Impact of aphid colony size and associated induced plant volatiles on searching and oviposition behaviour of a predatory hoverfly

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Abstract

Volatile chemicals emitted by aphids or aphid-infested plants act as kairomonal substances for several aphid natural enemies, and are therefore considered as indirect defence for the infested plants. In the present study, the foraging and oviposition behaviour of the aphid specific predator, *Episyrphus balteatus* DEGEER (Diptera: Syrphidae), was investigated with respect to the aphid colony size, using a leaf disc bioassay. Female *E. balteatus* exhibited pronounced searching and acceptance behaviour, leading to egg laying, in response to large *Myzus persicae* Sulzer (Homoptera: Aphididae) colony sizes. Behavioural impacts of synthetic aphid alarm pheromone and geranyl acetone toward *E. balteatus* female foraging and oviposition behaviour were also demonstrated in this work. These results highlight the role of aphid semiochemicals in predatory hoverfly attraction and provided an opportunity to elucidate some mechanisms of decision-making by female syrphid predators during their foraging and egg-laying behaviour.

Keywords: *Episyrphus balteatus*, foraging behaviour, *Myzus persicae*, (*E*)-β-farnesene, geranyl acetone

Introduction

Volatile chemical signals released by herbivore-infested plants serve as olfactory cues for parasitoids (DU *et al.*, 1998; DE MORAES *et al.*, 1998; VAN LOON *et al.*, 2000) and predators (EVANS & DIXON, 1986; DICKE, 1999; NINKOVIC *et al.*, 2001). According to CHANDLER (1968a), aphidophagous syrphid species have been divided into two groups: (1) phytozetric syrphid
species, such as *Melanostoma mellinum* (L) (Diptera: Syrphidae), that rely more on plant-derived stimuli than on aphid stimuli, and (2) aphidozoetic species, such as *Episyrphus balteatus*, that use aphid-derived chemicals to locate their prey and subsequent oviposition sites. Several studies have demonstrated that specific blend of odours produced by injured plants or released by the pests are attractive to certain predators and parasitoids (Vet & Dicke, 1992; Dicke, 1994). It is likely that aphid-associated odours are interpreted by female *E. balteatus* as olfactory cues orientating them toward aphid-infested sites (Shinoda et al., 1998). Honeydew, for example, acts as an oviposition stimulant for syrphid females and as an olfactory cue used in the location of aphid colonies (Budenberg & Powell, 1992; Bargen et al., 1998; Sutherland et al., 2001). (E)-β-Farnesene (EβF) is the main component of the aphid alarm pheromone and it was identified in 16 aphid species, alone or associated with other molecules (Francis et al., 2005a). Previous behavioural studies have demonstrated the kairomonal role of EβF in various aphid natural enemies, including ladybeetles, hoverflies and parasitic hymenoptera (Du et al., 1998; Al Abassi et al., 2000; Francis et al., 2004; Harmel et al., 2007; Verheggen et al., 2007, 2008). EβF, associated with other natural molecules such as α-pinene, β-pinene and limonene, has also been used efficiently as reliable olfactory cues by syrphid larvae in prey location (Francis et al., 2005b).

Because of its high reproductive rate, voracity and degree of adaptation, *E. balteatus* is considered as an efficient aphid natural enemy (Chambers, 1988). Moreover, its high mobility enables it to lay eggs over large areas (Schneider, 1984; Chambers, 1988, 1991) and to locate aphid colonies earlier in the season than other aphidophagous predators (Hagen & van den Bosch, 1968; Horn, 1981; Dixon, 2000). Previous studies have demonstrated that the oviposition rate of hoverfly *E. balteatus* females was a function of aphid densities (Chandler, 1968b; Itô & Iwao, 1977; Geusen-Pfister, 1987; Bargen et al., 1998; Scholz & Poehling, 2000; Sutherland et al., 2001; Almohamad et al., 2006). However, behavioural responses of predatory hoverflies to odour cues induced from aphid colony size have received little attention (Laubertie et al., 2006; Almohamad et al., 2008a; Verheggen et al., 2008).

In the previous study of Almohamad et al. (2008b), there was a significant quadratic relationship between the amount of released EβF and *M. persicae* colony sizes. Geranyl acetone was also identified and its amount slightly decreased with increasing aphid colony size. The present study aims to assess the foraging and oviposition behaviour of *E. balteatus* females with respect to different densities of *M. persicae* colonies. The behavioural impacts of the volatiles EβF and geranyl acetone were also evaluated toward females of predatory hoverfly.
Materials and Methods

Plants and insects

Broad bean plants (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic trays filled with a mix of perlite and vermiculite (1:1) and maintained in controlled environment growth rooms (16:8 Light:Dark; 20 ± 1°C). Two aphid species, namely *M. persicae* and *Megoura viciae* BUCKTON were taken from stock rearing on *V. faba*, in separate air-conditioned rooms under the same conditions as described above. Adult *E. balteatus* were reared in 75 x 60 x 90 cm cages 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 two days to allow oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm) and were fed daily *ad libitum* with *M. viciae* as standard diet.

Leaf disc system

The leaf disc-system consisted of (1) a circular piece of *V. faba* leaf, (2) aphids and (3) 7 ml of an agar diet (agar 1% solution w:w), placed in a 25 mm diameter Petri dish, to reduce desiccation. Leaves were infested with different quantities of *M. persicae* and were kept for 24 h in a controlled conditions incubator (16:8 Light:Dark; 20 ± 1°C) before testing. The leaf disc system was placed on a Plexiglass holder at a height of 20 cm before being exposed to a hoverfly predator. This setup was previously shown as an efficient method to evaluate the oviposition behaviour of the hoverfly *E. balteatus* in response to aphid-infested plants (ALMOHAMAD et al., 2006).

Influence of aphid colony size on syrphid behaviour

In no-choice experiments, a single *E. balteatus* female was placed in a net cage (30 x 30 x 60 cm) in presence of the leaf disc system previously described and supporting a *M. persicae* colony made of 25, 75 or 125 individuals. A non-infested leaf was used as a control. Their foraging behaviour was recorded for 10 minutes using the Observer® (Noldus Information Technology, version 5.0, Wageningen, The Netherlands). Four behavioural events were observed. 1) Immobility: the hoverfly stays on the net cage without moving. 2) Searching: the syrphid flies in the cage around the infested plant, either in an extensive or intensive way. 3) Acceptation: the hoverfly lands on the plant, stays immobile or walks on it, makes proboscis extension on the plant surface. 4) Oviposition: the syrphid shows abdomen bending and lays eggs.

Behavioural observations were conducted in an air-conditioned room at 21 ± 1°C. Hoverfly females were approximately 20-30 days old and no oviposition had occurred for 24 h prior the experiment. This experiment was repeated 10 times for each treatment, including the control and three aphid densities.
Impact of (E)-β-farnesene and geranyl acetone on syrphid behaviour

We assessed the infochemical role of EβF and geranyl acetone toward E. balteatus female behaviour by placing 100 μl of a 400 ng/μl solution of EβF or geranyl acetone (diluted in paraffin oil) in a rubber septum placed itself on a leaf disc system without aphids, on the top of a Plexiglass holder. A treatment control was also run in parallel and consisted of leaf with only a rubber septum with only paraffin oil. A single hoverfly female was introduced in the cage and its behaviour was observed for 10 min. Ten replicates were performed for each treatment.

Fig. 1. Mean frequencies and durations (± SE) of behavioural observations of E. balteatus females in response to increasing aphid M. persicae colony size on leaf disc in no-choice experiment. Different letters above bars indicate significant differences between treatments (ANOVA, Tukey’s test, P < 0.05).
Statistical analysis

Behavioural sequences in response to different aphid colony sizes were compared using Tukey's test, and Dunnett's test was also performed to compare behavioural responses to tested chemical compounds. All analyses were performed using Minitab® software (14.2 version, Minitab Inc, State College, PA, USA).

Results

Influence of aphid colony size on syrphid behaviour

The mean frequencies and mean durations observed for each E. balteatus behavioural event are presented in (Figure 1-A) and (Figure 1-B), respectively. Higher aphid densities lead to an increase of searching ($F_{1,39} = 3.94; P = 0.019$), acceptance ($F_{3,39} = 20.95; P < 0.001$) and oviposition behaviours ($F_{3,39} = 24.95; P < 0.001$) in terms of frequencies (Figure 1-A). In addition, E. balteatus females did not exhibit abdominal protractions or egg-laying behaviour in the absence of aphids.

While the hoverfly predator increases its mobility in presence of large aphid colonies, the duration allowed for searching was not affected ($F_{1,39} = 1.96; P = 0.193$). Time taken for acceptance and oviposition behaviours were however increased with larger aphid colonies ($F_{1,39} = 16.98; P < 0.001$ and $F_{3,39} = 30.03; P < 0.001$, respectively)

Influence of (E)-β-farnesene and geranyl acetone on syrphid behaviour

Compared to the control, EBF induced higher frequencies of E. balteatus female searching ($t_{obs} = 3.49; P = 0.003$) and acceptance behaviour ($t_{obs} = 3.44; P = 0.004$), while the behavioural responses of E. balteatus female was not affected by the presence of geranyl acetone in term searching ($t_{obs} = -1.26; P = 0.358$) and acceptance behaviour ($t_{obs} = -0.25; P = 0.957$) (Figure 2-A). Hoverfly female also exhibited abdominal protraction and egg-laying behaviour in response to EBF, while this behaviour was not observed in the presence of geranyl acetone.

In the presence of EBF, females E. balteatus spent more time searching ($t = 2.98; P = 0.012$) and accepting the host plant ($t = 2.73; P = 0.021$). The time spent for searching ($t_{obs} = -0.53; P = 0.821$) and accepting the host plant ($t_{obs} = -0.08; P = 0.995$) did not significantly change in presence of geranyl acetone when compared to the control (Figure 2-B). E. balteatus was more mobile in response to the odour of EBF ($t = -4.55; P < 0.001$). As a result, EBF played an effective kairomonal role on E. balteatus female foraging behaviour and acted as an oviposition stimulant.

Discussion

Our experiments demonstrated that the foraging and oviposition behaviour of individual E. balteatus females was dependent of prey colony size. In previous experiments of SUTHERLAND et al. (2001), hoverfly females failed to
Fig. 2. Mean frequencies and durations (± SE) of behavioural observations of *E. balteatus* female in response to three sources of odour: (1) (E)-β-farnesene, (2) geranyl acetone and (3) paraffin solvent (as control), when presented in a rubber septum placed itself on a leaf disc system without aphids. Significant differences among the treatments when compared with the control: (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ (ANOVA, Dunnett's test).

exhibit enhanced behavioural responses, in terms of approaches and landings, to artificial leaves with the highest numbers of aphids. Our results show that *E. balteatus* females exhibited pronounced searching, acceptance (landing, walking, and proboscis extension) and abdominal protraction leading to egg-laying behaviour in response to increasing aphid colony size. These differences of behavioural responses may be due to the dispersion way of
individual aphids on the leaf disc. In our experiments, females were allowed to forage in an optimal way as they could access all oviposition cues, including visual, olfactory and gustatory stimuli (i.e. aphid-, and leaf-produced volatiles), in opposition to the study of SUTHERLAND et al. (2001), where females were unable to see the colony after landing because individual aphids were contained in clip-cages with a bean leaf. These positive behavioural responses of E. balteatus females (ovipositional tactics) to increasing aphid colony sizes, suggests an adaptive oviposition behaviour that allows the emerging larvae to locate immediately sufficient food resources and may be operating at higher colony sizes. Conversely, previous field observations showed that syrphid females preferentially oviposit in young (apterous) aphid colonies and that they avoid heavily infested plants (KAN & SASAKAWA, 1986; KAN, 1988a;b; KAN, 1989). It is possible that females avoid crowded aphid colonies, since they are subjected to increased migration of the prey and therefore may not support the full development of several syrphid larvae. We think therefore that females E. balteatus could select quantitatively and qualitatively their oviposition site in a way that assures and optimises the developmental conditions of their offspring.

According to BARGEN et al. (1998), the number of eggs laid by female hoverflies depends also on the presence and quantity of oviposition-eliciting substances emitted by the prey and the infested plant. Previous laboratory experiments have demonstrated that the aphid colony size influences the quantity of volatile compounds, such as EBF, emitted by aphids (Almohamad et al., 2008b), and of their liquid secretions such as honeydew (BUDENBERG & POWELL, 1992; SUTHERLAND et al., 2001). In our experiments, E. balteatus females responded positively to aphid volatiles, i.e. EBF emission from M. persicae. Indeed, our behavioural observations demonstrated that EBF has an attractive effect on E. balteatus females and acts as an oviposition stimulant. Previous experiments have shown that honeydew acts as a contact kairomone and oviposition stimulant for hoverfly females (BUDENBERG & POWELL, 1992; SUTHERLAND et al., 2001). SUTHERLAND et al. (2001) also reported that syrphid females demonstrated more gustatory and oviposition responses to honeydew-treated area. These may explain our finding that E. balteatus females exhibited more proboscis and ovipositor extensions in response to increasing aphid colony size. With aphid alarm pheromone (EBF), honeydew might also provide hoverfly females with information about aphid colony size. E. balteatus females did not respond positively to the presence of geranyl acetone and no egg-laying behaviour was observed in response to this molecule, neither on healthy V. faba leaf. This is consistent with the behavioural experiments of FRANCIS et al. (2005b) using syrphid larvae. These authors showed that crushed aphids associated with V. faba plants were-attractive to E. balteatus larvae but the chemical cues from healthy V. faba plants did not carry any infochemical role for syrphid larvae. On the other hand, although geranyl acetone did not exhibit an attractive effect towards E. balteatus females, this molecule may have a repellent effect on other insects. HERN & DORN (2002) indicated that geranyl acetone, a volatile released by
healthy apple fruits, exhibits a repellent effect towards adult females of *Cydia pomonella* L. (Lepidoptera: Tortricidae).

**Conclusions**

The implications of this research are that the production of EBF in response to increasing aphid colony size (Almohamad et al., 2008b) and its effective kairomonal role in hoverflies attraction can potentially be used to enhance the numbers of aphidophagous syrphids in field situations. So, syrphids may be encouraged to remain in an area where EBF is supplied, for instance, by using a controlled septum release system, and to lay more eggs even when aphid numbers are low. This can have a significant effect even when aphid densities are low.

In conclusion, *E. balteatus* females demonstrated a positive density-dependent response to aphid colony size in terms of foraging and oviposition behaviour. Our results also provided an opportunity to understand the mechanisms of the response to aphid colony of different sizes. Thus, we may be able to better utilise aphidophagous syrphids within an integrated pest management strategy.

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