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Full Length Research Paper

The importance of a single floral visit of *Eucara macrognatha* and *Tetralonia fraterna* (Hymenoptera: Apidae) in the pollination and the yields of *Abelmoschus esculentus* in Maroua, Cameroon

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Experiments were made to determine the effects of a bee visit on the pollination and the productivity of okra, *Abelmoschus esculentus* (L.) Moench (Malvaceae). Three treatments were used on 30 randomly-selected plants each. These included Autonomous Self-Pollination (ASP) with flowers from which insects visit, with airborne pollen flow excluded, flowers that received a single bee visit (SBV) of *Eucara macrognatha* (SBV1) and *Tetralonia fraterna* (SBV2). All flowers produced fruits with or without insect visits. The two main visitors *E. macrognatha* and *T. fraterna* were effective pollinators, and of course their visits increased the proportion of ovules that developed into seeds (pollination rate), which led to longer fruits. The domestication of the two wild bees is recommended to improve the pollination and the productivity of okra in Maroua.

Key words: *Abelmoschus esculentus*, *Eucara macrognatha, Tetralonia fraterna*, Maroua, Cameroon, pollination rate, effective pollinator.

INTRODUCTION

Many insects visit flowers from which they obtain carbohydrate and protein food (Pesson and Louveaux, 1984). During this, they pollinated the visited flower (Vaissière and Froissart, 1996; Cane, 2002). The role of pollinators of many plants is well known throughout the world and their activities are essential to ecosystem functioning and agriculture (Jacob-Remacle, 1989). In many crop plant species, the honey bee (*Apis mellifera*) seems to be the main pollinator insect (Klein et al., 2007). Many techniques are now developed on habitat conservation to maintain the population of natural bees (Torchio, 1990) for the pollination services (Carreck et al., 1997; Velthuis and Van Doorn, 2006), welfare (Borneck and Bricout, 1984; Gallai et al., 2009) and wildlife (Jean, 1987; Ricketts et al., 2004; Gallai et al., 2009).

Abelmoschus esculentus (L.) Moench (Malvaceae), commonly called okra, lady's finger or gumbo, is an important vegetable in the tropics and sub-tropics that probably originated from the Ethiopian region of Africa, but now widely grown throughout Africa; particularly in Sudan, Egypt and Nigeria (George, 1989).

It is also very important in other tropical areas including Asia, Central and South Americas, and recently, there has been an interest in its growth in heated Greenhouse in northern Europe (George, 1989).

Okra is of considerable economic importance following the usefulness of its seeds and pods as food (Sawadogo et al., 2006). In Cameroon *A. esculentus* occupies a prominent place in the diet, culture and life of many ethnic groups in the Far North Region. Here, the importance of okra makes the crop a potential source for

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the improvement of the income and livelihood of the cultivators.

Okra is self-compatible, and passive self-pollination can take place in its hermaphrodite flowers (Al-Ghzawi et al., 2003). Its pollen grains are very large and echinate, 156 µm in diameter with spines over 20 µm in length (Vaissière and Vinson, 1994) so that pollination with both self and cross-pollen is possibly achieved by insects (Hamon and Koechlin, 1991; Al-Ghzawi et al., 2003). Anthesis takes place at dawn, and the flower remains open all morning and closes by noon or early afternoon.

It is wilted in the evening and the petals usually fall the next day along with the staminal column, consequently providing the insect with the possibility of efficiency in the process between 7: 00 and 10: 00 am (Azo'o et al., 2011).

In Maroua, *Apis mellifera* was poorly observed on the floral entomofauna of *A. esculentus* while *Eucara macrognatha* and *Tetralonia fraterna* were found as the main anthophilous insects of okra in 2009 and 2010 cultivating seasons (Azo'o et al., 2011). The relative high abundance of both *E. macrognatha* and *T. fraterna* observed since the cultivating season of 2009 enabled our study on parallel experiment, in 2010, on the contribution of each of the two wild bee species on the pollination and the productivity of okra via their individual pollination effectiveness. This aspect allows us to complete usefully data on the pollination of *A*. *esculentus*. In fact, foragers, mainly Apoidea, exhibit the strongest biological relationship to flowers and provide substantial economic and ecological benefits to entomophilous crops and wildlife (Klein et al., 2007; Bozena, 2009). Generally, in a given region, the mastery of the relationships between anthophilous insects and cultivated crops can permit to keep or shield certain insect species for pollination and plant production (Dequesne, 1996).

The main objective of our research, therefore, was to qualify and quantify the importance of two wild bees, namely *E. macrognatha* Gerstaecker, 1870 and *T. fraterna* Friese, 1911, shown to be the most prevalent for the pollination of okra, its pod and seed productions in Maroua. Specifically, our study's aim was to measure the impact of a single visit of both *E. macrognatha* and *T. fraterna* on *A. esculentus* pollination, fruit size and seed yield.

MATERIALS AND METHODS

Our 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 from July to October 2010. 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 carried out on an area of 2500 m^2 during the rainy season in 2010. Cleaning and ploughing of the experimental area were done prior to sowing. On 20 July 2010, three okra seeds of "Clemson spineless" variety were sown in holes of 10 cm of depth and 10 cm of diameter half-filled with a mixture of ash and soil. These holes were separated by 50 cm within and apart on rows. Two weeks after sowing (5 August, 2010), plantlets were trimmed to one per hole.

On this day, chemical fertilizer (NPK 20-10-10) was applied around each conserved plant of each hole. The weeding of the plants was manually performed for it was necessary to maintain weed-free plots. Watering was done when it seldom rained with one litre of water measured per hole at the dawn and at the twilight. Direct observations on flowers were made daily during 8 days of the blooming period and between 6:00 and 8:00 am (local time) since preliminary observations indicated that okra flowers were fully visited by the two wild bee species between 7:00 and 10:00 am (Azo'o et al., 2011). At least, five specimens of each bee species were captured with the pliers and were conserved in a box containing 70% of ethanol for future taxonomy. Bee identifications were done by Dr. Alain Pauly, Department of Entomology Royal Belgian Institute of Natural Sciences of Brussels in Belgium.

To assess individual pollination effectiveness, we used random samples of 30 experimental plants for each of the 2 treatments which are: (1) Autonomous self-pollination (ASP) in which flower buds were isolated with hydrophilic bags (12 × 16 cm; Osmolux®, Pantek France, Montesson) a day before anthesis to prevent anthophilous insect visitation and airborne pollen flow the following day. These bags were removed on the day following anthesis. The flower and the equivalent plant were tagged and (2) Single bee visit (SBV) treatments, in which our 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 bee foragers. Each flower was monitored until it received a single visit by *E. macrognatha* or *T. fraterna*. The floral product harvested (nectar or pollen) was registered for each bee species, and this was done on the basis of the foraging behaviour of each species. Nectar harvesters were seen going on the bottom of the flower and gathering this product at the level of the nectary while pollen gatherers directly scratched the anthers with mandibles or legs. Moreover, the duration of bee visits was recorded by the observer using a stopwatch.

After a bee 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; Gingras et al., 1999), after which the flower and the equivalent plant were also tagged.

For all treatments, only the first flower at the base of the plant was considered. Two weeks after anthesis, each fruit was harvested and tagged for future analysis. The total length of each fruit was measured with a soft tape to assess overall fruit size, a yield component for the fresh market, and it was then 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 mature seeds in the fruit (Gingrass et al., 1999).

During the study period, flowers of several other plant species including *Commelina benghalensis* (Linnaeus, 1753); *Cucurbita maxima* (Duchesne, 1786); *Cassia obtusifolia* (Linnaeus, 1753); *Kyllinga tenuifolia* (Steudel, 1855); *Corchorus obtorius* (Olivier, 1868); *Panicum anabaptismum* (Steudel 1854); *Pennisetum pedicellatum* (Trinius, 1834); *Acacia seyal (*Delaplane, 1970); *Ageratum conyzoides (*Linnaeus, 1753), a 1 ha plantation of *Zea mays* (Linnaeus, 1753) and a 2 hectares of *Gossypium barbadense* (Linnaeus, 1753) in bloom in the vicinity of the experimental plot were observed to attract *E. macrognatha* and *T. fraterna.*

Normal-theory statistical analysis was used on continuous variables by applying standard analysis of variance (ANOVA). The

Table 1. Parameters related to the production of okra as a function of treatments.

Parameter	ASP	SBV		*F*
		SBV1	SBV2	
Fruiting rate (%)	100[a]	100[a]	100[a]	
Pollination rate	71.01 ± 7.44[a]	81.43 ± 5.85[b]	76.60 ± 7.40[c]	16.66
Matured seeds per pod	79.83 ± 8.97[a]	94.26 ± 6.99[b]	84.13 ± 9.37[a]	22.74
Immature seeds per pod	32.43 ± 7.01[a]	21.13 ± 6.87[b]	26.60 ± 7.01[c]	15.68
Fruit length (cm)	15.08 ± 1.22[a]	16.19 ± 1.19[b]	15.21 ± 1.47[b]	08.54

Means ± SD within a column followed by the same letter are not significantly different.

method used to discriminate among the means between two treatments was the Honestly Significantly Difference (HSD) procedure. The means are given with their standard deviation (SD) and in all cases, the significant probability was computed. Means are reported as significantly different if *P* was less than or equal to 0.05, except where indicated. The contribution of the two wild bee species to the increment of the pollination rate, the number of developed ovules, the length of the fruit and to the reduction of the number of non-developed ovules was estimated based on the relative difference in these values between fruit issued from treatment 1 ASP and treatment 2 SBV1 or treatment 3 SBV2.

RESULTS

Table 1 shows the fructification rate, the pollination rate, the mean number of developed ovules per fruit, the mean number of non-developed ovules per fruit and the mean length of fruit in treatment 1 ASP, treatment 2 SBV1 and treatment 3 (SBV2). It appeared from this table that each flower turned into a fruit, regardless of the treatment it received. The pollination rate ranged from 71.01% in treatment 1 to 81.43% in treatment 2 and 76.60% in treatment 3. The difference between all the treatments was significant (F = 16.66; df = 2, 87; P < 0.05). The difference was significant between treatments 1 and 2 (P < 0.05), 1 and 3 (P < 0.05) and 2 and 3 (P = 0.02). The contribution of *E. macrognatha* to the pollination rate is 12.80% and that of *T. fraterna* is 7.29%. The mean number of developed ovules per pod ranged between 79.83, 94.24 and 84.13, and was affected by the pollination treatment. The difference between all the treatments was significant (F = 22.74; df = 2, 87; P < 0.05). The difference was significant between treatments 1 and 2 (P < 0.05), 2 and 3 (P < 0.05), but not significant between 1 and 3 (P = 0.13). The contribution of *E. macrognatha* to the increment of the matured seeds is 15.31% and that of *T. fraterna* is 5.12%.

The mean number of aborted seeds differed with the treatments (F = 15.68; df = 2, 87; P < 0.05); it was higher in the fruits from flowers that were isolated for autonomous self-pollination (mean = 32.43) than those that received a single visit of *T. fraterna* (mean = 26.60) and *E. macrognatha* (mean = 21.13). A statistical difference between the mean number of immature seeds was found between treatments 1 and 2 (P < 0.05), treatments 1 and 3 (P < 0.05) and treatments 2 and 3 (P = 0.03). The contribution of *E. macrognatha* to the reduction of the abortive seeds is 34.84% and that of *T. fraterna* is 14.90%.

The mean length of fruit per treatment was between 15.08, 16.19 and 15.21 cm. The difference between all these treatments was significant (F = 8.54; df = 2, 87; P < 0.05); there was no statistical difference observed only between treatments 1 and 3 (P = 0.90). The contribution of *E. macrognatha* to the increment of the fruit length is 6.85% and that of *T. fraterna* is 0.90%.

Overall, the pollination rate, the number of matured seeds per pod and the mean length of fruits were higher in the flowers that received a single visit of *E. macrognatha* than those that received a single visit of *T. fraterna* and those that were isolated for autonomous self-pollination. At the same time, the activity of *E. macrognatha* was higher than that of *T. fraterna* as concerns the reduction of aborted ovules.

The activity of the wild bees observed foraging on okra flowers was mainly for pollen gathering base to their foraging behaviour. All foragers of both *E. macrognatha* and *T. fraterna* were found on the flower between 6:30 and 7:30 am time period.

Moreover, *E. macrognatha* and *T. fraterna* made long visits on okra flowers. The mean duration of a bee visit was 892.00 ± 666.89 s (n = 30) for *E. macrognatha* and 345.33 ± 273.30 s (n = 30) for *T. fraterna*. These values were significantly different (F = 9.99; df = 1, 58; P = 0.002). During their activity, Neither *E. macrognatha* nor *T. fraterna* were observed foraging on the neighbouring plant species. *Gossipium barbadense*, which is in the same botanic family than *A. esculentus*, was predominantly visited by *Megachile* sp. The latest bee species however was not seen foraging on *A. esculentus.*

DISCUSSION

Autonomous self-pollination was the main pollination mode in okra at our study site, which permitted fruiting without any pollen deposition by anthophilous insects. Our results agreed with other reports (Purewal and Rhandawa, 1957; Al Ghzawi et al., 2003; Azo'o et al., 2011). As pollen grains of okra are very large (Vaissière

and Vinson, 1994) and are not wind borne (Mc Gregor, 1976), it is the natural contact between the uppermost anthers and the lower part of the stigma that enables self pollination (Hamon and Koechlin, 1991). Thus, even in the absence of pollen vector, enough ovules were fertilised to insure pod set, as was already found by Al Ghzawi et al. (2003) and Azo'o et al. (2011), on the "Clemson spineless" variety of *A. esculentus* in Jordan and in Maroua, respectively. Yet, anthophilous insects played a significant role in the pollination and the production of okra.

The two wild bee visits on *A. esculentus* flowers were prior to pollen harvesting following the deepness of the flowers; then the nectary is not easily accessible by them (Azo'o et al., 2011). In this case, the studied bee species harvested preferentially pollen and then lose low energy than that displayed for nectar gathering, as anthers were accessible to them. Moreover, the mean value of the sugar contains of the nectar of *A. esculentus* we obtained is equal to 21.74%. According to Proctor et al. (1996), the sugar contains of the nectar lower than 30% is suggested to be unable to allow a net energy gain for bee foragers.

Individual from both species of bees could spend > 30 min visiting a flower. During this time, they frequently contacted the anthers, got the echinate pollen grains in the body hair (Hamon and Koechlin, 1991) and could thereby do some pollination with this self pollen before flying off.

Generally, before flying to the flower, the studied wild bees started grooming; the pollen fell from the foragers in small clumps and could induced self-pollination. Moreover, the daily period of the bee activity on flowers, in the morning, coincided with the period of the maturation of anthers and the optimal receptivity of stigmas of *A. esculentus*. According to Srivastava and Sachan (1973), pollen fertility of okra is at maximum in the period between an hour before and two hours after the opening up of the flower.

The difference in the duration of the visits between *E. macrognatha* and *T. fraterna* could explain the difference in the pollination rate, the number of seeds and the fruit length among the fruits that resulted from a single visit of each bee species. The duration of a bee visit on *A. esculentus* flower is then decisive as it allows for the bee to increase the number of pollen grains deposited on the stigma. In fact, during their activity, the two wild bee species frequently go forth and back between the anthers and the stigma of the same flower and then could induced during their time visit self-pollination. The fact that an individual bee foraging on *A. esculentus* was not observed visiting another plant species indicated that *E. macrognatha* and *T. fraterna* show floral constancy for *A. esculentus* flowers (Pesson and Louveaux, 1984).

The difference in the body sizes between the two bee species can also explain the difference of parameters related to fruit and seed sets between treatments 2 and 3 (Azo'o et al., 2011). According to Jacob-Remacle (1989), the longer and bigger a bee is, the greater its pollination effectiveness.

The difference of the mean length of fruit between treatments 1 and 2 and between treatments 2 and 3 is related to the difference of the mean number of normal seeds between these treatments. Villières (1987) reports that, in crop plants fruit enlargement requires growth-promoting hormones, which the developing seeds release. The higher the number of developed ovules, the longer or bigger the fruit formed. Similarly, Al-Ghzawi et al. (2003) and Azo'o et al. (2011) obtained a significant difference in the number of seeds and the mean length of pods on plants isolated in a cage without insect pollinator compared to open pollinated in okra of the "Clemson spineless" variety in Northern Jordan and in Maroua, respectively.

Indeed, a floral visit by each studied bee species 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.

Conclusion

In the absence of honey bees, *Apis mellifera*, in our experimental field, *E. macrognatha* and *T. fraterna* played an important role in the pollination and probably in the production of *A. esculentus*. The two wild bee species are considered as potential pollinators, but mainly with self-pollen as they frequently go forth and back between the anthers and the stigma of the flower in question. Amongst the two wild bee species, the contribution of *E. macrognatha* is higher than that of *T. fraterna* on the pollination, the pod length and the seed number of okra. Therefore, *E. macrognatha* and *T. fraterna* could serve as backup or alternate pollinators of *A. esculentus* in Maroua. Then the conservation of the two wild-bee nests in areas surrounding okra plantations in bloom is recommended.

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