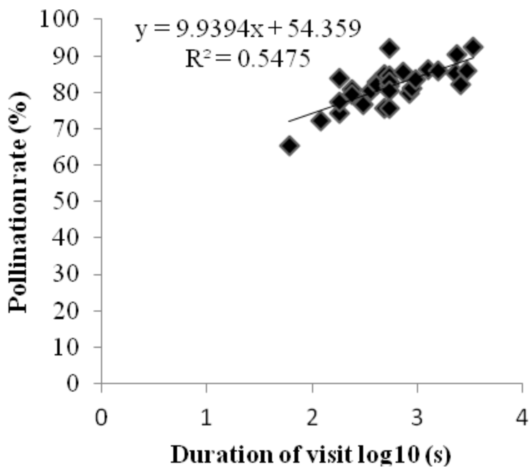

Figure 1: Linear regression between the duration of visit and the pollination rate.

Figure 1 shows the linear relationship between the pollination rate and the duration of a visit of thirty flowers having benefited each of a single visit of *E. macrognatha*. It follows from this figure that the pollination rate increases with increasing duration of *E. macrognatha* visits. The linear regression between these two variables is positive and significant ($R^2 = 0.55$; n = 30; *df* = (1; 28); $P < 0.001$). Duration of visit accounted for 55% of the variation in pollination rate.

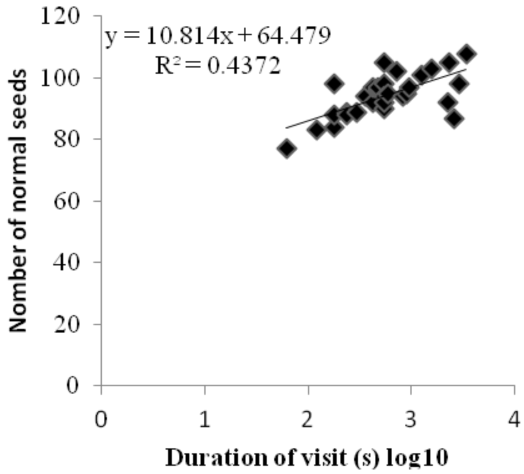

Figure 2: Linear regression between the duration of visit and the number of normal seeds.

Figure 2 shows the linear relationship between the number of normal seeds and the duration of a visit of *E. macrognatha* for thirty *A. esculentus* flowers. It follows from this figure that the number of normal seeds raises with the duration of *E. macrognatha* visits. Linear regression between the two variables is positive (R^2 = 0.44; n = 30; *df* = (1; 28); P < 0,001). Duration of visit accounted for 44% of the variation in number of normal seeds.

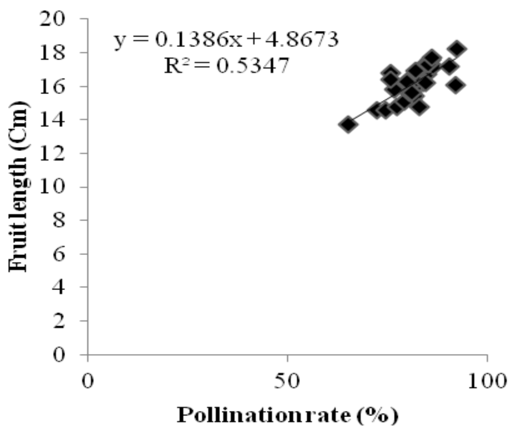

Figure 3: Linear regression between the pollination rate and the length of Okra fruits.

Figure 3 shows the linear relationship between the pollination rate and the length of a fruit of *A. esculentus* issued from thirty flowers investigated. It follows from this figure that the fruit length increases with the pollination rate imputed to *E. macrognatha*. Linear regression between the two variables is positive and significant (R^2 = 0.53; n = 30; *df* = (1; 28); P < 0,001). Pollination rate accounted for 53% of the variation in fruit length.

5 DISCUSSION

It was previously shown that self-pollination, which characterized flowers of Okra, allows the production of seeds and fruiting without any pollen deposition by anthophilous insects on the stigma (George 1989; Hamon and Koechlin, 1991; Al Ghzawi *et al*., 2003; Azo'o *et al*., 2011; 2012). As pollen grains of Okra are very large (Vaissière and Vinson, 1994) and are not wind borne (Mc Gregor, 1976), there exist on *A. esculentus* flowers, in the natural state, contact between the uppermost anthers and the lower part of the stigma. This primitive genetic trait makes *A. esculentus* a plant species that reproduces by passive self-pollination (Hamon and Koechlin, 1991). Naturally, the pollination of *A. esculentus* flowers begins prior to the opening of the flower as the stigma is already receptive and anthers dehiscent (Srivastava and Sachan, 1973; Chandra and Bhatnagar, 1975). Thus, even in the absence of pollen vector, the ovules are fertilized by self-pollen to insure pod set. Despite passive self-pollination that characterizes *A. esculentus*, the Apidae play a vital role in the pollination and productivity of this plant species (Free, 1993). This role falls especially to *E. macrognatha* that appeared as one of the most active flower-visiting insects of Okra in our study area (Azo'o *et al*., 2011; 2012). This wild bee, mainly devoted to *A. esculentus* pollen, contributed directly and efficiently to the pollination of *A. esculentus*. Indeed, a floral visit of *E. macrognatha* has appeared to have a significant additional supply of pollen grains on the stigma of the flower visited. The higher duration of *E. macrognatha* visit during pollen harvesting improved the potentiality of self-pollination in *A. esculentus* visited flower. That could be explained by the foraging behaviour of the wild bee for pollen gathering. In fact, *E. macrognatha* foragers got dusted with the *A. esculentus* pollen grains, and frequently go forth and back between the anthers and the stigma of the same visited flower. When not interrupted by a competitor insect for pollen harvesting, a predator, the wind strength or the rainfall an individual, could spend up to 30 minutes visiting a flower. During this time, its frequently contacted the anthers, got the echinate pollen grains in the body hair and could thereby do some pollination with this self-pollen before flying off. In addition, before flying away to the flower, these foragers took off and started grooming; the pollen fell from the foragers in small clumps. Overall, the latter behaviour was observed, in the main, on the top of the stigma and induced the deposition of conspecific pollen grains of the visited flower. It is known that in entomophilous plants for most pollination to be effective, it involves pollen transferred on the body of insects (Vaissière *et al*. 1996). Figure 4 illustrates the pollination activity of an individual of *E. macrognatha* on the flower of *A. esculentus* in our field study. The additional input of pollen grains deposition on the stigma of *A. esculentus* flower by the forager is quite perceptible in the latter photography.

Figure 4: Self-pollination activity by *E. macrognatha* on the flower of Okra.

The duration of a visit of *E. macrognatha* appears as an important factor in the pollination of *A. esculentus* flowers. It brought a supplementary number of pollen grains on the stigma, which had already received a quantity of pollen grains from autonomous self-pollination. This additional pollen deposition had a significant impact on the pollination rate, the number of developed seeds per pod and the length of the fruit. Thus, the higher the duration of wild bee visits, the higher the rate of pollination; therefore the number of normal seeds and the length of the fruit ensuing are maximized. This justifies the positive and significant correlations between the duration of visit, the pollination rate and the number of mature seeds. It is known that the more a flower

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is receiving pollen grains, the higher the potentiality for her to produce many seeds (Jacob-Remacle, 1989). The number of seeds determines the variation of the length of the fruits produced. Pollinic hormones, relayed by the stimulating action of the growth-promoting hormones produced by the seeds favour the length or size of fruit formed; the greater the pollination rate, the higher the fruit formed is long or large (Gingrass *et al.*, 1999); This therefore justifies the positive correlation obtained between the two variables.

6 CONCLUSION

Eucara macrognatha plays an important role in the production of *A. esculentus* crop because the duration of its visits correlated positively to the pollination rate and the number of seeds produced; the length of fruits is also correlated positively with the number of seeds per fruit from flowers benefited to a single visit of *E. macrognatha*. Therefore, the conservation of the nests of *E. macrognatha* in areas surrounding Okra in bloom is recommended to improve on the pollination rate, seed yields and fruit length of *A. esculentus*.

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