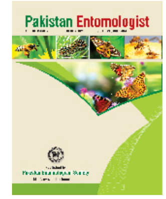
Pakistan Entomologist

Journal homepage: www.pakentomol.com



VARIATION IN POLLINATOR ASSEMBLAGE AND REPRODUCTIVE PERFORMANCE OF WHITE CLOVER (*TRIFOLIUM ALEXANDRINUM* L.)

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ARTICLE INFORMATION

Received: April 4, 2014
Received in revised form: November 6, 2014
Accepted: December 17, 2014

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ABSTRACT

The study was conducted to understand the variation in pollinator assemblage due to three different land use types (forest edge, hilly rain torrent and agricultural land) and its ultimate impact on reproductive performance of *Trifolium alexandrinum* at district Dera Ghazi Khan (Punjab) Pakistan. Two parameters of pollinator assemblage i.e. abundance and species richness and four parameter of reproductive success of *T. alexandrinum* were studied i.e. number of seeds/head, seed weight/head, number of abnormal seeds/head and germination. Abundance and species richness were the highest near forest edge and in hilly rain torrent, respectively while both were the lowest in agricultural land. Reproductive performance of *T. alexandrinum* was the best near forest edge in terms of number of seeds / head, seed weight/head and germination while it was the poorest in agricultural land in terms of number of abnormal seeds and germination. Species richness failed to predict any of the four parameters of reproductive success however; abundance was strongly related with the germination. For better assessing the role of assemblage on plant reproductive success, future studies should explicitly categorize pollinators into functional groups on the basis of their relative pollination efficiencies and then assess their role in plant reproductive success.

Keywords: *Trifolium alexandrinum,* pollinators, Abundance, Species richness, Reproductive success

INTRODUCTION

Plants and pollinators have been coevolved through millions of years and each interactive unit has evolved certain traits for better exploitation of the other (Stebbins, 1970). This view mainly expresses more specialized nature of interactions between the corresponding plants and their pollinators. However, numerous empirical studies have proved more generalized interactions, in which most of the plant species are visited by array of pollinators, known as pollinator assemblage (Gomez and Zamora, 2006; Herrera, 1996; Waser *et al.,* 1996).

The pollinator assemblage mainly varies due to habitat fragmentation and change in land use i.e. from urban to rural gradient (Lopes and Buzato, 2007, Sajjad *et al*., 2012). As the both, ultimately affect the availability of nesting places and of food resources. Different pollinator species may respond differently to landscape changes (Steffan-Dewenter *et al*., 2002). In consequence, there might be reduction in abundance of pollinators (e.g., SteffanDewenter and Tscharntke, 1999), shift in pollinator guilds (e.g., Donaldson *et al*., 2002) and favor of non-native species to access floral resources (e.g., Aizen and Feinsinger, 1994).

The diverse pollinator assemblage overwhelms the pollination limitation phenomena in many self-incompatible plant species (Ashman *et al.,* 2004; Knight *et al.,* 2005) as reproductive success could be pollinator limited (Burd,1994; Johnson and Bond, 1997). However, different pollinator species may vary in their pollination effectiveness because of variation in pollen load they transfer to the stigma (Herrera, 1987; Pacini, 1992) and number of visitation frequencies (Schmitt, 1980; Bosch and Blas, 1994). For example numerous pollination systems have confirmed Hymenoptera pollinators as more effective than the Diptera and Coleoptera

Cite this article as: Latif, A., S. Saeed, A. Sajjad and S.A. Malik, 2014. Variation in pollinator assemblage and reproductive performance of white clover (*Trifolium alexandrinum* L.). Pak. Entomol., 36(2):89-95.

pollinators (O'Brien, 1980; Hippa, *et al.,* 1981).
The spatial variations among the pollinator species, in terms of abundance and diversity, not only maintain the generalized pollination system but are also responsible for unrestricted gene flow between the populations in close proximity (Herrerea, 1996; Fenster *et al.,* 2004). In this way, spatial variation in pollinator assemblage often favors the variation in pollination effectiveness (Fishbein and Venable, 1996; Gomez and Zamora, 1999). This variability in pollinator assemblage is especially prevalent in intensive agricultural systems because the ephemeral nature of floral resources can alter pollinator foraging behaviours (Diekotter *et al*., 2010) and reduce the presence of habitat specialists (Tylianakis *et al*., 2005).
The abundance-richness relationship in pollinator assemblages confirms that richness is the function of pollinator abundance and plant population size (Steffan-Dewenter, 2003). Numerous studies have proved spatial variation in pollinator abundance (Price *et al.,* 2005, Gomez and Zamora, 2006; Ollerton *et al.,* 2006) while a few numerical studies have confirmed the spatial variation in pollinator richness or diversity.
The empirical evidences of the local variability of pollinator assemblage due to change in local landscape and its ultimate impact on plant reproductive success has not been reported previously from agricultural ecosystems of Pakistan. This study was planned to investigate the impact of local variability of pollinator assemblages due to different land use types (forest edge, rain torrent and agricultural land) and their ultimate impact on reproductive performance of *T. alexandrinum*.

MATERIALS AND METHODS

Study Site

The study area was located in the village 'Jallowali' about 150 km in north of Dera Ghazi Khan City. The village is characterized by three distinct types of landscapes i.e. a forest of 25 acres, plain agricultural land and hilly rain torrents (Table 1). The most frequently sown crops are rice, wheat, sugarcane, cotton and pulses. We selected three study sites, one in or near each type of landscape, separated by at least 2 km from each other.
The location 'a' was situated near undisturbed forest edge with a plant community dominated by xerophytes i.e. *Acacia modesta, Zizyphous nummularia, Alhagi marurum, Prosopis cineraria, Capparis aphylla, Tamarix dioca, Rhizia stricta, Prosopis specigera, Salvadora oleoides, Calotropis procera, Sueda fruticosa, Solanum xanthocarpum* and *Peganum hermala*. Location 'b' was surrounded by agricultural land i.e. *ca*. 1 km from all sides. Location 'c' was situated near hilly rain torrents in a small ravine. This landscape is characterized by rill and gully erosion which has been suppressed at many places by plantation of different plant species i.e. *Tamarix dioca, Prosopis cineraria* and *Capparis aphylla.*
Climate in the zone is Sub-tropical with extreme environmental conditions having hot summer and cold winter. The mean monthly maximum and minimum temperatures ranges from 35˚C to 40˚C and 10˚C to 20˚C respectively, while mean monthly summer rainfall is *ca*. 250 mm (Khan *et al.,* 2010).

Experimental material

Experimental material was white clover (*Trifolium alexandrium* L.). It is grown mainly as fodder crop and fixes about 118-125 kg nitrogen per hectare annually (Mowad *et al.,* 1998). This crop is highly cross-pollinated and tripping agencies like bees are required to improve seed set (Yamada *et al.,* 1989; Bukheit, 1989).
Trifolium alexandrium was sown in pots having soil media composed of silt and farmyard manure in ratio of 1:1. Two sets of pots (one hundred in each) were kept in each of the three locations at distance of *ca.* 150 m, from each other. In each set, the pots were placed one foot away from each other in the form of a compact square block during 1st week of April, before the onset of the bloom.

Visitor census

Pollinator abundance was recorded throughout the flowering season (mid-April to mid-May) by randomly observing 30 pots for 60 seconds each and counting the number of visiting individuals. Six observations were made along the day at 09:00, 10:00, 12:00, 14:00, 16:00 and 17:00 hrs with three days interval.
All the bees were identified up to genus level using the keys of Michener (2000). Butterfly species were identified by the specialist (Acknowledgements). Voucher specimens were submitted to the Agricultural Museum of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan.

Reproductive success

Upon maturity, 30 individual heads were randomly selected, each from 30 randomly selected pots in each location. Healthy and abnormal seeds were counted and weighted separately. The seeds were further subjected to germination test at room temperature (25-30 °C) in pure silt.

Data analysis

Pollinator assemblage was calculated in terms of their abundance, species richness and diversity (Magurran, 2004). Species Richness was measured as number of insect species observed visiting flowers in each location. Diversity was calculated as Shannon-Wiener index (Colwell, 2005). We used pollinator rank-abundance curves in order to visualize the structure of the pollinator communities (Magurran, 2004).
Among-location differences in plant reproductive success (number of seeds per pod, seed weight per pod, abnormal seeds per pod and germination percentage) were analyzed using one-way ANOVA. Means were compared by using Least Significant Difference (LSD) test at p=0.05. Individual based rarefaction curves were used to estimate the number of species (S) expected in a random samples of 'n' individuals taken from a larger collection made up of 'N' individuals and 'S' species (Gotelli and Entsminger, 2005) and graph was drawn in PAST software (Hammer *et al.,* 2001).

RESULTS

In total, 684 insects representing 32 species in three orders were observed visiting the flowers of *T. alexandrinum* in three isolated locations. The majority of the insect visitors belonged to order Lepidoptera and Hymenoptera (57% and 40%, respectively in call the three landscapes) (Fig.1). Most of the visitation was restricted to only seven species comprising more than 65% of the total visits in all the three landscapes. These species include two long-tongued large bees (*Apis dorsata* and *A. florea*), three butterflies (*Taracus venosus, Danus chrysippus* and *Anaphais aurota*), a moth (*Helicoverpa armigera*) and a wasp (*Vespa* sp.). All the seven bees recorded in the census belonged to Family Apidae i.e. *A.dorsata*, *A. florae*, *Amegilla* sp., *Xylocopa* sp.1, *Xylocopa* sp.2, *Thyreus* sp. and *Ceratina sexmaculata*.

Pollinator assemblage structure exhibited similarity among the three locations with a few abundant species and large number of scarce species (Fig. 2). However, the species composition differed at three sites. A butterfly '*T. venosus*' was the most abundant floral visitor among the three locations. *A. dorsata* and *A. florea* (both comprising 74% of the total bees) were common bees among the three locations while its abundance was highest near forest edge followed by rain torrent and agriculture land (Fig. 2).

There was no significant difference among the three locations in terms of Shannon-Wiener ($F= 0.15$, $P=0.86$, $df = 2$). However, the three locations differed significantly with respect to species richness ($F= 2.59$, $P < 0.08$, $df = 2$) (Table 2). The individual based rarefaction curves of three locations suggest that species richness is expected to be increase with sampling effort as have not yet reached an asymptotic level (Fig. 3).

There was significant difference among the three sites in terms of reproductive success parameters i.e. number of seeds, seed weight / head, abnormal seeds and germination percentage (Table 3). Plants near forest edge proved to be the best in terms of number of seed ($F= 12.89$, $P < 0.0001$, $df = 2$), seed weight / head ($F= 17.11$, $P < 0.0001$, $df = 2$) and germination ($F= 16.91$, $P < 0.0001$, $df = 2$). Agricultural land and rain torrent were statistically non-significant with respect to number of seed and seed weight / head. The Maximum abnormal seeds and the minimum germination were recorded in agricultural land (Table 3).

Species richness did not predict any of the four parameters of reproductive success however; abundance was strongly related with the germination (Table 4). The correlation between number of seeds and seed weight per head was significantly positive ($P = 0.05$). Contrary to this, the correlation between number of abnormal seeds and germination was significantly negative ($P = 0.05$). Germination was only positively related with number of seeds and seed weight per head (Table 5).

DISCUSSION

The pollinator community of *T. alexandrium* was dominated by long-tongued insects i.e. butterflies and long tongue bees (Apidae). Butterflies and moths can penetrate their long proboscis in the flower while the short or long-tongued bees have the additional advantage of their ability to manipulate the flower. Although a flower visitor may not necessarily be an effective pollinator yet this type of study is rather easy in alfalfa in terms of tripping of the flower i.e. release of the staminal column from the restraining process on the wing and the keel petals and is mainly performed by the insects (Bolton, 1962).

Based on this tripping mechanism, a previous study (Ahmad, 1976) from Punjab, Pakistan highlights the promising pollinator fauna of alfalfa including a short tongued *Halictus* sp. (Hymenoptera) and a long tongued *Megachile cephalotes* (Hymenoptera). The same study also reports a negligible contribution of butterflies, syrphids and beetles towards tripping of alfalfa flowers. Some other studies also signify Hymenoptera (bees) as the most abundant and efficient pollinator group of alfalfa than the Leipdoptera (butterflies and moths) and Diptera (flies) (Goodman and Williams, 1994). This is because of their high efficiency in pollen deposition on stigma, high searching, and visitation activity (Rodet *et al.,* 1998). In this study, *A. dorsata* and *A. florea* comprised 74% of the total bee abundance and therefore supposed to be the most responsible for predicting reproductive performance of *T. alexandrium*. As *A. dorsata* was the most abundant near forest edge followed by rain torrents and agricultural land, the reproductive success of *T. alexandrium* was heights near forest edge as compared to rain torrents and agricultural land (Table 3).

Pollinator assemblage structure in rank abundance curves revealed butterflies as the most abundant floral visitor at all the three locations. The spatial structure of variation in pollinator abundance and community composition can have important implications for plant reproductive performance (Gomez and Gonzalez-Megias, 2007). This spatial scale variation in pollinator communities remains less clear. Flower visitation activity of pollinators is often strongly affected by climatic conditions and climate varies on both large and small spatial scales (McCall and Primack, 1992). The size and quality (availability of nesting places and floral resources) of any natural habitat also determines the richness and abundance of pollinator species (Cunningham, 2000). The reason being *A. dorsata* was the most abundant near forest edge since it is social in origin and build nest on trees (Qayyum and Ahmad, 1967). All the three locations had different pollinator assemblage structures. The pollinator assemblage of an area may also vary on temporal scale (Hammer *et al.,* 2001). To understand the spatial variability in abundance and diversity of butterflies in any area it is also important to know the larval and floral host plant range of each butterfly species.

Species richness was the highest in rain torrent followed by forest edge and agricultural land. Habitat quality and fragmentation has several implications on pollinator abundance, diversity, foraging behavior along with having negative effect on seed set through genetic degradations (Oostermeijer *et al.,* 1994; Matthies *et al.,* 1995). Ockinger and Smith (2007) have reported positive relation between quality and quantity of surrounding habitat and abundance or richness of visiting insects. Habitat isolation and quality affects pollinator diversity by variety of ways e.g. patch size and density of flowering plants, occurrence of competing alternative flowers (Kleijn and Langevelde, 2006) and genetic variability (Donaldson *et al.,* 2002).

Abundance, in this study was positively related with the germination while species richness had no impact on reproductive success of *T. alexandrium*. In this study majority of the community was composed of butterflies which are known to be the less effective pollinators of *T. alexandrinum* (Goodman and Williams, 1994). Irrespective of the butterflies, bees (e.g. *A. dorsata* and *A. florea* in this study) can increase fertilization by 90 percent (Rodet *et al.,* 1998). In conclusion, it is hard to estimate plant reproductive success merely on the basis of abundance and richness since these do not consider functional diversity by categorizing pollinators on the basis of their relative effectiveness. For better assessing the role of pollinator assemblage on plant reproductive success, future studies should explicitly categorize pollinators into functional groups on the basis of their relative pollination efficiencies and then assess their role in plant reproductive success.

ACKNOWLEDGEMENTS

This study was funded by Higher Education Commission of Pakistan. We are grateful to Dr. Claus Claussen (TwedterHolz 12 D-24944 Flensburg, Germany) for his help in identification of Syrphid fly species and Dr. Ather Rafi (National Agriculture Research Centre, Pakistan) for butterfly species.

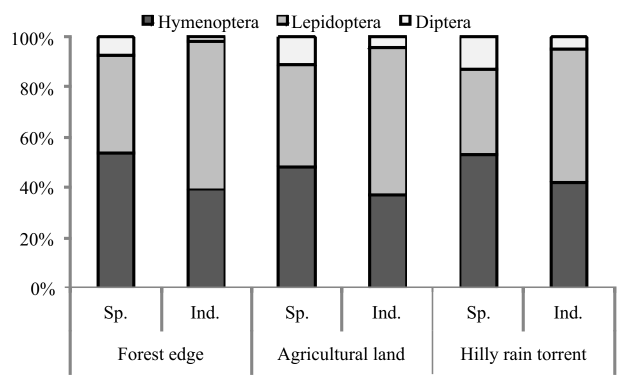
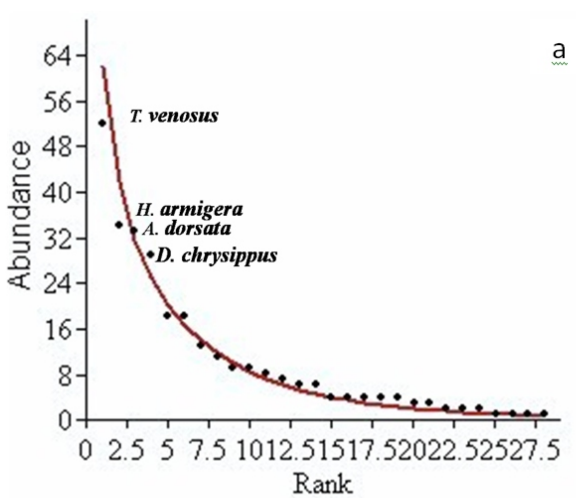
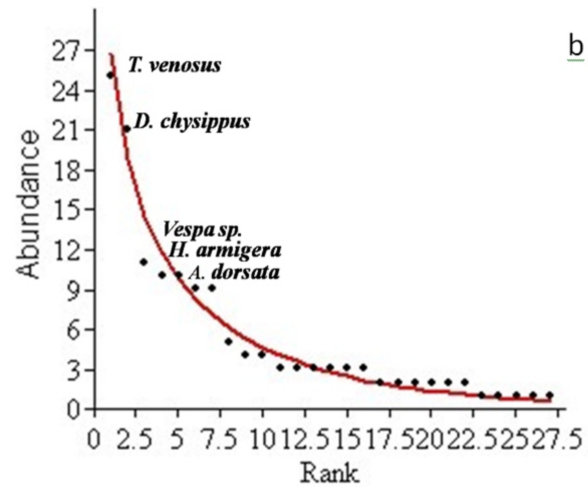


Fig. 1
Percentage of species and individuals of related insect orders at three locations during 2009 (ind.=individuals: sp.= species)





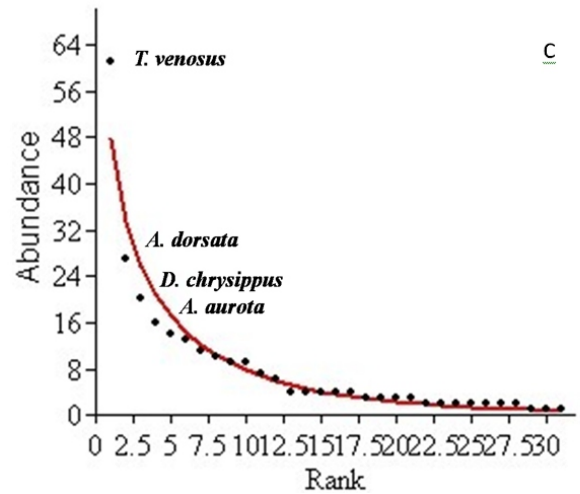


Fig. 2
Rank-abundance curves of pollinator species visiting the three locations (a: Forest edge; b: Agricultural land; c: Hilly rain torrent) at Dera Ghazi Khan (Punjab), Pakistan.

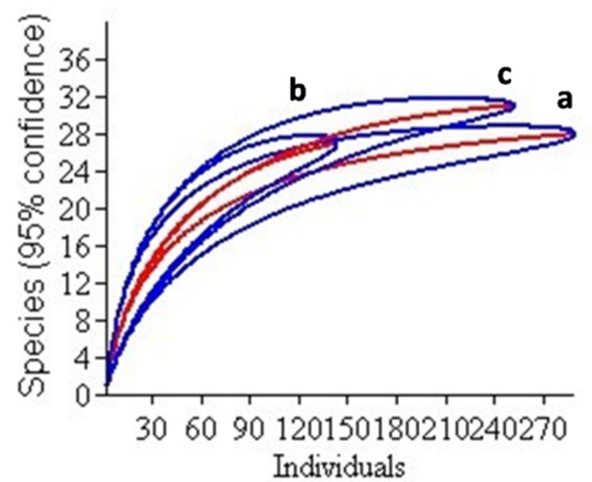


Fig. 3
Rarefaction curves of three locations i.e. (a) Forest edge (b) Agricultural land (c) Hilly rain torrent, based on individual rarefaction method showing the expected number of species as a function of sample size.

Table 1

Properties and types of three studied landscapes at Dera Ghazi Khan (Punjab), Pakistan.

Experimental locations	GPS	Altitude feet
Forest edge	31°13.51" N, 70°36.88" E	565±17
Agricultural land	31°12.77" N, 70°37.31" E	587±16
Hilly rain torrent	31°11.62" N, 70°37.54" E	575±16

Table 2

Variation in pollinator abundance, diversity and richness among the locations. Mean sharing similar letters have statistically no difference at alpha=0.05 level.

Locations	Abundance	Shannon-Wiener	Species richness
Forest edge	289	2.788 a	28 ab
Agricultural land	143	2.840 a	27 b
Hilly rain torrent	252	2.838 a	31 a

Table 3

Comparison of reproductive success of *T. alexandrinum* among three locations. Mean sharing similar letters have statistically no difference at alpha=0.05 level.

Locations	No of seeds/head	Seed weight/head (gm)	Abnormal seeds	Germination (%)
Forest edge	50.889±0.007 a	0.161±2.152 a	2.806±0.251 b	64.759±0.768 a
Agricultural land	35.00±0.008 b	0.112±2.596 b	8.061±0.887 a	58.414±1.151 c
Hilly rain torrent	38.245±0.006 b	0.106±2.051 b	3.694±0.337 b	62.120±0.831 b

Table 4

Linear regression analysis between yield attributing components of *T. alexandrinum* and pollinator assemblage.

Linear Model	r^2	p	F
Germination = 25.11 + 0.17 Abundance	0.904	0.004	37.551
Abnormal seeds = 6.95 – 5.16 Abundance	0.017	0.807	0.068
No. of seeds = 22.76 + 8.06 Abundance	0.532	0.100	4.546
Seed weight = 7.14 + 2.314 Abundance	0.259	0.320	1.401
Germination = -18.03 + 2.83 Richness	0.197	0.378	0.980
Abnormal seeds = 28.00 - 0.77 Richness	0.285	0.275	1.595
No. of seeds = 58.22 – 0.59 Richness	0.022	0.780	0.089
Seed weight = 0.32 – 6.99 Richness	0.178	0.405	0.866

Table 5

Pearson correlation coefficient matrix among yield attributing components.

	Seed weight (gm)	No. of seeds	No. of abnormal seed
No. of seeds	0.887*		
No. of abnormal seed	-0.209	-0.115	
Germination %	0.221	0.100	-0.509*

* Significant correlation at 0.05 level

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