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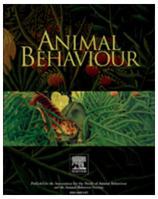

Assessment of oviposition site quality by aphidophagous hoverflies: reaction to conspecific larvae

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Aphidophagous predators adapt their foraging behaviour to the presence of conspecific and heterospecific larvae. We studied the effect of the presence of conspecific larvae and their tracks on the oviposition site selection of an aphid-specific predator, *Episyrphus balteatus* DeGeer (Diptera: Syrphidae), in two-choice experiments using a leaf disc bioassay. Gas chromatography – mass spectrometry analysis was used to identify the volatile chemicals released from odour extracts of *E. balteatus* larval tracks. The behavioural effects of these volatile substances on hoverfly females were also evaluated. Our experiments demonstrated that *E. balteatus* females were deterred from ovipositing when presented with a *Vicia faba* leaf with aphids and conspecific larvae. The oviposition-deterring stimulus was also active when females were presented with a leaf that contained conspecific larval tracks. A mixture of chemical compounds was found in the volatile pattern of odour extracts of larval tracks. The main volatile chemicals were 3-methylbutanoic acid, 2-methylbutanoic acid, 2-methylpropanoic acid, 3-hydroxy-2-butanone, hexanoic acid and phenol. Females also laid significantly fewer eggs in response to odorant volatiles emitted from larval extracts. These results highlight that predatory hoverfly females avoid ovipositing in aphid colonies in which conspecific larvae or their tracks are already present, suggesting that this behaviour constitutes a strategy that enables females to optimize their oviposition site and reduce competition suffered by their offspring.

Female insects, foraging for suitable oviposition sites, often face many stimuli from their environment that ultimately influence their offspring's performance (Schoonhoven et al. 1998). Prior occupation by a conspecific individual has been found to influence females'_ oviposition decision in various insect species (Price 1970; Van Lenteren & Debach 1980; Hemptinne et al. 1993; Janssen et al. 1995; Nufio & Papaj 2001). Typically, females avoid laying eggs on hosts that are already being exploited, which constitutes a behavioural strategy to improve the survival, growth and reproductive potential of their offspring (Kindlmann & Dixon 1993; Peckarsky et al. 2000; Almohamad et al. 2008a), and to reduce competition suffered by their offspring (Doumbia et al. 1998). It has been also demonstrated that the stimuli permitting females to distinguish between the occupied and unoccupied hosts are chemical cues (Dempster 1992; Mudd et al. 1997; Seeley 1998; Li et al. 2001), which derive from conspecific eggs (Anbutsu & Togashi 1996, 1997; Scholz & Poehling 2000), larvae (Williams et al. 1986; Anbutsu & Togashi 1996; Růžička 1997; Doumbia et al. 1998; Růžička 2001; Fréchette et al. 2003) or larval tracks (Dittrick et al. 1983; Anderson et al. 1993; Yasuda et al. 2000; Michaud & Jyoti 2007).

Aphidophagous hoverflies are well-known aphid natural enemies that can have a significant impact on the suppression of aphid populations (Chambers 1988). However, the stimuli that allow hoverfly females to evaluate a suitable oviposition site are still unclear. Field and laboratory observations indicate that syrphid eggs are laid close to aphid colonies (Dixon 1959; Chandler 1968a, b; Evans & Dixon 1986; Hemptinne et al. 1993). These studies showed convincingly that there is an optimal number of hoverfly eggs that should be laid in an aphid colony to maximize the number of surviving offspring, and argued that this number is typically much less than the number required to provide adequate control of aphids. Furthermore, syrphid eggs are targets for both cannibalism and intraguild predation (Branquart et al. 1997; Hindayana et al. 2001; Fréchette et al. 2007). Therefore, adult females must select oviposition sites that not only provide their offspring with sufficient resources to complete development, but also minimize their exposure to predictable sources of mortality.

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Based on our previous studies (Almohamad et al. 2006, 2007, 2008; Harmel et al. 2007; Verheggen et al. 2008), we wanted to determine the cues and behavioural mechanisms that enable *E. balteatus* females to locate and select a suitable oviposition site, taking into consideration that the ‘quality’ of an oviposition site may depend not only on the presence of aphids and their numbers, but also on the presence of inter- or intraspecific competitors. The oviposition avoidance of occupied hosts by parasitoid females and phytophagous insects has been studied intensively (Price 1970; Rothschild & Schoonhoven 1977; Janssen et al. 1995; Kanno & Harris 2002; Nakashima & Senoo 2003; Li & Zhang 2006; Nakashima et al. 2006). It has been recently discovered that the oviposition behaviour of aphidophagous predators is often modified by the presence of conspecific and heterospecific larvae. Subsequently, reports appeared on the oviposition repellence of larvae or their tracks in various aphidophagous insects, initially in green lacewings (chrysopids: Růžička 1994, 1996), then in coccinellids (Hemptinne & Dixon 1991; Růžička 1997; Doumbia et al. 1998; Yasuda et al. 2000; Agarwala et al. 2003; Michaud & Jyoti 2007) and in *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae; Růžička & Havelka 1998). Nevertheless, the oviposition response of syrphid females to the presence of conspecific and heterospecific competitors has received little attention~~s~~. We aimed to investigate the behavioural reaction of the aphidophagous hoverfly *E. balteatus* to the presence of conspecific larvae and their tracks in aphid patches.

METHODS

Plants and Insects

Broad bean plants, *Vicia faba* L., were grown in plastic trays (30 × 20 cm and 5 cm high) filled with a mix of perlite and vermiculite (1:1) and maintained in controlled-environment growth rooms (16:8 h light:dark; 20 ± 1 °C). Two aphid species, namely *Myzus persicae* Sulzer and *Megoura viciae* Buckton, were reared on *V. faba* in separated air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in cages (75 × 60 cm and 90 cm high) and were provided with bee-collected pollen, sugar and water. Broad beans infested with *M. viciae* were introduced into the cages for 3 h every 2 days to allow oviposition. Hoverfly larvae were mass reared in aerated plastic boxes (110 × 140 mm and 40 mm high) and were fed daily ad libitum with *M. viciae* as standard diet.

Experiment 1: Effect of Conspecific Larvae on Oviposition

We assessed the oviposition response of *E. balteatus* females to the presence of conspecific larvae. To do so, we placed a circular piece of *V. faba* leaf, 2.5 cm in diameter, on a petri dish containing an agar solution to reduce desiccation. An excess of aphids (125 individuals) were then placed on the leaf along with two *E. balteatus* third-instar larvae. A control treatment was also run in parallel and consisted of a leaf disc infested with the same numbers of aphids without larvae. In two-choice experiments, both petri dishes containing their aphid-infested leaves (one with larvae and the other without larvae) were positioned on two Plexiglas holders, of 20 cm height, and separated by 15 cm in a net cage (30 × 30 cm and 60 cm high). This set-up was previously described and shown to be an efficient method to evaluate the oviposition behaviour of *E. balteatus* in response to aphid-infested plants (Almohamad et al. 2006). A single female was then introduced into the cage and allowed to lay eggs for 3 h. The eggs laid on each leaf disc were then counted. Experiments were conducted in an air-conditioned room at 21 ± 1 °C. *Episyrphus balteatus* females were approximately 20–30 days old and no oviposition had been induced for 24 h prior to the experiments. Ten replicates were performed for this experiment.

Experiment 2: Effect of Larval Tracks on Oviposition

In this experiment, two third-instar larvae of *E. balteatus* were supplied with an excess of *M. persicae* aphids (125 individuals) on a circular piece of *V. faba* leaf, 2.5 cm in diameter, placed on agar in a petri dish. Aphid-infested leaf discs were then covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL, U.S.A.) and kept in the controlled incubator (16:8 h light:dark; 20 ± 1 °C) for 24 h. After 24 h, the larvae and all aphid material were carefully removed. The leaf discs (either containing larval tracks or not) were then infested with a new excess of aphids. In similar two-choice experiments as described above, a single female was introduced into the net cage (30 × 30 cm and 60 cm high) in the presence of the two aphid-infested leaf discs (one with larval tracks and the other without larval tracks as control) presented on similar 20 cm-high holders and then allowed to lay eggs for 3 h. The eggs laid on each leaf disc were then counted. Experiments were conducted in similar conditions as described above. This experiment was replicated 10 times for each treatment.

Experiment 3: Effect of Larval Tracks on Foraging

Aphid-infested leaf discs with larval tracks were prepared as described for experiment 2. In no-choice experiments, a single *E. balteatus* female was placed in a net cage with each aphid-infested leaf disc treatment (one with larval tracks and the other without larval tracks as a control). We recorded their foraging and oviposition behaviour for 10 min using Observer version 5.0 (Noldus Information Technology, Wageningen, The Netherland). Descriptions of the four behavioural events that were observed were grouped as follows: (1) immobility: the hoverfly female was immobilized on the cage without moving; (2) searching: the hoverfly female hovered in the cage close to the infested plant; (3) acceptance: the hoverfly female landed on the plant, stayed immobile or walked on it, with proboscis extension on the plant surface; (4) oviposition: the hoverfly female showed abdomen bending and laid eggs.

The behavioural observations were conducted in an air-conditioned room at 20 ± 1 °C. Tested *E. balteatus* females were approximately 20–30 days old and no induction of oviposition had been realized for 24 h prior to the experiment. This experiment was replicated 10 times for each treatment.

Experiment 4: Volatile Chemicals Released by Larval Tracks

Volatile collection system

We collected volatile chemicals using a push/pull volatile collection system consisting of a glass air-collection chamber (Schott, 10 cm base diameter, 12 cm high) placed in an air-conditioned room at 21 ± 1 °C, and previously washed with hot water and hexane. Ten third-instar larvae of *E. balteatus* were placed in a closed glass petri dish 5 cm in diameter without aphids, and kept inside an incubator (16:8 h light:dark; 21 ± 1 °C) for 24 h. After 24 h, the larvae were carefully removed from the glass petri dishes. Three petri dishes containing odour extracts of larval tracks were then placed in the glass air-collection chamber. Incoming air was pulled through an in-line activated charcoal filter before entering the glass chamber at a flow of 200 ml/min. The volatile-enriched air was then pulled through an adsorption trap containing 40 mg SuperQ (Alltech, Deerfield, IL, U.S.A.). Volatiles were collected during 3 h and the filters were eluted with 150 μl of diethylether.