



P-ISSN: 2349-8528
 E-ISSN: 2321-4902
 IJCS 2018; 6(3): 3166-3169
 © 2018 IJCS
 Received: 17-03-2018
 Accepted: 20-04-2018

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International Journal of Chemical Studies

Investigations on the antennal role in mating and host selection behaviour of *Spodoptera litura* (Fabricius)

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Abstract

Experiments were carried out with male and female adults of *Spodoptera litura* to ascertain the role of antennae in their mating and host selection behavior. The olfactory responses of *S. litura* larvae to odour emitted by different hosts viz., castor, cauliflower, sunflower, tomato and lab lab were tested in multiarm olfactometer. The experiments to know the role of antennae in mate and host selection were carried out with the adults with antennae and without antennae showed that the treatments having adult males with antennae laid fertile eggs, whereas, male without antennae laid sterile eggs. As far as the host preference for oviposition was concerned, the treatments involving male without antennae and female with antennae and, both male and female with antennae preferred castor and sunflower, respectively. The results of multiarm olfactometer experiments showed that the larvae had more preference for castor and least preference for lab lab, while, adult female preferred sunflower for oviposition.

Keywords: *Spodoptera litura*, antennae, ovipositional preference, olfactometer

1. Introduction

The tobacco caterpillar, *Spodoptera litura* is a serious polyphagous pest distributed throughout the world causing damage to more than 290 plants species (Wu *et al.*, 2004) [21]. Management of this insect is very difficult owing to its polyphagous nature. Understanding the host preference of plant-infesting insect like *S. litura* is important for its management (Xue *et al.*, 2010) [22]. The antennae of adult insects have various types of sensilla with different functions which play a crucial role in host-finding and mating (Schneider, 1964; Zacharuk, 1985) [18, 23]. Very little research works have been carried out on the mechanisms that drive both host and mate selection. In particular, there are no data on the antennal sensory equipment of *S. litura*, despite the crucial role that the antennae plays in insect's host location and recognition (Anderson *et al.*, 2000; Isidoro *et al.*, 2001; Kristoffersen *et al.*, 2006) [1, 11, 13], as well as mating (Bartlet *et al.*, 1994; Romani *et al.*, 2008) [4, 16]. Keeping this in mind, the investigations were conducted to know the influence of antennae on host and mate selection behaviour in adults and host preference in larvae of *S. litura*.

2. Materials and Methods

1. Mass culturing of *S. litura*

Mass culturing of *S. litura* was carried out at the Insectary Unit of Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore by following the procedure detailed by Subramanian (1998) [17].

2. Experiment to analyze the role of antennae in mate and host selection

An experiment was carried out with both male and female adults of *S. litura* to analyze the role of antennae in their mate and ovipositional selection behaviours. Different hosts viz., nerium, castor, cauliflower, tomato and sunflower were placed inside the insect cage (45 x 45 x 60 cm). Newly emerged male and female adults of *S. litura* were taken separately and then the experiment was carried out with the following treatments.

- T1 - Male with antennae + female without antennae
- T2 - Male without antennae + female with antennae
- T3 - Both male and female without antennae
- T4 - Both male and female with antennae (control)

For each treatment five pairs of adults were released and the observations on the mating status and oviposition status were recorded based on the hatchability of the eggs.

3. Multiarm olfactometer experiment

The olfactory responses of *S. litura* to odour emitted by hosts were tested in multiarm olfactometer (Fig. 1). The airflow was pumped into the odour chambers connected to the olfactometer at a rate of 700 ± 10 ml/m at the entrance. Device was placed on a table at a vertical distance to avoid experimental error. The experiment was conducted at $24 \pm 1^\circ\text{C}$ temperature and 55 to 65 per cent relative humidity. In order to avoid positional bias, the odour chambers were rotated for every replicate. Olfactometer was cleaned after every trial session by using laboratory glassware cleaning solution, acetone (purity >90%) and hexane (purity >90%). The 6 arm olfactometer was designed to expose the different types of stimulus to the test insects.



Fig 1: Multiarm olfactometer with different hosts

Five different hosts *viz.*, castor, cauliflower, sunflower, tomato and lab lab were used in five arms and the sixth arm was left without any host. The opening at the centre for releasing the test insects was closed with a lid or through a sliding door. Air was drawn from the centre with specified speed. The larvae were starved for 4 hours prior to bioassay. The test insects of fifteen *S. litura* larvae at different instars were released separately at the centre of arena and the instrument was covered with black cloth to avoid the host preference by their visibility and to create darkness (Fig. 2). The experiment was repeated for five times. The number of insects reaching the different arms was counted after 10 minutes for further analysis. For oviposition preference, fifteen mated female adults were released and the instrument was covered with black cloth. The number of insects reaching different arms was counted after 10 minutes for further analysis.



Fig 2: Host preference analysis for *S. litura* using multiarm olfactometer

4. Free choice test

A circular plastic tray of 75cm in diameter was used as the experimental arena. Five different hosts *viz.*, cauliflower, castor, sunflower, tomato and lab lab were placed at the inner edges of the tray. Five leaves from each host were used for the experiment. A group of 15 larvae of *S. litura* at first, second, third, fourth and fifth instars were gently introduced separately at the centre of the tray with a camel hair brush and the number of insects reaching different hosts was counted after 15 minutes. The experiment was repeated for five times.

Statistical Analysis

Completely Randomized Design was followed for the host preference studies. Analysis of variance was performed for various treatments and means were compared by the least significant difference (Gomez and Gomez, 1984) [9].

3. Results and Discussion

1. Experiment to analyze the role of antennae in mate and host selection

The experiment carried out with the male and female adults of *S. litura* to know the role of antennae in their mate and host selection showed that the treatments *viz.*, both male and female without antennae, and male without antennae and female with antennae laid sterile eggs whereas the treatments *viz.*, both male and female with antennae and male with antennae and female without antennae laid fertile eggs. As far as the host preference for oviposition was concerned, the treatments involving the male without antennae and female with antennae and both male and female with antennae preferred castor and sunflower, respectively (Table 1). Insect learning can significantly change the host selection of ovipositing insects; previous experience of a host plant can lead to an increased preference for that host species (Papaj, 1986; Landolt and Molina, 1996; Cunningham *et al.*, 1998) [15, 14, 7].

Table 1: Role of *S. Litura* adult antennae in mating and oviposition

S. NO.	Treatment	Insect Pairs Released*	Insects Laid Eggs Mass*	Mating Status	Oviposition Status
1.	Male with antennae + Female without antennae	5	4	+	Laid fertile egg mass
2.	Male without antennae + Female with antennae	5	5	-	Laid sterile egg mass
3.	Both without antennae	5	3	-	Laid sterile egg mass
4.	Both with antennae	5	5	+	Laid fertile egg mass

* Nos; + Mated; - Unmated

2. Multiarm olfactometer experiment

2.1 Host preference by the larvae

The larvae had more preference for castor leaves and lab lab was the least preferred. First instar larvae were gregarious and did not show any specific host preference. Second instar larvae have shown slow movement towards castor and cauliflower, which was observed beyond the set time period of 10 minutes. Whereas, other instars were active and showed more preference for castor (6.23, 5.33 and 5.20 Nos., respectively for 3rd, 4th and 5th instars) followed by cauliflower and sunflower. In general, all the larvae showed

less preference for tomato and lab lab (Table 2). Experience-based preferences were thought to confer a certain degree of behavioural plasticity on host location and feeding cues in individual generalist larvae that feed preferentially on specific hosts. Carlsson *et al.* (1999) [6] demonstrated that either linalool or geraniol could serve as olfactory attractants to third instar *S. littoralis*, provided that the larvae had previous feeding experience in the presence of the volatile. Olfactory preferences of polyphagous noctuids were strongly influenced by the odours during feeding (Anderson *et al.*, 1995; Carlsson *et al.*, 1999; Glendinning, 2002) [2, 6, 8].

Table 2: Host Preference of *S. Litura* Larvae Under Multiarm Olfactometer

S. No	Host	III RD Instar (NOS.)	IV TH Instar (NOS.)	V TH Instar (NOS.)	Ovipositional Preference By Adult (Nos.)
1.	Castor	12.25 (3.57) _a	11.25 (3.43) _a	11.75 (3.50) _a	4.00 (2.11) _a
2.	Sunflower	6.25 (2.59) _c	6.50 (2.64) _b	6.25 (2.59) _b	1.75 (1.49) _b
3.	Cauliflower	9.00 (3.08) _b	10.25 (3.27) _a	10.75 (3.35) _a	3.25 (1.93) _a
4.	Tomato	1.00 (1.18) _d	1.25 (1.27) _c	0.75 (1.10) _c	0.75 (1.10) _c
5.	Lab lab	0.75 (1.10) _d	0.75 (1.10) _c	0.50 (0.97) _{cd}	0.25 (0.84) _{cd}
6.	Control	0.25 (0.84) _d	0.00 (0.71) _d	0.00 (0.71) _d	0.00 (0.71) _d
SE(d)		0.172	0.172	0.149	0.135
CD (0.5)		0.361	0.361	0.314	0.284

* Mean of four replications

2.2 Ovipositional preference

Among the five host plants tested, the adult female preferred sunflower (6.02 Nos.) followed by castor (4.15 Nos.) and cauliflower (2.70 Nos.) for oviposition. The tomato and lab lab were least preferred for oviposition (Table 2). Among the various senses perceived by the insects, the olfaction often was the most important in mate and host selection (Hildebrand and Shepherd, 1997; Bernays and Chapman, 1994) [10, 5]. Olfaction was critical to execute innate behaviours such as recognition of mates, location of food sources, and selection of suitable host plants for oviposition, that were crucial for survival and reproduction in phytophagous insects, (Bernays and Chapman, 1994; Jaenike, 1990; Visser, 1986) [5, 12, 20].

3. Free choice test

The free choice test was also conducted to ascertain the host preference of the *S. litura* larvae. The results revealed that the third, fourth and fifth instars larvae highly preferred castor (12.05, 11.33 and 11.00 nos., respectively) followed by cauliflower for feeding and the lab lab was least preferred by the larvae for feeding (Table 3). This might be due to higher quantity and more diverse accumulation of free amino acids in castor, in combination with physiochemical and mechanical attraction (Showler, 2001) [19]. Free choice test for host selection by *S. frugiperda* showed that the insects preferred cotton bolls for feeding, and larvae fed on cotton bolls exhibited longer larval and pupal development, and longer adult life span with similar egg production (Barros *et al.*, 2010) [3]. These findings fall in line with the present observations.

Table 3: Host Preference of *S. Litura* Larvae Under Free Choice Test

S. No	Host	Instars		
		III (Nos.)	IV (Nos.)	V (Nos.)
1.	Castor	13.50 (3.74) _a	13.75 (3.77) _a	13.25 (3.71) _a
2.	Sunflower	6.00 (2.55) _c	6.50 (2.64) _b	7.25 (2.78) _b
3.	Cauliflower	7.25 (2.78) _b	7.75 (2.87) _b	8.00 (2.91) _b
4.	Tomato	2.00 (1.58) _d	1.25 (1.27) _c	1.00 (1.18) _c
5.	Lab lab	1.25 (1.31) _e	0.25 (0.84) _d	0.50 (0.97) _c
SE(d)		0.097	0.194	0.175
CD (0.05)		0.207	0.414	0.372

* Mean of four replications

4. Conclusion

From the present experiments, it could be concluded that the male adults without antennae did not mate with opposite sex and the female adults without antennae laid fertile eggs, whereas in case of both sex without antennae, the female moth laid sterile eggs. Multiarm experiment and free choice test for the host preference revealed that the castor leaves were mostly preferred by third, fourth and fifth instar *S. litura* followed by cauliflower and sunflower. Present investigation clearly revealed that the insect uses the olfactory sensilla located on the antenna for finding its mate and hosts for oviposition which was evidenced by the failure of mating in

treatments including male without antennae and also male and female without antennae.

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