INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
08–142/CCHS/2009/11–1–77–80
http://www.fspublishers.org

Full Length Article

Role of Insects in Cross-pollination and Yield Attributing Components of *Sesbania sesban*

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ABSTRACT

This experiment was performed in an effort to know the pollination biology and ecology of *Sesbania sesban*. Different yield attributing components were measured in three types of pollination treatments i.e., wind pollination, self pollination and cross pollination by insects. Cross pollinated inflorescences produced maximum number of pods (4.41) and maximum seed weight (1.1 g) as compared to wind pollinated (2.42 pods & 0.72 g seeds inflorescence^{-1}) and self pollinated inflorescences (2.25 pods & 0.46 g seeds inflorescence^{-1}). Germination was also better in cross pollinated inflorescences as compared to self and wind pollinated inflorescences. *Megachlie bicolor* and *Apis dorsata* exhibited most efficient foraging behavior, whereas *A. floera* proved to be the most abundant pollinator with highest visitation index of 0.74. Most of the pollinator activity took place in afternoon (1530 h–1830 h).
Key Words: *Sesbania sesba;* Pollination biology and ecology; Bee pollinators; Reproductive success

INTRODUCTION

Perennial *Sesbania sesban* is used in various agro-ecosystems around the world in a variety of ways. It can be used as fodder crop, green manure and fuel wood (Evans & Rotar, 1987; Macklin & Evans, 1990; Weigand *et al*., 1995) different parts are used in medicines for human and livestock (Woodward, 1988). It is native to Africa but subsequently distributed to almost every country of the world (Evans, 2001). Despite of its wide adaptation and multiple uses, little research has been done on the mating behavior, pollination biology and ecology of this species (Brewbaker, 1990; Gebermariam *et al*., 2002). Knowledge of pollination biology, ecology and breeding system is essential to determine genetic structure of population (Brown & Allard, 1970; Heering, 1994). *Sesbania sesban* was one of the five species included in the genetic improvement programme for agroforestry development in bimodal rainfall highlands of Eastren and Central Africa agro-ecological zones (Owuor & Owino, 1993). It is important to know the degree of self and cross pollination in *S. sesban* as it helps in developing some sampling techniques helpful for getting maximum information about genetic recombinations. Cross pollination in *S. sesban* takes place by a variety of bee species (Evans & Rotas, 1987).

Plant species relying on animal (*e.g.*, insects) vectors for pollination may evolve specialized flowers as a result of mutualistic interactions with certain species (Herrera, 1989). If specialized visitors are sometimes scarce or absent (*e.g.*, Herrera, 1990; Ågren, 1996) these plant species may be at a greater risk of pollinator limitation to seed production (Jordano, 1990; Sun, 1997). Native bees are very important and specialized visitors of papilionoid flowers of *S. sesban* (Evans & Roter, 1987) therefore the knowledge of native bee diversity visiting on *S. sesban* flowers and their role in crop reproductive success is very important in order to maximize the seed yield of *S. sesban*.

The current study aimed to provide some basic information about reproductive biology and ecology of *S. sesban* by finding the diversity of flower visiting bees, their relative abundance, population dynamics and ultimately their role in crop reproductive success.

MATERIALS AND METHODS

Study site. The study was carried out from August to November, 2007 vegetative season (August-November). The study site is located at research farm of University College of Agriculture, Bahauddin Zakariya University Multan, Pakistan (30.255º North Latitude & 071.513º East Latitude). Climate of the area is sub-tropical with hot summer and cold winter; the mean daily maximum and minimum temperatures are in the range of 38 to 50°C and 8 to 12°C, respectively with the mean monthly summer rainfall is *ca.* 18 mm.

Pollinator community. Pollinator community on *S. Sesban* was recorded in whole flowering season. To identify the pollinator species, collection was done throughout the

To cite this paper: Sajjad, A., S. Saeed, W. Muhammad and M.J. Arif, 2009. Role of insects in cross-pollination and yield attributing components of *Sesbania sesban*. *Int. J. Agric. Biol.,* 11: 77–80

flowering season using hand net. The collected pollinators were identified to the lowest taxonomic level by using standard identification keys developed by Department of Biology, Valdosta State University. These keys are available at http://chiron.valdosta.edu/jbpascar/Intro.htm.

Population dynamics of pollinators. Population dynamic and attendance (mean activity rate) of different pollinators was recorded through out the flowering period with seven days interval. Data was recorded from 1500 h to 1800 h at time of opening of flowers. Twenty plants were selected randomly for this purpose and observed for 40 seconds each and counted for the number of individuals of each insect species per plant and the observation were repeated with one hour of interval. Visitation rate of an insect species was also estimated in a relative fashion. We used visitation Index (*I*VR) (Talavera *et al*., 1999), defined as the product of its frequency at the flower (*F*) and its mean activity rate or attendance (AR) i.e., *I*VR= *F* x AR. Meteorological data of average temperature and relative humidity was also measured. Insect frequency at the flower (F) is defined as number of individuals of an insect species relative to the total number of insects included in the census.

Foraging behavior. Foraging behavior (time stayed by an individual insect specie per inflorescence per visit) was counted by using a stop watch in such a way that when the insect sat on the inflorescence, the time was started and counted for number of flowers visited by that insect specie. Linear regression analysis was done between number of flowers visited per inflorescence per visit and time spent per inflorescence per visit *Xylocopa* sp. was very less frequent visitor therefore it was omitted from this study.

Pollination biology. For the comparison between different pollination methods i.e., self pollination, wind pollination but no insect pollination and open pollination (wind & insect pollination) an experiment was performed. For this purpose 60 un-opened individual inflorescences of the same age were selected and counted for the number of flowers per inflorescence. Out of these 60 inflorescences, 20 individual inflorescences were veiled with butter paper bags (for self pollination). Other twenty individual inflorescences were veiled with nylon mesh bags (wind pollination but no insect pollination) and remaining 20 inflorescences were only tagged and kept exposed for insects and wind pollination.

After two days, the inflorescences were un-veiled and counted for number of flowers shed and number of fertilized flowers. Then these were set to their fate up till crop matures. On the maturity of crop, pods were harvested and counted for number of pods matured per inflorescence, number of seeds per pod, pod length, pod weight and seed weight. To get more reliable yield contributed by the three pollination treatments, seed weight per inflorescence was calculated by the product of number of mature pods per inflorescence and seed weight per pod. Seed produced by the three pollination methods were tested for germination.

The data of number of average number of flowers per inflorescence, number of fertilized flowers, number of seeds per pod, pod length, pod weight, Seed weight per pod and seed weight per inflorescence were subjected to the statistical analysis using Analysis of Variance. Means were separated by Least Significant Difference (LSD) test at P= 0.05 using MSTAT-C software (Steel & Torrie, 1890).

RESULTS

Pollinator community of the *S. sesban* was composed of six bee species. These include *Apis florea*, *A. dorsata*, *Megachile bicolor*, *Megachile* sp., *Xylocopa* sp. and a *Nomia* sp. *Nomia* sp. was very rare therefore excluded from our studies.

A. florea proved to be the most frequent pollinator (Table II) having average population of 1.28 individuals per plant per 40 sec and maximum visitation index (0.74). *A. dorsata* remained the second most frequent visitor (0.59 individuals per plant per 40 sec). The two *Megachile sp.* also remained active in low attendance. *Xylocopa* sp. was very less frequent pollinator of *S. sesban* i.e., 0.06 individuals per plant per 40 sec with visitation index less than 0.01.

The flowers visited–time spent per inflorescence relationship (Table III) was linearly significant for four pollinator species. This suggests that each pollinator species visited different number of flowers with different stay times. *M. bicolor* proved to be the most efficient visitor having higher explicative value (R^2= 0.87) followed by *A. dorsata* (R^2= 0.36) and *Megachile* sp. (R^2= 0.26). *A. florea* was very lazy forager (R^2= 0.04) and covered only 1.34 flowers on average in 16.71 seconds of visit per inflorescence (Table II).

As the flowers opened in afternoon, most of the insect visitation took place in afternoon (1530 h to 1830 h). Therefore data of population dynamics was recorded in this interval of the day.

Results showed (Fig. 1) that *A. florea* started its activity earlier (1530 h) than *A. dorsata* (1630 h) and attained its peak at 1630 h. However, *A. dorsata* got its peak activity at 1730 h, which was the end point of *A. florea* activity. *A. dorsata* observed feeding in later hours (1830 h), near and shortly after sunset. Other three bees i.e., *M. bicolor*, *Megachile* sp. and *Xylocopa* sp. remained active in low population from 1630 h to 1730 h.

There was no fluctuation in temperature and relative humidity around the flowering period and average temperature and relative humidity remained 28.25ºC and 73%, respectively. Weather also remained clear in all the dates of data record.

There was no significant difference between numbers of flowers per inflorescence among the three pollination treatments i.e., nylon mesh, butter paper and open pollinated. This is because inflorescences of the same age and same size were selected for the experiment. Flower and pod shedding is a common phenomenon of leguminous crops including *S. sesban*. Flower shedding was also on the

Table I. Yield attributing components of *S. sesban*

Treatments	Flowers Inflorescence^{-1}	Fertilized flowers inflorescence^{-1}	Mature pods inflorescence^{-1}	Seeds pod^{-1}	Pod length (cm)	Pod weight (g)	Seed weight (g pod^{-1})	Seed weight (g inflorescence^{-1})	Germination (%)
Nylon mesh (Self/wind pollinated)	*11.00±2.0 a	8.00±3.46a	2.42±1.38b	23.74±4.94a	7.07±2.60a	0.48±0.14a	0.30±0.07a	0.72±0.38b	10±2.7
Butter paper (Self pollinated)	12.00±2.08 a	8.50±2.77a	2.25±0.75b	18.83±5.01b	130.71±3.04b	0.39±0.11a	0.21±0.07b	0.46±0.15b	9±2.1
Cross-Pollinated (Insect pollination)	1.42±1.17 a	8.75±1.71a	4.41±1.83a	3.73±3.70	16.85±2.11a	0.38±0.11a	0.25±0.07ab	1.1±0.54a	16±4.2

*Mean±SD; n=12 for each treatment. Mean values sharing same letter in respective columns show non-significant differences (P<0.05)

Table II. Average pollinator attendance, visitation index (IVR), and visitation behavior of different insect pollinators

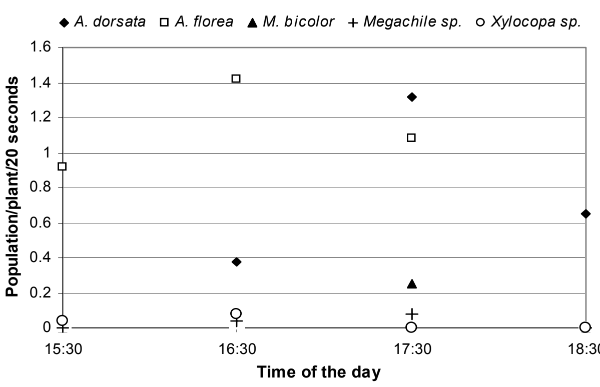
Pollinators	Pollinator attendance (Population plant^{-1} 40^{-1} sec)	Visitation Index IVR	Visitation behavior	
			Time stayed/inflorescence visit^{-1}	No. of flowers visited inflorescence^{-1} visit^{-1}
Apis dorsata	0.59 ± 0.21	0.16	12.8 ± 10.18	2.14 ± 0.53
A. florea	1.28 ± 0.63	0.74	16.71 ± 8.88	1.34 ± 0.80
Xylocopa sp.	0.06 ± 0.02	<0.01	-	-
Megachile bicolor	0.24 ± 0.18	0.01	4.13 ± 2.03	1.73 ± 0.88
Megachile sp.	0.17 ± 0.04	0.01	4.20 ± 1.93	1.87 ± 0.74

Table III. Linear regession analysis between Number of flowers visited and time spent

Pollinators	Linear model	R²	F	P	n
Apis dorsata	y = 1.42 + 0.05x	0.38	20.80	<0.0001	35
A. florea	y = 1.16 + 0.01x	0.04	1.54	0.224	35
Megachile bicolor	y = 0.048 + 0.40x	0.87	93.02	<0.0001	15
Megachile sp.	y = 1.03 + 0.19x	0.26	4.73	0.049	15

Y=Number of flowers visited inflorescence^{-1} visit^{-1}, X= Time spent (seconds) inflorescence^{-1} visit^{-1}

Fig. 1 Population dynamics of different pollinators in afternoon (1530 to 1830 h)



same pattern in all the three pollination treatments as numbers of fertilized flowers were also statistically similar for the three treatments. This means that all the flowers, which did not receive insect visitation (nylon mesh) or air current (butter paper) were able to self pollinate as well.

Comparison of means of number of mature pods per inflorescence (*F*= 10.34; *P*<0.001) revealed that the maximum numbers of pods (4.41) were successfully matured in open pollinated inflorescences (Table I). Pod maturation success in other two treatments i.e., Nylon mesh and butter paper were statistically at par i.e., 2.42 and 2.25 pods per inflorescence.

Statistically similar seeds per pod (*F*= 3.49; *P*= 0.048) were observed in nylon mesh and open pollinated treatments i.e., 23.74 and 23.73, respectively. Whereas only 18.83 seeds per pod were produced in inflorescences covered with butter paper. Likewise, pods produced in nylon mesh bags and open pollination were statistically similar in lengths (*F*= 6.64; *P*=0.0055) having mean lengths of 17.07 and 16.85 cm, respectively followed by pods produced in butter paper i.e., 13.71 cm.

On the other hand, there was no significant difference in pod weights per inflorescences (*F*= 2.23; *P*=0.1311) were recorded in all the three treatments. Whereas a significant difference was observed in seed weight per pod among all the three treatments (*F*= 4.83; *P*=0.0182). The maximum seeds weight (0.30 g) per pod was recorded in nylon mesh cage followed by open pollinated and butter paper i.e., 0.25 and 0.21 g, respectively.

Open pollinated inflorescences produced maximum number of seeds per inflorescences (*F*= 8.60; *P*=0.0017) i.e., 1.1 followed by other two treatments, which were statistically similar as well. Germination percentage was maximum (16%) in the seeds produced by open pollination as compared to the seeds produced in butter paper and nylon mesh bags i.e., 9 and 10%, respectively.

DISCUSSION

We recorded only the bee species i.e*., Apis dorsata, A florea, Megachile bicolor, Megachile* sp.*, Nomia* sp. *and Xylocopa* sp. Our this finding is in accordance with Gebremariam (2002); Heering (1994); Arroyo (1981);

Brewbaker (1990); Evans and Roter (1987) who found that *S. sesban* flowers are visited by a wide range of bee species including *A. mallifera*, *M. bituberculata* (mason bee), *Chalicodoma* sp. (leaf cutting bee), *Bombus canariensis*, *Xylocopa flavorufa* and *X. somalica*. This is because floral structure of *S. sesban* with pollen and nectar concealed in the papilionoid flowers (Burbidge, 1965) seems to be suited for cross pollination by bees.

Abundance of pollinators may increase the out-crossing rate (Gebremariam *et al*., 2002), which may vary greatly among and within population (Brown *et al*., 1989). In our study most of the pollinator visitation took place in afternoon (1530–1830 h). This is because *S. sesban* flowers open in afternoon (Heering, 1994) and pollinators are only attracted when the flowers are opened.

Flower and pod abortion is a common phenomenon in *S. sesban*. Out-crossing is probably the preferred method of reproduction under natural conditions but selfing is clearly possible (Brewbaker, 1990; Heering, 1994). According to FAO (1961) *S. sesban* does not require isolation for pure seed production due to its self-compatibility. Our results are also in agreement with this as there was no significant difference between number of fertilized flowers in cross pollinated (open pollinated) and self-pollinated (butter paper & mesh bags) inflorescences. Self-pollination may lead to inbreeding depression (Seavey & Bawa, 1986) and it may affect the latter stages of lifecycle. As in our study, pod abortion was greater in self-pollinated inflorescences (Nylon mesh & butter paper) as compared to open pollinated treatment. Contrary to our findings, Heering (1994) suggested that pod abortion is a common phenomenon and self or cross pollination is not a limiting factor in it.

There were 23.73 seeds produced per pod under open pollination, where as self pollinated pods produced 18.83 seeds. Similar results were obtained by Heering (1994) who found 26 seeds from open pollinated pod and 20 seeds from self pollinated pod. Selective abortion is also indicated by the pattern of seeds produced within the pod. Bawa and Webb (1984) observed greater seed settings in the distal end of the pod. Self pollination occurs latter in the flowering period (Gebremariam *et al*., 2002) when pollinators fail to visit the flowers. Such a "delayed selfing" favors the plant when pollinators are limiting.

In conclusion, bees played very important role in cross pollination of *S. sesban* and ultimately crop reproductive success by increasing seed weight per inflorescence (1.1 g) and germination (16%) as compared to other two treatments of self and wind pollination. In addition to cross pollination, further studies should be done on pollination potential of different native bee species, which may influence cross pollination by having different abilities of depositing pollen on stigma based on body size and shape.

Acknowledgement. We thank Terry Griswold, USDA-ARS Bee Biology and Systematics Laboratory Utah State University, Utah for identification of *Megachlie bicolor*.

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(Received 19 May 2008; Accepted 26.June 2008)