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Attraction of neonate *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae to different host plant volatiles

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Abstract

The orientation responses of 1st instar grampod borer *Helicoverpa armigera* larvae to the green leaf volatiles of three leguminous crops; pigeonpea, *Cajanus cajan*; mungbean *Vigina radiate* and blackgram *Vigina mungo* were determined in no-choice and two-choice tests under laboratory conditions. The larvae showed positive orientational responses to the leaves of all the three test plants. However, larvae attracted more towards the pigeonpea as compared to blackgram and mungbean in no-choice and two choice conditions. The results clearly indicate that the first instar *H. armigera* larvae showed higher attraction towards the volatiles of pigeonpea followed by blackgram and mungbean. Role of such host plant volatiles in eliciting the orientational responses of pod borer larvae will be useful in understanding the establishment and subsequent damage by the larvae which further helps in developing efficient management strategies.

Keywords: *H. armigera*, host plants, larval orientation, green leaf volatiles.

1. Introduction

The gram pod borer, *Helicoverpa armigera* (Hüb.), is a major constraint to crop production worldwide. This pest has wide host range and feeds on uncultivated and cultivated plants belonging to Asteraceae, Leguminosae, Solanaceae, Malvaceae and Poaceae families. In India it has been recorded on more than 181 plant species from 45 plant families and out of these 96 species are agriculturally important crop plants [7]. Yield loss due to outbreak of *H. armigera* on semiarid crops, may exceed US $2 billion annually, and an additional US $500 million on the pesticides used for controlling this pest [14]. In India, total losses in both pulses and cotton exceed $530 million annually, and insecticides applied for the control of this pest cast nearly $127.5 million on cotton and pulses. The extent of losses in chickpea and pigeonpea worldwide has been estimated over $927 million annually [13]. The management of this pest is mainly by synthetic pesticides but the pest status of this insect has risen due to the spread of strains resistant to conventional insecticides. This has led to several out breaks of *H. armigera* in Indian subcontinent, Australia and other parts of world [1, 6]. Moreover, the extensive use of pesticide leads to environmental problems, pest resurgence and secondary pest out breaks. Therefore, alternative control methods such as host plat resistance and the use of behaviour modifying chemicals for the management of this pest, are gaining interest.

Semiochemicals play a significant role in selection of host plants by insects. Knowledge regarding the role of kairomones in the host selection by insect could substantiate highly worthwhile in insect pest management programs. Studies on the orientation behaviour of larvae could be of huge aid in various insect control tactics, including interruption of larval orientation, attraction of larvae to toxic baits or cultivation of crop varieties lacking larval attractants [8]. Several studies addressed orientation of adults and larvae of *Helicoverpa* species to the kairomones emanating from different surface of the plants [2, 3, 4, 9, 10, 12, 15, 16, 17], but information is needed regarding the orientation of *H. armigera* larvae to the volatiles of leguminous host plants. Hence, the present study was carried out to evaluate the orientation responses of neonate gram pod borer among selected leguminous crop plants.

2. Materials and Methods

H. armigera larvae obtained from a stock culture maintained at Department of Zoology University of Delhi, on chickpea flour diet. The leaves of test plants, i. e. pigeonpea (*Cajanus* *cajan* var. ICP 1691), mungbean (*Vigina radiate* var. PUSA vishal) and blackgram (*Vigina mungo* var, T9) used in the studies were obtained from plants, grown in departmental field plots under pesticide free condition.

2.1. Bioassays

The orientation experiment with neonate *H. armigera* larvae was conducted in a straight glass tunnel olfactometer (150 X 40 mm), which was divided into three equal sectors S, M and B, i.e. stimulus, middle and blank by a marker. Each side of the glass tunnel was provisioned with removable glass dish (45 X 50 mm deep) placed properly for air circulation through the tunnel. The olfactometer was enclosed in a test arena (85 x 72 x 92 cm), made of white sun mica sheets with its front side open. The arena was uniformly illuminated (250Lux) through translucent light diffuser by two fluorescent light (L 18, W/25 white, 60 cm length), which were placed over the center of arena.

In no-choice tests, volatile stimuli from individual test plant foliage were stuck at the bottom of glass dish and provided at stimulus end of the tunnel, while the other side was kept blank. The neonate larvae were taken from the stock culture. These larvae were released on wet cotton placed in a petridish for 15 min, so as to bring them in identical physiological condition. Ten water satiated first instar larvae were released gently in to the central sector of glass tunnel with the help of a fine hairbrush. Movement of the larvae towards stimulus side or blank side was continuously observed for 5 minutes. The larvae reaching the end walls of the tunnel were removed, so as to avoid the interference with the observation of the other larvae.

Same procedure was followed for two choice tests, where foliage of different plant was provided at opposite end of tunnel. The experiments were conducted in a dark still air room where only source of light was that of test arena. Each test was repeated 5 times, using ten larvae per replicate. To avoid positional error, the test stimuli holder along with glass tunnel was rotated at 180^0 after each replicate.

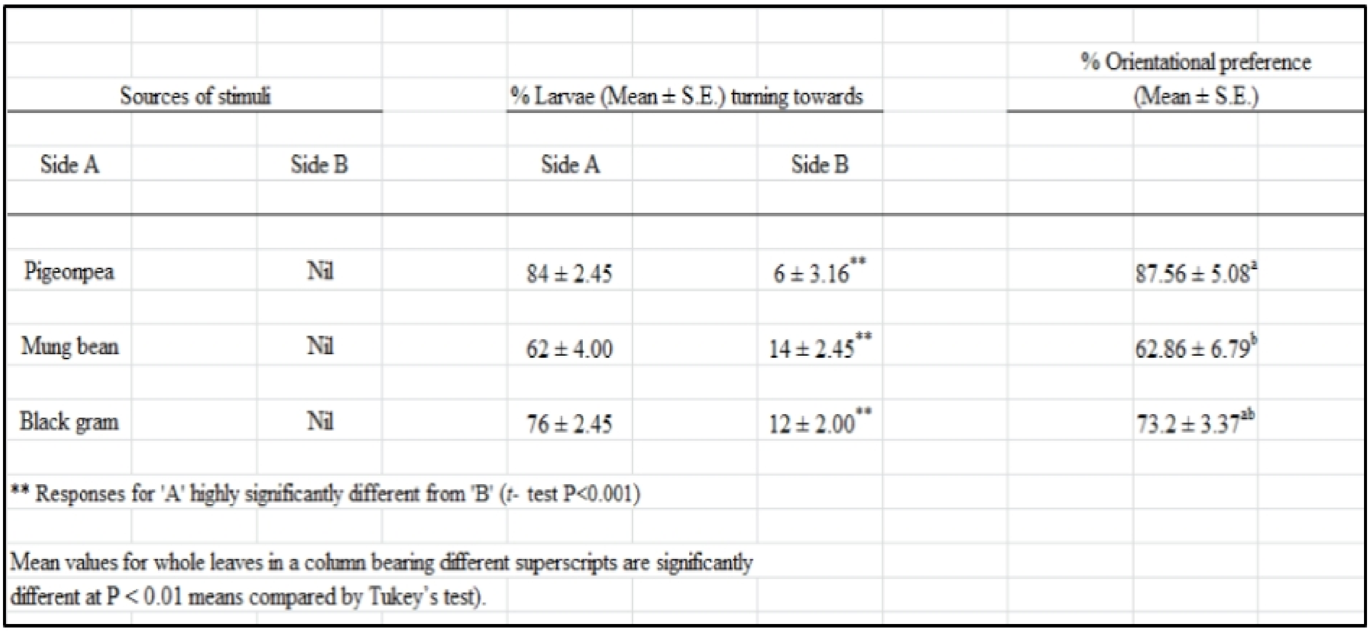
2.2. Statistical analysis

All statistical analyses were based on counts of the larvae without transformation. The percentage orientational preference (%OP) of the larvae for the stimulus was calculated as A-B/*n* in no-choice and A-M/*n* in two choice tests. Where 'A', 'B' and 'M' indicate the number of larvae present in stimulus, blank and middle parts, respectively, whereas 'n' denotes the total number of larvae used for each replicate. The significance difference between the mean responses of larvae under two different conditions was determined by student t-test or paired t-test. One-way ANOVA were performed to detect effects of host plant leaves on the two measures of orientation preference of larvae. When significant *F* value were found, means were separated using Tukey's test at P 0.05 level. All the analysis was performed with the help of statistical packages Jandel's Sigma Stat [5].

3. Results and discussion

Neonate larvae showed positive orientational response for the leaves of all the test plants in no choice test. The mean percentage of larvae turning towards stimuli emanating from the leaves of pigeonpea, mungbean and black gram was higher than those turning towards blank side. Orientational preference of first instar *H. armigera* larvae towards pigeonpea leaves were significantly higher than mungbean (P<0.05), but statistically equal to black gram leaves. Difference in mean orientational preference of larvae for black gram and mungbean did not differ significantly (P>0.05) (Table 1). Orientational preference of larvae was highest for pigeonpea leaves (87.56) and lowest for mungbean leaves (62.86) (F= 5.60; df= 2,12; P<0.019) (Table 1.).

Table 1: Orientational responses of neonate gram podborer larvae to whole leaves of pigeonpea, blackgram and mungbean in no-choice tests



Sources of stimuli		% Larvae (Mean ± S.E.) turning towards		% Orientational preference (Mean ± S.E.)
Side A	Side B	Side A	Side B	
Pigeonpea	Nil	84 ± 2.45	$6 \pm 3.16^{**}$	87.56 ± 5.08^{a}
Mung bean	Nil	62 ± 4.00	$14 \pm 2.45^{**}$	62.86 ± 6.79^{b}
Black gram	Nil	76 ± 2.45	$12 \pm 2.00^{**}$	73.2 ± 3.37^{ab}

** Responses for 'A' highly significantly different from 'B' (*t*- test P<0.001)

Mean values for whole leaves in a column bearing different superscripts are significantly different at P < 0.01 means compared by Tukey's test).

The results of the bioassays of 1st instar larvae under two-choice conditions, having pigeonpea or mungbean leaves at one end and blackgram or mungbean leaves at the opposite end of the glass tunnel, have been given in (Fig.1). It was observed that pigeonpea leaves elicited significantly higher orientational responses of larvae compared to black gram and mungbean leaves (P=<0.001). Orientational response of larvae for black gram was similar to that for mungbean leaves (P=0.001) (Fig. 1).

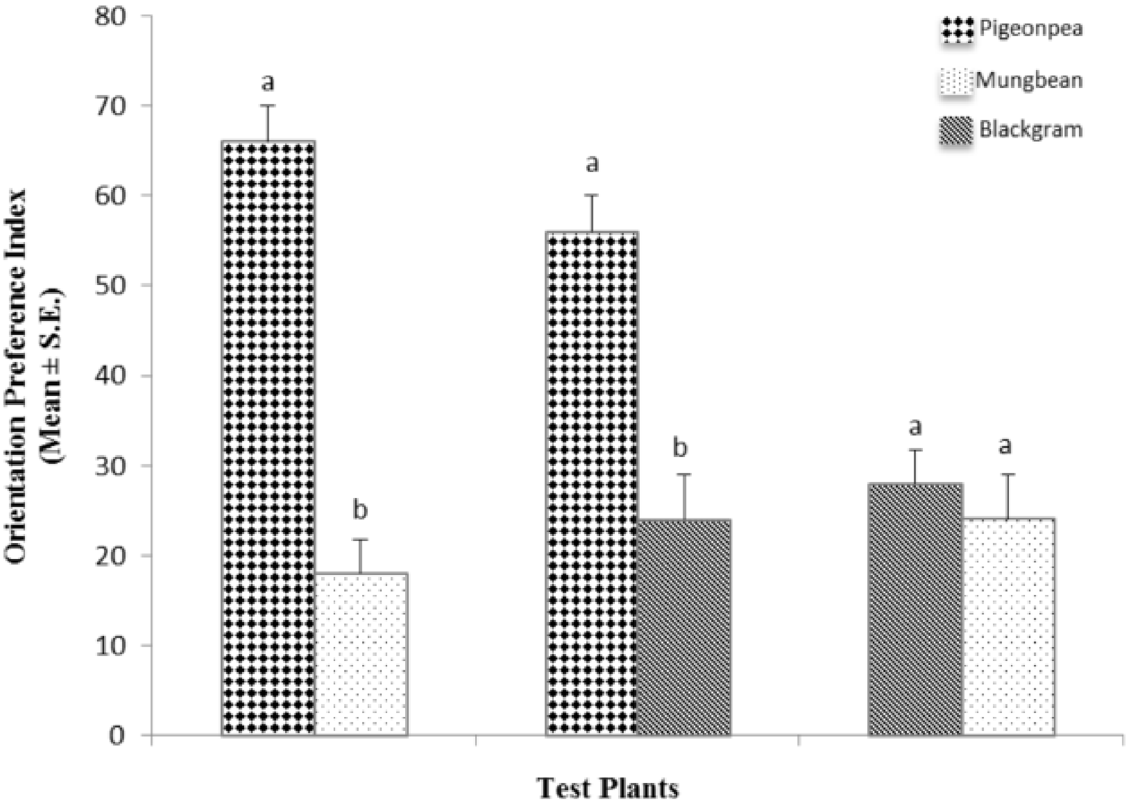


Fig. 1: Orientational preference of neonate gram podborer larvae to whole leaves of pigeonpea, blackgram and mungbean in two-choice tests

Leaves continuously emanate a small group of C_6 alcohols, aldehydes and esters call as “green leaf volatiles” (glv), which provide characteristic background odour to a plant surface. These volatiles disperse in environment in the form of “aerial bouquest”, enabling larvae to reliably detect host plants [18, 19]. The volatiles emanating from the leaf can exert their effect directly through chemotaxis or indirectly by affecting the expression of phototaxis and therefore influenced larval movement to locate food resources [11]. Our results clearly show that the neonate *H. armigera* larvae responded positively to pigeonpea, black gram and mung bean leaves. This suggests that leaves of three leguminous plants contain some common blend of volatiles that guide the neonate larvae to find their host.

In no-choice and two choice tests, larval orientation were higher for pigeonpea whole leaves compared to mung bean and black gram. This might be due the presence of active volatiles in pigeonpea, which are either present suboptimally or absent in mung bean and black gram leaves. Differences in attraction of larvae may also be due to the difference in the quality of volatiles emanating from their surfaces. It is ubiquitous that the amount of “glv” varies between plant species [18]. The attraction of larvae to whole leaves of black gram and mung bean were statistically identical. This might be due to the emission of same or identical volatiles or they release volatiles inducing equivalent responses. Attraction of neonate *H. armigera* larvae to the pigeon pea leaf volatiles has also been reported by Saxena and Rembold [12] and Singh and Mullick [16]. Hartlieb and Rembold [3] identified a mixture of six sesquiterpene kairomonal compounds in pigeonpea, which elicited positive orientational resposes in neonate *H. armigera* larvae. The volatiles emanating from the leaf and flower can exert their effect directly through chemotaxis or indirectly by affecting the expression of phototaxis and therefore influenced larval movement to locate food resources [11].

Our study clearly shows that pigeonpea, blackgram and mung bean leaves contains certain allelochemicals that are attractive for *H. armigera* larvae. Knowledge of such biological interactions of insect and its hosts will be useful in designing behavioural manipulation strategies, which may include interruption of larval orientation and trap cropping. Further study is required for the successful exploitation of such volatiles in the different management programs of grampod borer and other noctuids.

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