

Lysiphlebus testaceipes on *Aphis gossypii*: studies on remote host discrimination

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Abstract

The ability of *Lysiphlebus testaceipes* (Cresson) females to discriminate between parasitised and unparasitised *Aphis gossypii* Glover, and the mechanisms of host discrimination was studied. By comparing the resting time of parasitoid females on leaves with parasitised and unparasitised aphids their ability to host discriminate was evidenced. Y-tube olfactometer tests and direct observations of the searching behaviour of *L. testaceipes* females showed that the markers released during the parasitisation (at the insertion of the ovipositor and during egg laying) are perceived neither over long nor over short distance, but they are probably responsible of contact host discrimination. However, the parasitoids use their antennae to perceive olfactory cues and thus recognise parasitised hosts. The cues that act over short distance are directly or indirectly induced by the egg or the larva of the parasitoid developing inside the host.

Key words: *Lysiphlebus testaceipes*, parasitoid, *Aphis gossypii*, host discrimination, searching behaviour.

Introduction

The ability of parasitoids to discriminate between parasitised and unparasitised hosts is not always a sign of reproductive success. In some cases the younger larva can compete with the older one and may be able to complete its development. It thus can be biologically advantageous to parasitise an already parasitised host, as in superparasitism. However, this phenomenon should be avoided in biological control because it leads to a waste of reproductive potential and to a lower efficacy of parasitoids (Mackauer, 1990).

Host discrimination in *Lysiphlebus testaceipes* (Cresson) has been studied by van Steenis (1994). He states that *L. testaceipes* females are not able to discriminate between parasitised and unparasitised aphids, because "the distribution of the larvae in aphids parasitised during 1 day does not significantly deviate from a random distribution". In his experiment van Steenis offered *L. testaceipes* females a determined number of *Aphis gossypii* Glover individuals for one day, controlling if any larvae were present inside the aphids after 4 days of incubation. This method of analysis is widely used (Shirota *et al.*, 1983; Cloutier *et al.*, 1984, Micha *et al.*, 1992), but may not be accurate (van Lenteren *et al.*, 1978). However, a non-random larvae distribution does not necessarily indicate that the parasitoid is able to host discriminate. For example, a non-random distribution may be due to the fact that parasitised hosts have a lower probability to be attacked again by the parasitoid, because they are more mobile than unparasitised aphids, or because they leave the area patrolled by the parasitoid. A random parasitoid larvae distribution as in van Steenis (1994) may not necessarily correspond to a lack of host discrimination: there might be other reasons, such as aphids' behaviour (van Lenteren *et al.*, 1978; van Lenteren, 1981). Moreover, sometimes the parasitoid can superparasitise, regardless of its ability to dis-

criminate (van Alphen and Jervis, 1996). Thus, only on the basis of larvae distribution it seems impossible to state that a parasitoid can or cannot discriminate between parasitised and unparasitised hosts (van Lenteren *et al.*, 1978; van Alphen and Jervis, 1996).

The ability to host discriminate depends on host marking and marker perception (figure 1). The existence of a marker left by the parasitoid has been demonstrated: it may be either released during the attack or produced by the larva or, even, by the egg developing inside the host. This marker could be external, of chemical origin (Strand, 1986), or internal, linked to internal variations of the host (Chow and Mackauer, 1986). To host discriminate the cue must be perceived by another parasitoid in a second encounter.

The aim of this study was to analyse the ability of *L. testaceipes* to discriminate between parasitised and unparasitised aphids, to avoid superparasitism, and to identify the mechanisms of host discrimination.

Materials and methods

Rearing. The parasitoids were reared in a cage (80 x 50 x 70 cm) inside an incubator. The temperature was 25±1°C during the day and 20±1°C during the night, relative humidity was 70±10%, and photoperiod was L:D=16:8h. The light was provided by sodium lamps and fluorescent tubes. *A. gossypii* was used as host. Only mated, expert and age known *L. testaceipes* females were used in the experiments.

Experiment 1. Resting time of parasitoid females on leaves with parasitised and unparasitised aphids. If the parasitoid were able to host discriminate, it would show a response in terms of resting time on leaves. It would take off from leaves with parasitised hosts more quickly than from those

with unparasitised ones (Hart *et al.*, 1978).

Two leaves of *Cucurbita pepo* L. 'Greyzini' (surface: 20 cm²) were used in each trial. Each leaf was infested by 20 aphids [10 (adults) + 5 (1st and 2nd instar nymphs) + 5 (3rd and 4th instar nymphs)]. One leaf was infested by unparasitised aphids, whereas the other leaf was infested by parasitised aphids. To obtain parasitised aphids, a heavily infested leaf was exposed to some *L. testaceipes* females for 12 hours. Each leaf was placed in a Petri dish of 10 cm diameter, with its bottom covered with wet cotton, in order to keep the leaf fresh.

One young, mated and expert *L. testaceipes* female was released on each leaf (20 replicates per leaf type). To allow the parasitoid to leave at every moment, only the bottom of the dish was used. Each female was observed continuously and the resting time on the leaf was recorded. The bioassay was considered over when the female left the leaf. If the female rested for more than 45 minutes on the leaf, the trial was not considered. Each female was used twice: females, first tested on a leaf with parasitised aphids, were transferred on a leaf with unparasitised aphids in the second trial, and *vice versa*.

For each female the following parameters were measured:

- resting time on the leaf with parasitised aphids (t_p).
- resting time on the leaf with unparasitised aphids (t_u).

The index of relative difference T_{p-u} was defined as follows:

$$T_{p-u} = \frac{t_p - t_u}{t_u}$$

The mean T_{p-u} with its standard deviation was calculated. Friedman non-parametric test was applied to determine statistical differences between resting times on the two different leaf types.

Experiment 2. Long distance host discrimination. The ability of *L. testaceipes* females to use their antennae in host discrimination over long distances was investigated. A Y-tube olfactometer was used (Bazzocchi and Maini, 2000). The air flow (1.0 ± 0.1 l/min.) was produced by an electric pump and passed through a container filled with deionised water, in order to purify the air flow. Then, the air flow passed through a forked tube and, through two flowmeters, it reached two chambers, that contained the odour sources. A glass Y tube linked the two chambers to the release point of the parasitoid.

Two *C. pepo* leaves, one with unparasitised aphids, and the other one with parasitised aphids, were placed in the odour chambers of the olfactometer. Only 2nd and 3rd instar aphids were used. To obtain honeydew free leaves, in the first trial the leaves were prepared as in experiment 1. In the second trial the leaves were kept at $25.0 \pm 0.1^\circ\text{C}$ for 12 hours, to allow honeydew production. A young, mated and expert *L. testaceipes* female was freed at the release point. It could stay there or it could show a response to the odours, going towards one of the two odour chambers (with unparasitised or parasitised aphids). The percentage of females that responded to the air flow and, among them, the

percentage of females that made a particular choice, was calculated. 17 trials with honeydew free leaves and 20 trials with honeydew leaves were carried out.

Experiment 3. Short distance host discrimination. To investigate the existence of a marking signal perceptible only over a short range, the host searching behaviour of *L. testaceipes* females was observed accurately. It was analysed whether the encounters of the parasitoid with the parasitised and unparasitised hosts were random. If parasitised hosts were encountered less frequently than unparasitised ones, the existence of a short distance host discrimination by olfactory cues should be postulated, because the females would avoid parasitised and thus marked aphids. Finally, the possible variation over time of the deterrent effect of parasitised aphids was investigated.

Seven unparasitised and five parasitised aphids were placed on a *C. pepo* leaf inside a Petri dish (diameter: 10 cm). According to the trial, the parasitised aphids pertained to the three different classes: attacked by a parasitoid female 1, 6 and 24 hours before the beginning of the trial. A different young, mated and expert *L. testaceipes* female was then released. The aphids were numbered and their exact position was recorded. Their movements were continuously tracked and the behaviour of the parasitoid was recorded with a video camera connected to a stereomicroscope. The parasitoid could encounter every aphid that was on the leaf and, being the Petri dish without lid, it could leave the leaf, if it was not advantageous any more to search there. During the trial, every female could encounter different classes of aphids:

- 1) unparasitised,
- 2) attacked a) 1 hour, b) 6 hours, c) 24 hours before the trial,
- 3) just attacked (initially unparasitised, then attacked during the trial),
- 4) previously attacked, that were attacked again during the trial.

The term "attacked aphids" includes both pseudoparasitised and parasitised aphids.

The recordings were reviewed and, at every encounter, the number of aphids belonging to each class and the outcome of the encounter (attack or else) was registered. An aphid belonging to the first or second class, automatically passed to class 3 or 4 after an attack. Because of too low a number of data available, the 4th class was not considered for further analysis.

Data analysis

The following null hypothesis was postulated:

"The encounter frequency of each *L. testaceipes* female with aphids of a certain class does not depend on the class, but it is proportional to the fraction: [number of aphids of the class] / [total number of aphids]"

The *u test* for fractions (Czerminski *et al.*, 1990) was used for statistical analysis.

For each encounter, the theoretical probability (p_i) to encounter an aphid of a certain class, if there were no preferences for any class, was calculated:

$$p_i = \frac{\text{number of aphids of a certain class}}{\text{total number of aphids on the leaf}}$$

For each class, the expected fraction (p_0) of encounters with aphids of a certain class on the total number of encounters was calculated:

$$p_0 = \sum_{i=1}^N \frac{p_i}{N}$$

where N is the total number of encounters during a trial.

The null hypothesis could therefore be defined as:

$$H_0: \frac{m}{N} = p_0$$

where m is the observed number of encounters with aphids of a particular class.

For aphids of the 2nd class, the three series of trials (a, b, and c) were analysed separately, whereas for the 1st and the 3rd class, all trials were combined, thus dividing all the aphids in 5 classes.

For each class the following value was calculated:

$$u = \frac{\frac{m}{N} - p_0}{\sqrt{\frac{p_0 \times q_0}{N}}}$$

where $q_0 = 1 - p_0$.

If the null hypothesis is true, this function has an asymptotically normal distribution $N(0, 1)$. For the bilateral critical zone (two tail test) the critical value is: $u_{0.05} = 1.96$. If the empirical value u was $|u| \geq u_{0.05}$, the null hypothesis was rejected.

Results and discussion

Experiment 1. Resting time of parasitoid females on leaves with parasitised and unparasitised aphids. As shown by the relative difference index, the mean resting time of *L. testaceipes* females on leaves with parasitised aphids corresponded to 31% of the time spent on leaves with unparasitised aphids (table 1).

The parasitoid was able to discriminate between unparasitised and parasitised hosts. In fact, in presence of many parasitised aphids, the females left the searching area. Our results are in accordance with what reported by van Alphen *et al.* (1987): "...when an experienced parasitoid arrives in an exploited patch, she may reject

the parasitised hosts and leave the patch...". Nevertheless, from this experiment the mechanisms involved in host discrimination do not emerge (figure 1).

Table 1. Mean and standard deviation of relative difference index and results of Friedman test.

N	Mean T _(p-u)	Std. Dev.	H ₀	p
20	-0.69	0.18	t ₀ =t _i	< 0.05

Experiment 2. Long distance host discrimination. Almost all *L. testaceipes* females (94%) did not respond to the olfactory cues from leaves without honeydew (figure 2A), whereas when honeydew was present, 40% of the parasitoids did make a choice, without distinguishing between leaves with parasitised and unparasitised aphids (figure 2B).

L. testaceipes females were not able to discriminate between parasitised and unparasitised aphids, and thus the markers released during the parasitisation (i.e. at the insertion of the ovipositor and during egg laying) are not perceived by the antennae over long distances (figure 1). However, the parasitoid seems to perceive other olfactory cues with its antennae. In fact, females were attracted by leaves with honeydew. The presence of volatile kairomones in the honeydew may therefore be hypothesised, even though several Authors report that the kairomones in the honeydew act only by contact (Bouchard and Cloutier, 1984; Ayal, 1987; Budenberg, 1990; Hågvar and Hofsvang, 1991). Moreover, further studies are warranted in order to investigate the existence of other mechanisms, such as SOS signals emitted by the plant (Dicke, 1994).

Experiment 3. Short distance host discrimination. No significant differences between expected and observed fraction of encounters emerged, neither for unparasitised hosts nor for those just attacked, parasitised 1 and 6 hours before the test (table 2). Therefore, with unparasitised aphids and up to 6 hours from the attack, no deterrent effects seem to exist. Over short distance the antennae of the parasitoid do not perceive markers released both at the insertion of the ovipositor and during egg laying, which anyway may be involved in contact host discrimination (figure 1).

Table 2. Number of encounters observed, expected and observed fraction of encounters, and results of the u test for the five different classes of aphids.

Aphid class	no. encounters observed	Expected fraction (p_0)	Observed fraction (m/N)	Statistic
Unparasitised	219	0.35	0.38	ns
Just attacked	129	0.23	0.22	ns
1 hour	84	0.34	0.35	ns
6 hours	42	0.34	0.29	ns
24 hours	53	0.35	0.27	$p < 0.05$

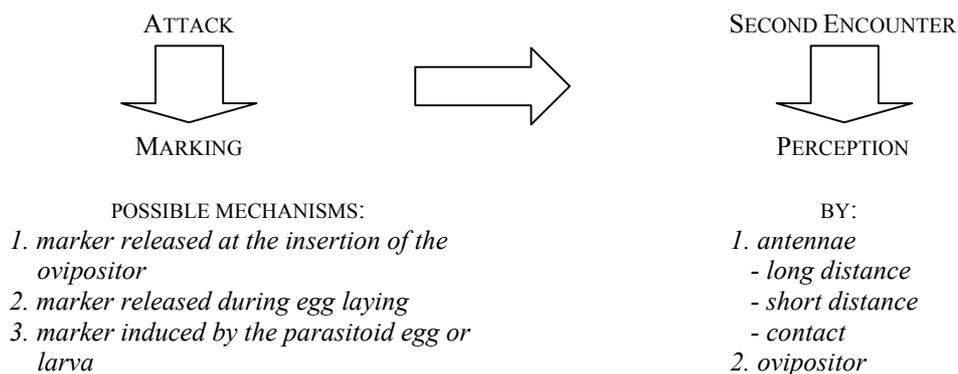


Figure 1. Possible mechanisms of marking and perception of the marker.

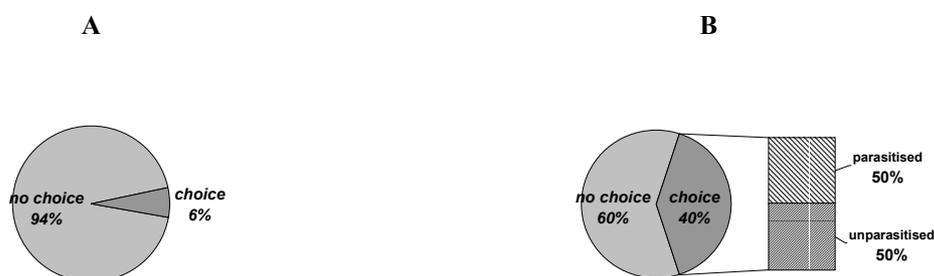


Figure 2. Reaction of *L. testaceipes* females to olfactory cues from leaves infested by parasitised and unparasitised aphids (A: leaves without honeydew; B: leaves with honeydew).

For aphids parasitised 24 hours before the trial, the observed fraction of encounters was significantly lower than the expected one (table 2). The female apparently avoids aphids that have been parasitised 24 hours before. Short distance olfactory cues, such as markers released by the egg or the larva of the parasitoid, or by the parasitised host, could cause this phenomenon.

Conclusions

L. testaceipes females are able to discriminate between parasitised and unparasitised hosts, hence both host marking and host perception occur.

Olfactory sensilla located on the antennae allow to recognise the difference between parasitised and unparasitised hosts.

The markers released during the parasitisation (at the insertion of the ovipositor and during egg laying) are perceived neither over long nor over short distance, but they are probably responsible of contact host discrimination.

Markers released by the egg or the larva of the parasitoid, or by the parasitised host, are perceived 24 hours after the attack by the antennae of *L. testaceipes* females over short distance, thus allowing host discrimination.

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