

Host and non-host plant volatiles on oviposition and orientation behaviour of *Trichogramma chilonis* Ishii.

Pathipati Usha Rani*, Y. Jyothsna and M. Lakshminarayana¹

ABSTRACT

In the process of host location and selection several cues associated with host's play a major role. Volatile compounds emitted by plants as a consequence of herbivore activities are often attractive to insect natural enemies including the Hymenopteran egg parasitoids, *Trichogramma* species. We studied the plant - parasitoid interactions where the plant surface chemicals act as infochemicals that attract or arrest the parasitoids for egg parasitization and strategically help in preventing the pest infestation. The leaf surface chemicals of *R. communis*, damaged due to the feeding of the host, *Achaea janata* (L) (castor semilooper) (Lepidoptera: Noctuidae), and a non - host, Serpentine leaf miner *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) were evaluated for their influence on host location, host acceptance and ovipositional behavior against the egg parasitoid, *T. chilonis* in laboratory bioassays. The *A. janata* damaged leaf emissions had synomonal effects on the parasitoid and induced orientation and oviposition, whereas, the surface chemicals from the plant infested with non-host *L. trifolii* ceased to produce any such effects. The maximum egg parasitization was observed in *A. janata* infested *castor* leaf extracts compared to the leaf miner infested or normal healthy castor leaf extracts. The results are interesting in the context of tritrophic interactions between the pest, parasite and the host plant and are useful in biological control of insect pests.

Keywords: *Ricinus communis*, *Trichogramma chilonis*, Host and Non -host synomones.

INTRODUCTION

Plant infochemicals play a major role in the tritrophic interactions of the plant -host - parasitoid interactions, especially during host selection process by parasitic wasps in general, including *Trichogramma* (Vinson, 1976; Nordlund, 1994). Host-habitat location, host location, host recognition and host acceptance are the important aspects of prey/host location (Nordlund *et al.*, 1988). Plant-derived stimuli are assumed to be more detectable from a distance (Jacqueline *et al.*, 1994). The volatiles emitted from the plants due to induction of herbivore feeding act as synomones, which are involved in indirect defense, because they attract their natural enemies (Dicke, 1999; Dicke and van Loon, 2000).

Plants produce and emit volatile organic chemicals in response to attract by feeding herbivores and often organisms. This area of research has become important in various contexts, primarily the utilization of plant generated volatiles for the attraction of the pest's natural enemies. The herbivore attack induce the plant to generate and emit organic compounds particularly terpenoids which are used as host location cues by wandering parasites or predators (Turlings *et al.*, 1990, 1995). *Trichogramma* the common egg parasitoid use the plant volatiles as host location cues from the damaged plant parts (Vet and Dicke, 1992).

The egg parasitoids are known to respond to the plants on herbivory by insects (Karbon and Baldwin, 1997). The volatile signals used by foraging parasitoids can originate from the plant, the host, or from an interaction between the two. In a few plants (Dicke *et al.*, 1990a; Turlings *et al.*, 1990; Dicke, 1994; McCall *et al.*, 1994; Takabayashi *et al.*, 1991) the latter comprises passive release volatiles as well as induced volatiles. These volatiles can be plant species specific and/or herbivore species specific (De Moraes *et al.*, 1998). Studies with plants like lima beans (Dicke *et al.*, 1990b, 1993), corn (Turlings and Tumlinson, 1992), and recently cotton (Turlings *et al.*, 1995; Rose *et al.*, 1996) has shown that induced volatiles were released not only locally by the damaged leaf, but also systemically in undamaged parts of the plant.

Trichogramma wasps are minute egg parasitoids of lepidopteran species having widespread application as biological control agents. They are widely used in an inundative release programme for lepidopterous pest control (Romeis *et al.*, 1999; Wang *et al.*, 1999). Different *Trichogramma* species/strains show different preferences for certain host eggs (Zhou, 1985; Ballal and Singh, 2003). Parasitism by *Trichogramma* spp. is also affected by host egg location on plants, including both leaf surface and plant height (Burbutis *et al.*, 1977; Wang *et al.*, 1997; Romeis *et al.*, 1999; Ballal and Singh, 2003). Additionally,

different levels of parasitism by *Trichogramma* spp. can be found on the same host on different plants (Zhou *et al.*, 1997; Kuhar *et al.*, 2004). Quite a few reports indicate that within an ecosystem *Trichogramma* spp. are able to distinguish between host-infested and uninfested areas (Vet and Dicke, 1992). It is interesting to know the behavioral responses of *Trichogramma* egg parasitoids towards the plant/leaf surface chemicals from the host and non-host infested plants

The Castor semi-looper, *Achaea janata* L. (Lepidoptera: Noctuidae) is a noctuid moth, which feeds on Castor *Ricinus communis* L. leaves. During population outbreaks, larvae consume most of the foliage leaving just the veins and petioles. As a measure to control the infestation caused by these insect-*Trichogramma* species are used as biocontrol agents. They have been reported to parasitize 50-83% of castor semilooper eggs (Salvador *et al.*, 1986). The serpentine leaf miner *Liriomyza trifolii* (Burgess) (Insecta: Diptera: Agromyzidae) damage by mining of leaves by larvae, which results in destruction of leaf mesophyll. It is a non-host for the *Trichogramma* spp. In this paper, we demonstrate the ovipositional and orientation behavior of *Trichogramma chilonis* towards the surface chemicals originating from plants infested with the host *Achaea janata* L. and non-host *Liriomyza trifolii* Burgess.

MATERIAL AND METHODS

Insect material

T. chilonis reared on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) at 28 ± 2 °C, $50 \pm 10\%$ r.h., L16:D8. The female parasitoids were 1–2 d old, mated females, inexperienced, and fed with honey were used in the experiments. Only fertile eggs, recognized by a horizontal brown ring, which develops after approximately 12 h, were used. Eggs were 24 h old and killed with UV-irradiation (60W, 50 min, 30 cm distance) or untreated. This short UV-irradiation does not affect the acceptance and suitability of the eggs for *T. chilonis*. UV-irradiated eggs have the advantage that no larvae emerge from unparasitized eggs and destroy the experiment. The castor semilooper, *A. janata* insects were reared on the castor leaves at 28 ± 2 °C, $60 \pm 10\%$ RH in the laboratory. Only third instar larvae were used for the experiments.

Plant material

The experimental plants, castor was grown in the laboratory green house under controlled conditions. Potted castor plants about 40cm in height, were selected for the experiments. The third instar larvae of *A. janata* were released on to the experimental plants at the rate of 1 larva/leaf and were allowed to feed for about 1hr. During

this period almost 50% of the leaf the larvae have consumed area. Another set of castor plants were infested with the larvae of leaf miner 1larva/leaf and the leaves having the mines all over the surface (10 days after infestation) was collected for extraction.

Extraction

The plant material was collected in the morning hours and was extracted using the organic solvent Dichloromethane (DCM) by leaf dip method (Varela and Bernays, 1988). 10 leaves of each *A. janata*, *L. trifolii* infested and healthy leaves were dipped in DCM solvent for 45seconds. The plant surface extracts obtained were filtered, concentrated by rotary evaporation and stored in refrigerator at -20°C .

Ovipositional Bioassay

All the bioassays on oviposition was carried out under laboratory conditions at $28 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ relative humidity and under a light bank with overhead fluorescent tubes (1200 Lux) as source of light. The procedure adopted for oviposition bioassay was similar to the one described by Jones *et al.* (1973) with suitable modifications Usha Rani *et al.*, (2007). Clean, healthy, 0 to 24 h old eggs of *Corcyra cephalonica* Stainton sterilized under UV lamp were washed twice in hexane to remove any traces of scales or kairomones present on the surface of eggs. They were then pasted on 1cm² white cardboard pieces at 50 eggs per piece (hereafter referred to as egg cards). The plant surface extracts were applied at 0.2mg/ μl concentrations on the egg cards at the rate of 10 μl of sample on each egg card. A control was maintained in which only DCM was used. Five such treated egg cards with surface extracts along with one control card were arranged equidistantly in the experimental arena, which consisted of a 150 mm diameter Petri dish, the base of which was covered with Whatman No. 1 filter paper, each piece of egg card containing 50 eggs was considered as one replication and each extract was replicated four times. Five healthy, fed 0 to 24-h-old *Trichogramma* mated females were released at the centre of each petri dish containing egg cards. The percentage parasitism was recorded on the fifth day based on the number of eggs that turned black due to parasitization.

Orientation Behavior

The response of parasitoids to the volatile components of the plant surface extracts were recorded in the laboratory using small glass tubes. Behavioral observations were conducted in small flat-bottomed culture tubes (9 cm long; 2.5 cm diameter). Sample material (20 μl) was applied with a micropipette to a localized spot on a piece of absorbent paper (4x0.7 cm). After evaporation

of solvent for about 2 min, paper strips were placed onto the inner side of the lid of the tube. For each treatment, controls were maintained in a separate tube with the same amount of DCM One single adult-mated female was released into the glass tube before closing the lid. Observations on landing behavior, time spent on each treated and control patch, probing, and antennation were recorded visually. Landing and antennation of the treated spot by the parasitoid denoted a positive response and assigned a highest activity. The observations were terminated after 20 min.

Behaviors were classified 0–4, with 0 as no reaction, no movement; 1 as moved upwards towards the paper strip; 2 as made circular movements around the paper strip; 3 as entered/ landed on the paper strip; and 4 as antennated and probed on the chemical spot. Parasitoids almost always responded quickly, either positively or negatively, to a treatment. Twenty females were tested for each treatment, and controls were tested at the same time. All treatments were repeated on three different days with 20 insects. Almost all parasitoids showed a response to a treatment, so hardly any nonresponders were observed. All experiments and observations were conducted under laboratory conditions.

Statistical Analysis

The data obtained by the ovipositional bioassay of the *T. chilonis* parasitisation was analysed by the paired t-test for comparison between the treatments (Sigma stat 3.5). Bars represent the % mean eggs parasitized ± SE.

RESULTS

Ovipositional behaviour

In the ovipositional assays *T. chilonis* highly preferred the host plant leaf surface extracts infested with *A. janata* over the surface extracts from the non host infested castor

Table 1. Effect of normal, host and non-host infested Castor Plant surface extracts on the Orientation behavior of *T. chilonis* .

Leaf surface extracts	Type of Response /Score	Initial time of response (in min)
Normal plant	2	10
<i>A. janata</i> infested plant	4	3
<i>L. trifolii</i> infested plant	1	15

The type of response shown by 20 individual parasitoids is presented. 0=No response, 1= moved upwards, 2=landed on the paper strip, 3=moving around the patch, and 4=probing on the patch.

plants or solvent treated controls. The host eggs parasitized by *T. chilonis* in all the three treatments were significantly different at $P < 0.001$ (Figure 1). Interestingly leaf miner infested castor leaf extracts were significantly less attractive to the parasitoid than the normal healthy castor leaf extract. In choice bioassays too parasitoids preference for *A. janata* infested plant extracts was high (Figure 1).

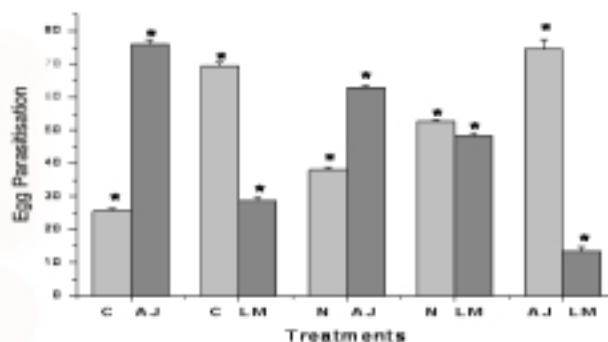


Figure 1. Effect of pest infested and uninfested Castor plant surface extract on parasitisation rates of *Trichogramma chilonis* on *C. cephalonica* eggs.

C-Control (Solvent), N- Normal Plant surface extract AJ- *Achaea janata* L. infested plant surface extract, LM- *Liriomyza trifolii* B. infested surface extract.

Orientation Behavior

In orientation assays also parasitoids response towards the extracts of castor plants infested with the host insect is very high. There was an immediate and quick movement of *T.chilonis* in the glass tube containing the extracts of host insect infested castor leaf extracts soon after their release. The parasitoid wasps had shown the probing and antennation on the strip having the host- plant surface extract. Parasitoids were highly active in these treatments, i.e. strips treated with *L. trifolii* infested castor leaf extracts and normal healthy castor leaf extracts there was only a slight upward movements by the parasitoids, *T. chilonis* did not even reach the paper strip on the treated patch (Table 1). However, the behavior of *T. chilonis* in solvent treated control treatments was entirely different. Parasitoids after their release into the glass tubes remained at the bottom of the tube without making any efforts to move or fly.

DISCUSSION

The observations on *T. chilonis*, behavior towards various host, non-host infested castor plant extracts indicated

the importance of type of herbivore feeding on a single host plant. It appears that the parasitoids were able to recognize certain chemicals or chemical combinations existing in the castor leaves infested with the host insect's *A. janata*. Presence of these chemicals in the host eggs increased their attractiveness/preference for the oviposition by *T. chilonis*. We presume these extracts contain some volatile as well as contact chemicals, which guide and stimulate the parasitoids to oviposit on the treated surface. A small number of host eggs treated with the extracts of non host (*L. trifolii*) infested leaves as well as Uninfested (Healthy) castor leaves were parasitized by *T. chilonis*. In orientation assays too parasitoids exhibited an upward movement (score-2) in the glass tube, thus acknowledging the presence of a few synomonal compounds in the extracts.

Herbivore feeding stimulates the production of volatile organic compounds and this has been demonstrated by several plant herbivore systems (Kessler and Baldwin, 2001; De Moraes *et al.*, 1998). Our experiments show that castor plants under the attack by *A. janata* larvae (which cause severe damage to the leaf by their voracious feeding), emit volatile and also contact chemicals that enhance the location/ acceptability of host eggs by parasitoid. These induced emissions of chemicals had occurred only in castor plant infested with *A. janata* larvae and not the *L. trifolii*.

We suggest certain organic volatile compounds might have been emitted by the *A. janata* damaged castor leaves which drew the parasitoids towards their source and certain contact chemicals that are also present in the extracts could have stimulated the oviposition. Because, though in less percentage, the host eggs treated with non-host insect infested as well as normal healthy castor plant extracts also has considerably stimulated parasitisation by *T. chilonis* in comparison with the solvent treated host eggs.

Our studies showed that the attractiveness of *A. janata* herbivore-induced synomones towards *T. chilonis* were specific at the herbivore level. The synomones induced by *L. trifolii* could not attract the egg parasitoids though they are from the same plant. Some specialist parasitoids of lepidopteran larvae and aphids could differentiate between volatiles emitted from host-infested plants and odor from plants damaged by non-hosts (De Moraes *et al.*, 1998; Powell *et al.*, 1998). Learning of distinct stimuli, which indicate the presence of suitable host species, has been shown to be a tool for parasitoids to cope with the variability of chemical signals available (Vet and Groenewold, 1990; Turlings *et al.*, 1992; Vet and Dicke, 1992; Dicke and Vet, 1999). Thus the parasitoids use the

plant synomones. Investigations of the preference of *T. chilonis* for oviposition on castor semilooper infested leaves are necessary for a comprehensive elucidation of the role of host specificity for host location of this parasitoid.

Here we found that the surface extracts from the same host plant infested with different insects had shown varied responses. Parasitoids, in particular, are highly susceptible to very small changes in the quality of the host's internal biochemical environment (Harvey, 2005). Other studies have shown that parasitoids can be more affected than their hosts by the quality of the host plant (Harvey *et al.*, 2003; Soler *et al.*, 2005). In plant both host and non-host larvae induce the plant to emit qualitatively and quantitatively the same synomones (Röse *et al.*, 1998). But in the case of castor *R. communis*, we found that the egg parasitoids preferred host insect infested surface extracts when compared to the non-infested plant surface extracts. At present there is not enough experimental evidence available to discern a general pattern of herbivore specificity of induced plant synomones. A better knowledge of the surface chemistry will help to understand interactions between plant parasitoid, insect-parasitoid interactions.

In conclusion, the specialist *A. janata* appears to be host that induces plant synomones which are the indicators for the *T. chilonis* during the entire host selection process from location of host habitats to location of hosts and host acceptance/recognition. The tritrophic system studied here is described the relationship of the oligophagous and monophagous on the second and the third trophic level by little variation of chemical cues for the parasitoid. These built in characteristics of the tritrophic system might be a prerequisite for the development of such selective responses of a parasitoid towards specific infochemicals.

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Pathipati Usha Rani*, **Y. Jyothsna** and **M. Lakshminarayana**¹

* Biology and Biotechnology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad - 500 007, ¹Directorate of oil Research, Rajendranagar, Hyderabad (AP), India, *e-mail: purani@iict.res.in.