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Host generated cues alter the foraging behavior of Cabbage butterfly, *Pieris brassicae* and its larval parasitoids, *Cotesia glomerata* and *Hyposoter ebeninus*

M. Debarma,[1] D.M. Firake[2]

[1]College of Post Graduate Studies, Central Agriculture University, Umroi road, Barapani, Meghalaya; [2]Division of Crop Improvement (Entomology), ICAR Research Complex for NEH region, Umiam, Meghalaya, India

Abstract

Effect of host-generated cues on foraging speed of herbivore as well as its natural enemies was studied under net house conditions in Meghalaya, India. Foraging speed of *P. brassicae* was significantly higher towards the healthy plants, whereas it was lowest towards the damaged plants along with herbivore cues. In contrast foraging speed of parasitoids *H. ebeninus* and *C. glomerata* was highest towards damaged plants along with herbivore cues and lowest towards healthy plants. It indicates that herbivore and its parasitoids respond to the volatiles generated by their host. In addition to host plants natural enemies also utilize herbivore-generated cues for their detection.

Introduction

Cabbage white butterfly, *Pieris brassicae* (Linnæus) is a major pest of several economically important brassicaceous crops in hilly regions of India including north eastern states (Sachan & Gangwar, 1980). Although chemical pesticides are very effective, several times they are hazardous to the human as well as natural enemies of crop pests. Therefore biological control based on parasitoids, predators and pathogens are gaining prime importance. Parasitoids play an important role in reducing the population density of *P. brassicae* as well as generalist predators such as spiders, Chrysopids, staphylinids and carabids also attack the eggs and larvae (Pfiffner *et al*., 2009). Ichneuomonid wasp, *Hyposoter ebeninus* (Gravenhorst) and braconid wasp, *Cotesia glomerata* (Linnæus) are two well known parasitoids of *P. brassicae* and attempts are being made to properly utilize them into the biological control programs against the crop pests. Though biocontrol is an old practice, it is however not much popular due to several limiting factors including knowledge on parasitoid ecology, behavior, plant-insect interactions and the role played by the parasitoids.

Correspondence: Dnyaneshwar Madhukar Firake, Division of Crop Improvement (Entomology), ICAR Research Complex for NEH region, Umiam, Meghalaya, India.
Tel./Fax: +91.364.2570372.
E-mail: dfirake@gmail.com

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Plants can protect themselves from insect pests by attracting their natural enemies with the help of different cues. These cues can be herbivore induced plant volatiles or herbivore emitted volatiles. Compounds released by the plants in response to insect damage permit parasitoids to differentiate between healthy and damaged plants and thus it helps in finding their host. The chemical defense mechanisms of plants are either direct or indirect (Dicke & Sabelis, 1988; Vet & Dicke, 1992; Cartesero *et al*., 2000; Dicke & Van Loon, 2000; Van Loon *et al*., 2000; Kessler & Baldwin, 2002; Shiojiri, 2002). Direct defense mechanism occurs when secondary metabolites produced by the plants repel or avoid insect pests from feeding on them or kills this herbivore after ingestion of a plant parts (Van Loon *et al*., 2000). Conversely indirect chemical defense mechanism utilizes provision of herbivore specific foraging cues to natural enemies of the feeding insects (Takabayashi *et al*., 1991; Hoballah & Turlings, 2001; Dicke *et al*., 2003; Turlings & Wackers, 2004; Schnee *et al*., 2006). Many studies indicating tri-trophic relationship in cole crops ecosystems exist, particularly with cabbage plants species *P. brassicae, C. glomerata* (Fatouros *et al*., 2012) and *H. ebeninus.* However, the basic knowledge on impact of different host generated cues on herbivore and their natural enemies is not available. Therefore the present investigation was carried out to generate the information on influence of different cues (plant and herbivore volatiles, herbivore excreta, etc.) on foraging behavior of herbivore and its natural enemies.

Materials and methods

All experiments were carried out inside the net house at entomology farm, Division of Crop Improvement (Entomology), ICAR Research Complex for NEH Region, Umiam, Meghalaya, India. Egg masses of the cabbage butterfly *P. brassicae* were collected from the cabbage fields of Division of Crop Improvement of the Institute and reared inside wooden cages (30×30×45 cm) on live cabbage plants. After pupation all the pupae were removed and kept separately inside the glass jar up to adult emergence. Similarly first and second instar larvae of *P. brassicae* were also collected from the same cabbage fields and reared inside the cages. After two weeks parasitized larvae were removed and reared separately in plastic vials (100 mL) up to the emergence of either gregarious larval parasitoid, *C. glomerata* or solitary larval parasitoid, *H. ebeninus*. Honey solution (10%) was provided as a food to the adult insects. Newly emerged insects after mating were used for the experiment.

One-month-old seedlings of cabbage plants (Hybrid: Wonder ball) were transplanted into the plastic pots (30×30 cm) and all the necessary horticultural practices (*i.e.*, irrigation, weeding and other intercultural operations) were followed for healthy growth of the crop. The experiment was conducted after 1.5 months of transplanting. Six different treatments were considered in this study (Table 1) considering the importance of host-plant, herbivore volatiles and herbivore products.

Accordingly six different groups of five plants were prepared inside the net house, each plant in a group was separated by 15 cm and each group was placed in different corners at 2 m distance (Figure 1). Wherever required the fresh excreta of first instar larvae (2 g) were used during the overall experiment. Five 3-day-old larvae were used in the experiment and kept in the specialized vial (the lid made up of very fine nylon net) during experiment. In mechanical damage treatment, the tender leaves of appropriate size (two in each plant) were mechanically damaged and they were exposed to the test insects after about one hour. Newly hatched adults (less than 12 h of emergence) were used for this study. After one hour, thirty adults of *P. brassicae* and both species of parasitoids were released from the center point of the net house in such a way that the distance between the release point to all the treatments should be similar (Figure 1). Six independent observers were appointed for taking readings during the experiments. The observations on foraging speed (number of visits per 5 min) were recorded in six different groups of plants. The experiment was conducted for three times from 9.00 a.m. to 4.00 p.m. (3 h interval) in each day and the whole experiment was repeated for three times at five days interval.

The data were processed with SPSS version 13.0 (StataCorp., College Station, TX, USA) for MS-Windows for all statistical analysis. The homogeneity of variances between different treatments was tested with Levene's test, differences between treatments were afterward tested using analysis of variance (ANOVA), at a P=0.05 significance level. Tukey's honestly significant difference (Tukey's HSD) test was used to find means that were significantly different from each other.

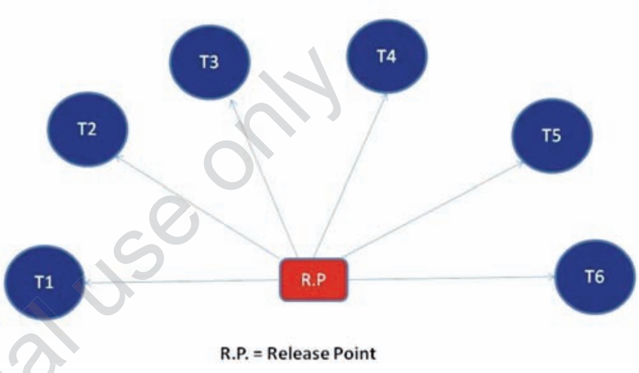

Figure 1. Arrangement of six treatments inside the net house.

Table 1. Six different treatments used in the experiment.

Treatment	Description
MDP	Mechanically damaged plants (similar to first instar larval damage)
MDP+LE	Mechanically damaged plants + larval excreta of first instar larvae
MDP+LE+L	Mechanically damaged plants + larval excreta + 3-day-old first instar larvae (L) (inside the insect rearing vial to restrict the movement)
HP+LE	Healthy plants + larval excreta
HP+LE+L	Healthy plants + larval excreta + 3-day-old first instar larvae (inside the insect rearing vial)
HP	Healthy plants

MDP, mechanically damaged plants; LE, larval excreta; L, larvae; HP, healthy plants.

Table 2. Behavioral response of *P. brassicae* (L.) and its natural enemies under the influence of plant and herbivore volatiles.

Treatments	Number of visits per 5 min		
	P. brassicae	*H. ebeninus*	*C. glomerata*
MDP	2.00±0.19	11.67±0.38	13.22±0.06
MDP+LE	1.70±0.12	17.33±0.19	16.67±0.13
MDP+L+LE	0.40±0.04	23.12±0.07	25.33±0.04
HP+L+LE	3.82±0.02	12.33±0.19	10.33±0.19
HP+LE	3.28±0.35	7.16±0.10	9.12±0.07
HP	7.60±0.23	3.33±0.19	4.67±0.10
F value	167.34	1087.37	3241.28
F test	<0.001*	<0.001*	<0.001*

MDP, mechanically damaged plants; LE, larval excreta; L, larvae; HP, healthy plants. *Significantly different at: P≤0.05.

Results

In Table 1 the different treatment are summarized with abbreviations. Foraging speeds during different treatment are reported in Table 2. Foraging speed of *P. brassicae* was significantly higher (F=167.34, P<0.01) comparing the healthy plants (HP) (7.60±0.23 visits/5 min) with HP+larvae (L)+larval excreta (LE) (3.82±0.02 visits/5 min), HP+LE (3.28±0.35 visits/5 min), mechanically damaged plants (MDP) (2.00±0.19 visits/5 min) and MDP+LE (1.70±0.12/5 min); foraging speed was lowest towards the treatment MDP+L+LE (0.40±0.04 visits/5 min). In contrast, foraging speed of *H. ebeninus* was highest (F=1087.37, P<0.01) towards MDP+L+LE (23.12±0.07 visits/5 min) followed by treatment MDP+LE (17.33±0.19 visits/5 min), HP+L+LE (12.33±0.19 visits/5 min), MDP (11.67±0.38 visits/5 min), HP+LE (7.16±0.10 visits/5 min) and it was lowest towards the healthy plant (3.33±0.19 visits/5 min). A similar trend was also observed in the case of gregarious larval parasitoid, *C. glomerata* (F=3241.28, P<0.01).

Discussion and conclusions

Insect pests use various cues to find out their host plants and several times these cues are the volatiles released by host plants in response to the herbivore damage. In the present study butterfly visits were significantly higher towards the healthy plants compared to damaged plants. Therefore it is possible that several factors including host plant damage, plant size, herbivore density and chemical composition of plants are responsible of egg laying preference by female butterfly. Higher visit frequency of butterfly towards undamaged and herbivore free plants might be due to avoid intra-specific competition or aggregation of the offspring's. Parasitoids also utilize different cues generated by the host plants to locate their hosts *i.e.* herbivore (Vinson, 1976; Turlings *et al*., 1990; Vet & Dicke, 1992; Dicke, 1999; Schnee *et al*., 2006; Heil, 2008; Fatouros *et al.*, 2012).

In the present study the parasitoids visited damaged plants with herbivores and their traces more frequently than healthy plants, indicating that parasitoids respond to the volatiles generated by host plants in an opposite direction respect to herbivores. Furthermore, likewise, damaged plants with larval excreta were visited by parasitoids with higher frequency respect to healthy plants with larval excreta. Therefore, in addition to host plants, natural enemies also utilize herbivore generated cues for their detection, this statement is supported by previous researches (Turlings *et al.*, 1993). Mattiacci *et al.* (1994) also reported that healthy plants treated with herbivore regurgitate, attract parasitoids. Further the number of visits by both the parasitoids was higher in damaged plants over healthy plants. These results are in agreement with other findings (Geervliet *et al.*, 1994; Potting *et al*., 1999), where it was observed that *C. glomerata* along with *C. rubecula* preferred herbivore-damaged plants to undamaged HP.

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