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FLORAL VISITS OF THE WILD BEE, *LITHURGUS ATRATUS*, IMPACT YIELD AND SEED GERMINABILITY OF OKRA, *ABELMOSCHUS ESCULENTUS*, IN SRI LANKA

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Abstract—Bee-pollinated crops in landscapes with a low abundance of bees suffer from insufficient pollination. The present study investigates the effect of wild bee pollination on fruit and seed production in okra, *Abelmoschus esculentus* (L.) Moench. The study was conducted in a home garden, where an okra field was established for three pollination trials each to include 40 okra plants. For each trial, three sets of 25 flower buds were selected and tagged. One set was covered to deter bees, another set was kept open to enable bee visits and the other set was cross-pollinated by hand. Of the two species of bees, *Tetragonula iridipennis* (Smith) visited flowers for nectar while *Lithurgus atratus* Smith collected and carried pollen grains. The period of stigma receptivity and pollen availability coincided with the highest activity of *L. atratus* from 10.00 a.m. to 12.20 p.m. Bee-pollinated flowers had significantly enhanced okra pod length and diameter, seed number and seed germinability compared to the trial with flowers covered to deter bees. Hand pollinated flowers also produced significantly longer pods and a higher number of seeds with higher germinability. Although there was no significant difference in pod length and diameter and seed number between hand pollination trial and bee-pollinated trial, germinability of bee-pollinated seeds was significantly higher. Present study highlights the importance of the wild bee, *L. atratus* to enhance pod size, seed number and seed germinability in okra in Kurunegala, Sri Lanka.

Keywords: Abelmoschus esculentus, Lithurgus atratus, cross-pollination, ladies' fingers

INTRODUCTION

Bees visit plants to collect pollen (source of protein) and nectar (source of carbohydrate) as food for them and provision their nests as food for the young (Michener 1974; Roubik 1989). Bees are the most important group of pollinators, visiting more than 90% of the leading 107 global crop types (Klein et al. 2007). Of the hundred principal crops that make up most of the world's food supply, only 15% are pollinated by domesticated bees (mostly honey bees, bumble bees, and alfalfa leafcutter bees), while at least 80% are pollinated by wild bees and other wildlife (Freitas & Pereira 2004). In nature, wild bees are highly diverse, differing widely in their forage preferences and flight times depending on weather conditions.

Bees have varied relationships with flowers based on the type of floral resource they specialize on. Many solitary bees are resource specialists for pollen, and rarely for nectar (Wcislo & Cane 1996). The pollen relationships of solitary bees, unlike those of social bees, vary from being general (visiting a wide variety of unrelated flower types belonging to several families) to a highly specialized. Based on the diversity of pollen gathered, bees are termed polylectic, oligolectic and monolectic. Although some bee species, such as honeybees, are polylectic and collect pollen from plants of different families, oligolectic bees restrict their pollen gathering to species in the same genus or family (Robertson 1925; Cane & Sipe 2006). Floral oligoleges are often touted as being highly efficient pollinators due to close evolutionary relationships with their preferred floral hosts and associated foraging specializations (Sampson et al. 2016). Their numerous foraging specializations often favour rapid synchronous flight, copious pollen harvest, and strong host fidelity (Strickler 1979; Liu & Pemberton 2009).

The bees belonging to the genus *Lithurgus* (Hymenoptera: Megachilidae: Lithurgini) show foraging specializations towards pollen of Convolvulaceae (Karunaratne et al. 2005a) and Malvaceae (Rust et al. 2004; Karunaratne et al. 2005a) and some on Asteraceae (Michener 2007). *Lithurgus* is a world-wide genus in tropical and warm to moderate temperate zones (Michener 2007). According to Michener (2007), *Lithurgus* nests in burrows excavated in dead, dry, often rotten wood or even in dry cow manure. Twenty-seven species of *Lithurgus* have been identified (O'Toole and Raw 1991), of which only the Old-World species, *Lithurgus atraus* is known from Sri Lanka (Karunaratne et al. 2005a).

Malvaceae is a worldwide family of herbs, shrubs and small trees with a major concentration of genera in the tropical regions (La Duke & Dobley 1995). Several members of the family are of economic importance, including Okra (*Abelmoschus esculentus* (L.) Moench) or ladies' fingers, which is native of West Africa, and is one of the important

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vegetable crops grown throughout the tropical and sub-tropical regions, and also in the warmer parts of the temperate regions (Ige & Eludire 2014). Pollination in okra, with both self and cross-pollen, is possibly achieved by insects (Hamon & Koechlin 1991; al. Ghzawi et al. 2003). Few studies (Njoya et al. 2005; Azo'oEla et al. 2011; Angbanyere & Baidoo 2014) demonstrate the need for cross-pollination by wild bees in okra to achieve optimum yields both in seed quality and seed set, though okra is capable of autonomous self-pollination (Azo'o Ela et al. 2012; Azo'o Elaet al. 2011; al. Ghzawiet al. 2003). The decline in wild bee pollinators has occurred as a result of the reduction in the provision of food and nesting resources for bees, as well as increasing fragmentation of natural habitats and floral resources. Free (1993) highlights the fact that pollination limitation results in reduced economic yields due to lower fruit set and seed set, reduced fruit or seed quality or a lower germination ability.

In Sri Lanka, okra is grown in all three zones; wet, intermediate and dry zones of the country. The extent of okra cultivation ranges from large-scale farms to small-scale home garden crop beds. Though Sri Lanka is a home range for 150 species of bees in 39 genera (Karunaratne 2004; Silva et al. 2018), no investigations have been carried out to evaluate the effect of wild bees on pollination of okra. Therefore, the present study was planned with a view to identify pollinators of okra and to investigate fruit set and seed set in the presence of bees compared to fruit set and seed set in the absence of bees and when flowers are hand pollinated.

MATERIALS AND METHODS

Study site

The study was conducted in a home garden (geographical coordinates 7^0 51'N, 8^0 33'E and 122m above sea level) with an area of 18.58×10^{-5} ha in Kurunegala, where the mean annual rainfall, temperature and day and night humidity are 2050.2 mm, 27.9°C, 71.5% and 74.4%, respectively. The study was conducted over a period of 11 months from August 2015 to July 2016.

Experimental planting design of okra

A sunny area of 6 m × 5 m was selected to cultivate120 plants of okra for the three pollination trials; open flowers to allow bee visits, covered flowers to stop bee visits, and for hand pollination. Weeds were removed manually and the soil was turned and mixed with homemade compost and dry cow dung mixed in 1:1 proportion. Sixty holes (10 holes × 6 rows) were prepared at an equal distance (45 cm apart from each other) and in each hole, two seeds of okra were planted and watered every morning and evening. No pesticides or chemical fertilizers were added to the plot. The study was started after plants reached the reproductive stage.

Recording time of flower opening, stigma receptivity and anther dehiscence

Ten mature flower buds were tagged and observed from 7:00 a.m. to record the time of flower opening, duration of open and time of wilting. The time at which each flower opened and the time of wilting were recorded.

On a sunny day, stigmas in 10 randomly selected flowers were checked for stickiness by touching the stigmas using a needle tip to determine the time and the period of stigma receptivity. Anther dehiscence of the same 10 randomly selected flowers was observed from 7:00 a.m. and the time of anther dehiscence which was evident by the presence of pollen on split open anthers was recorded.

Recording morphology and amount of pollen grains per flower

Ten flowers, in which the anthers were not yet split, were selected to investigate the number of pollen grains per anther and per flower. From each flower, one anther was removed and placed in a solid watch glass. The anther was dissected longitudinally using a fine needle point and pollen grains were removed into the solid watch glass after adding one millilitre of 50% alcohol. The number of pollen grains was counted and recorded. This number was multiplied by the number of anthers per flower to get the total number of pollen grains produced by each flower. Morphology of pollen grains was studied by mounting pollen grains using Glycerol gel (Wodehouse 1935) containing methyl green as the stain. The length was measured using an eyepiece graticule calibrated using a stage micrometer.

Identifying different species of bees visiting okra, their activity period and preferred floral resources

Before starting the pollination trials, two to three representative specimens of different species of bees visiting okra flowers were collected using a sweep net. Bees were curated using standard entomological techniques and the pinned specimens were identified using the key by Karunaratne & Edirisinghe (2008).

The activity period of bees was determined by observing their visits to two randomly selected flowers at a time, between 7.00 a.m. to 14:00 p.m. during five days of similar climate (from 11.03.2016 to 15.03.2016). The species of bees and their numbers that visit the two selected flowers during the first 20-minute period of each hour from 7.00 a.m. to 14.00 p.m. were recorded. The behaviour of bees at flowers; whether collecting pollen or nectar from flowers at the nectary was also recorded.

Determining the effect of Lithurgus atratus on fruit and seed set compared to hand pollination and pollination in the absence of bees

Pollination trials were conducted to study the effect of wild bee pollination on fruit and seed set in okra. During a period of one week, randomly selected 25 flower buds were selected and tagged for each of the three pollination trials. Randomly selected 25 flower buds about to open were tagged and kept open for bees to visit. Another set of randomly selected 25 flower buds about to open were tagged and covered by fine mesh bags to prevent visits of bees to flowers. Another set of randomly selected 25 flowers were hand pollinated as soon as the stigma becomes receptive. Pollen grains from another freshly dehisced anther of a flower from a different plant were collected onto a fine brush (No. 04 size) and were deposited on the receptive anthers of the selected flowers, after which were covered by fine mesh bags to prevent bee visits.

At full maturity, the pods produced from the three pollination trials were harvested and their length and diameter at the base were measured. Fruits were kept until they become dry to obtain the mature seeds. The seeds of each pod from the three trials were counted and recorded.

To test the germination ability of seeds obtained from the three trials, okra seeds obtained from the three trials were subjected to the standard germination test: performed in Petri dishes lined with filter papers sprinkled with water as the substratum at room temperature (Lotito & Quagliotti 1991). Water was sprinkled daily in the morning and evening until seedlings emerge. The number of germinated and ungerminated seeds from the three trials was counted.

Data Analysis

Data obtained from the study were analyzed using Minitab 14.0 and MS Excel-2013. Analysis of variance (ANOVA) was conducted to test any significant difference in length and diameter of fruits, the number of seeds per fruit and the number of germinated seeds per fruit among the three trials (open, bagged and hand pollinated flowers). In addition, two-sample t-tests were carried out to analyze where significant differences between trials lay.

RESULTS

Okra seedlings emerged in 3 days after seeds were planted. After 45 days of maturity, the plants reached the reproductive stage during which the flowers appeared. Flowers fully opened around 8.00 a.m. and the lifespan of a flower was about 6 hours. They wilted around 2.00 p.m. and fell on the following day.

Time of stigma receptivity, anther dehiscence and number of pollen grains per okra flower

Stickiness on stigmas started around 8.00 a.m. and remained until around 12.30 p.m. This period was considered as the stigma receptive period. Anther dehiscence of the 10 randomly selected flowers was observed around 8.00 a.m. on a sunny day and thereafter pollen was available in anthers. Pollen grains are sticky and of the pectinate type, being spherical in shape with spines around. The diameter of a pollen grain of okra with spines is 0.014 mm ($N = 7$) and excluding the spines is 0.010 mm ($N = 7$).

The mean number of pollen grains per anther, anthers per flower and the total number of pollen grains per flower were 31.7, 48.2 and 1520 respectively (Fig. 1).

Different species of bees visited okra flowers

Two species of bees, *L. atratus* of the Megachilidae family and *Tetragonul airridipennis* of the Apidae family visited okra flowers during the study period. A five-day field observation to count the number of bees visiting okra flowers at different time periods of the day (Fig. 2) revealed that *L. atratus* was the most abundant bee on okra flowers and the maximum mean abundance was found during 11.00-11.20 a.m. Its activity on flowers ranged from 9.00 a.m. to 1.20 p.m., which

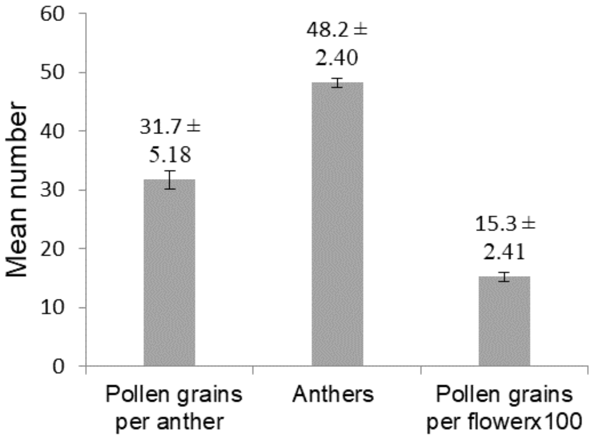

FIGURE 1. Mean number ± standard deviation of pollen grains per anther, anthers per flower and the total number of pollen grains per flower in okra.

coincided with the period of pollen availability and anther dehiscence. Compared to *L. atratus*, *T. iridipennis* was in low abundance and individuals were mostly observed from 9.00 a.m. to 12.20 p.m., with decreasing numbers through the morning. There were no bees before 8.20 a.m. and after 2.00 p.m.

The stingless bee, *T. iridipennis* collected nectar at the nectaries, while *L. atratus* collected and carried pollen from okra flowers. Close observations revealed the presence of large yellow colour pollen grains of okra on the ventral abdominal scopa of the female bees of *L. atratus*. *Tetragonula irridipennis* bees had no pollen grains on their body.

Effect of Lithurgus atratus on the pod and seed production and seed germinability in covered, uncovered/open and hand pollinated okra flowers:

Pod production

A total of twenty-five pods were collected from each of the three pollination trials. Pod length (Fig. 3(i)) and diameter (Fig. 3(ii)) were highest in pods produced by uncovered/open flowers, followed by pods produced by hand pollinated flowers, and were lowest in pods produced by closed flowers. Analysis of variance revealed a significant difference in length ($P = 0.009$) and diameter ($P = 0.005$) of pods among the three trials. Post-hoc tests revealed that the uncovered and hand-pollinated flowers produced bigger pods than covered flowers (Table 1).

Seed production and germinability

The number of seeds produced was highest in pods produced by the uncovered flowers and lowest in pods produced by covered flowers (Fig. 3(iii)). Analysis of variance revealed a significant difference in seed production ($P = 0.008$) and germinability ($P = 0.000$) of seeds among the three trials. Post-hoc tests revealed that uncovered flowers produced more seeds with greater germinability than covered or hand pollinated flowers (Fig. 3(iii), 3(iv) and Table 1).

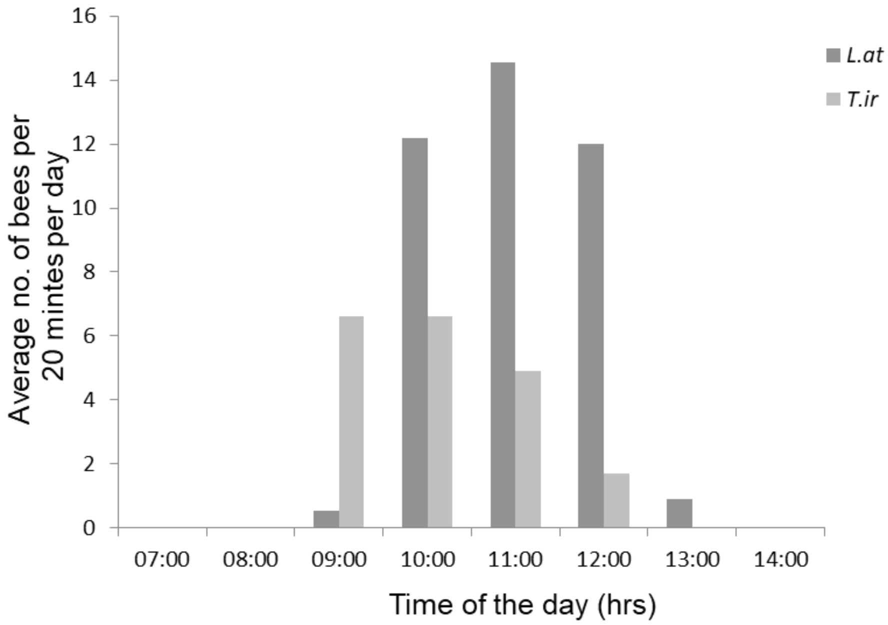

FIGURE 2. Average number of *L. atratus* and *T. irridipennis* bees that visited two okra flowers per 20 minutes at hourly intervals in five consecutive days of similar climate (Average temperature 30°C, 38% RH and no rainfall) from 11/03/2016 to 15/03/2016.

TABLE 1. Comparison of the three palliation trials; covered uncovered/open and hand pollinated okra flowers in relation to pod and seed production and seed germinability using paired sample t-test.

Paired samples	Pod length *P*-value	Pod diameter *P*-value	Seed set *P*-value	Seed germinability *P*-value
Covered – Open flowers	0.019	0.015	0.022	0.000
Open – Hand pollinated flowers	0.366	0.366	0.366	0.001
Covered - Hand pollinated flowers	0.025	0.030	0.038	0.000

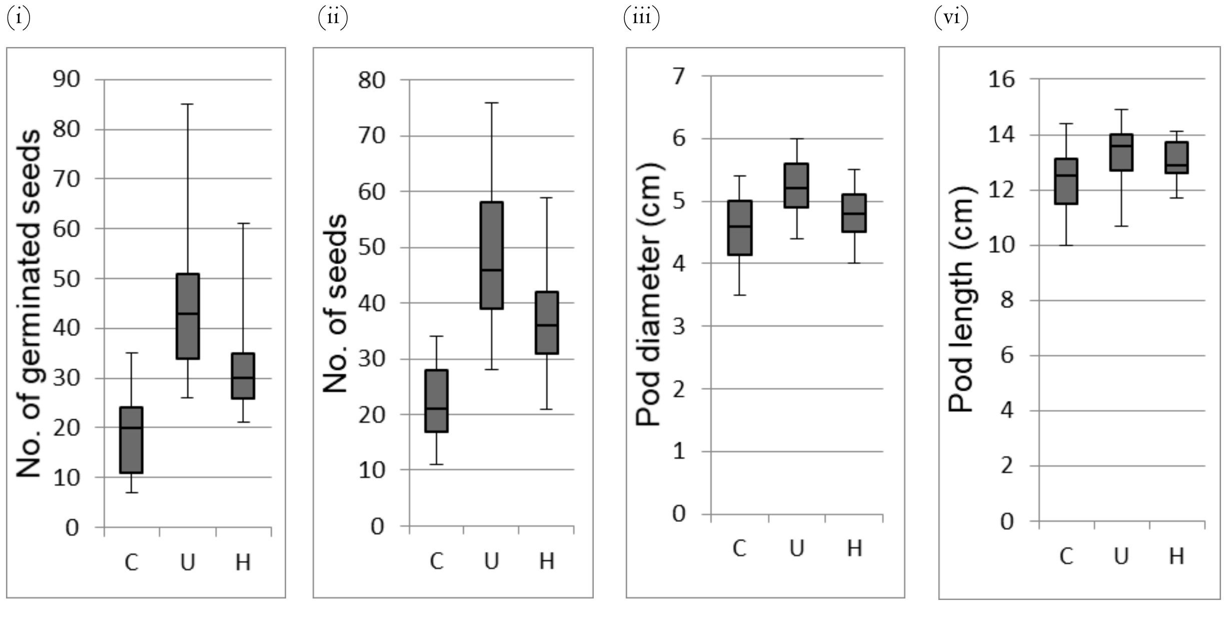

FIGURE 3. Boxplots illustrating (i) pod length and (ii) diameter and the (iii) no. of seeds produced per pod and the (iv) number of germinated seeds collected from okra pods produced from the three pollination trials: covered flowers (C), uncovered/open flowers (U) and hand pollinated flowers (H). Similar letters denote no statistically significant difference ($P > 0.05$) and dissimilar letters exhibit statistically significant differences ($P \leq 0.05$).

DISCUSSION

Wild bee visitors to okra flowers

In our study, of the two species of bees visiting okra flowers, *Lithurgus atratus* was the only species with the ability to collect and carry pollen on its body. *Lithurgus atraus* is a medium sized megachilid bee with scopal hairs on the metasomal sterna (Karunaratne and Edirisinghe 2008). These black hairs on the metasomal sterna are sparsely arranged enabling it to carry large spiny pollen grains of okra (Vaissière and Vinson 1994) that are not wind-borne (McGregor 1976). In contrast, the stingless bee, *T. iridipennis* was seen collecting only nectar from flowers. They did not collect and carry pollen from okra flowers, possibly due to their inability to collect, pack and carry large pollen grains with long spines. However, close observations of foraging behaviour of *T. iridipennis* is necessary to specify their role in pollinating okra. In a study conducted by Azo'o Ela et al. (2012) in Maroua, Cameroon, two species of wild bees, *Eucara macrognatha* (Gerstaecker) and *Tetralonia fraternal* Friese, (Apidae) were observed as the main pollinators visiting okra flowers. In Sri Lanka, two species of *Tetralonia* (Karunaratne et al. 2005a) of the three species found in Sri Lanka (*T. commixtana, T. taprobanicola, T. fumida;* Karunaratne et al. 2005b) have been observed visiting mostly Convolvulaceae flowers and other *Hibiscus* species. All these species are medium-sized bees adapted to carry large spiny pollen grains from Malvaceae and Convolvulaceae flowers.

In the present study, *L. atratus* visits to flowers coincided with the pollen availability and stigma receptivity in okra flowers enabling both self and cross-pollination by the bees. According to Azo'o Ela et al. (2012), daily activity of bees in the morning coincided with the period of the maturation of anthers and the optimal receptivity of stigmas of okra. Pollen fertility of okra is at a maximum in the period between an hour before and two hours after the opening up of the flower (Winfree et al. 2014), facilitating maximum pollination by the visiting bees. According to Winfree et al. (2014), pollination efficiency of any bee species is determined more importantly by overall foraging abundance. In the present study, the abundance of *L. atratus* was high when stigmas were receptive and anthers dehisced.

The Genus *Lithurgus* includes bees that excavate their own nests in woody substrates (Potts et al. 2005; Vitale & Vazquez 2017) close to their nectar and pollen sources. Their nutrient requirement is high as pollen is needed for the development of their young ones. Hence, they tend to nest near to food-rich habitats. Blaauw & Isaacs (2014) observed that the abundance of wild pollinators, pollination, and the yield was enhanced in high bush blueberry plantations that were adjacent to wildflower patches. Therefore, wildflower patches next to the crop field and the availability of nest substrates may increase the abundance of bees and hence the yield in okra.

The efficiency of visits of wild bees on pod and seed production and seed germinability in okra

Increased pod size, seed set and germinability, important aspects of yield in okra, were higher when flowers were exposed to bee visitation. Angbanyere & Baidoo (2014) also indicated that insect pollination helps to improve the yield of okra. We found that seed set and seed germinability were lower in hand pollinated flowers than open ones, possibly because damage was caused to pollen grains during the transfer of pollen using a fine brush, or because hand pollinators were less efficient compared to the bees, which may have specifically adapted to collect and carry pollen. A study conducted in Cameroon (Njoya 2005) to investigate the effect of wild bee pollination on the reproductive performance of okra revealed that hand and insect pollination of okra flowers gave seed sets varying between 73–84% per pod which differed significantly from that of the covered flowers that only allowed spontaneous self-pollination, with 57% seed sets per pod. Azo' o Ela et al. (2012) also showed a significant contribution by bees to produce longer fruits and more seeds. As okra is propagated by seeds, the next generation of the crop depends on the germinability of the seeds. Therefore, the present study emphasizes the importance of cross-pollination by *L. atratus* to produce okra seeds with a significantly higher germinability. This may be a result of more viable pollen deposition by bees. However, further studies to investigate the role of individual visits of pollen loaded different wild bee species to okra flowers enabling pollination would shed more light on this finding.

Previous studies (Al Ghzawi 2003; Azo'oEla et al. 2011) have concluded that autonomous self-pollination is the main pollination mode in okra, which permits fruiting without any pollen deposition by anthophilous insects. According to Hamon & Koechlin (1991), natural contact between the uppermost anthers and the lower part of the stigma enables self-pollination. In addition, the behaviour of stingless bees within flowers may drop pollen on stigmas of flowers facilitating self-pollination. However, many studies in different parts of the world, and our present study indicate significant additional increases in pod size, seed number, and seed germinability in the presence of wild bee pollinators in okra. However, the contribution of visits by different wild bee species in different ecosystems, during different seasons, to okra pollination should be further evaluated.

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