Role of (E)-β-farnesene in systematic aphid prey location by Episyrphus balteatus larvae (Diptera: Syrphidae)

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Abstract. The foraging behaviour of beneficials such as aphidophagous predators depend largely on volatile compounds emitted by potential preys. Even if polyphagous predatory species are considered, all the potential preys are not systematically localised and accepted. In this work, chemical cues from different aphids and plants, each alone or in association, were studied to elucidate their role in prey location. Using a four-arm olfactometer, attraction of combinations of three aphid (Megoura viciae, Acrystosiphon pismum and Aphis fabae) and one plant (Vicia faba) species for Episyrphus balteatus larvae was observed. Predatory hoverfly larvae were attracted by all tested stimuli in the presence of aphids, whatever the species. Whole or crushed aphids and also aphids on bean plant parts were attractive to syrphid larvae, but the host plant alone did not present any infochemical role for E. balteatus. Identification and quantification of the volatile releases from aphid and plant species, alone or in association, were performed using SPME and GC-MS methods. Aphid alarm pheromone, (E)-β-farnesene, was found in the volatile pattern of each aphid and was tested for its role as an effective kairomone for the hoverfly.

INTRODUCTION

With their importance in pest control, predators and parasitoids are fascinating subjects for ecological studies. Beneficial insects are sensitive to chemical aspects of the multitrophic environment, particularly with regard to host location (Poppy, 1997). To localise their preys in natural habitats, entomophagous beneficials use numerous chemical cues emitted by preys and host plants, each alone or in association (Vet & Dicke, 1992). Plenty of different chemical cues correspond to the diversity of associations between potential prey and host plant species. Recently, responses of Coleomegilla maculata L. (Coccinellidae), Coccinella septempunctata L. (Coccinellidae) and Chrysoperla carnea (Chrysopidae) were found to be related to semiochemicals released from potential prey species and their host plants (Zhu et al., 1999; Al Abassi et al. 2000); they use chemical cues to locate their preys. One molecule, (E)-β-farnesene, was identified to be effective kairomone for the predators. The importance of this molecule as kairomone was confirmed by Francis et al. (2004a) for the two-spot ladybird. Indeed, only aphids releasing (E)-β-farnesene alone were found to be attractive for Adalia bipunctata L.

Although A. bipunctata and C. septempunctata are abundant in many agro-ecosystems, several hoverfly species are the main effective predators during their larval stages in crops (Tenhumberg & Poehling, 1995). Among them, Episyrphus balteatus DeGeer is the most commonly recorded syrphid species on agricultural lands (Chambers et al., 1986) and was already studied to investigate predator prey location (Bargen et al., 1998). In order to study the relation between volatile emission from aphids and prey location by E. balteatus larvae, three aphid species were selected according to the volatile pattern they released. While Acrystosiphon pismum Harris and Aphis fabae Scopoli only emit (E)-β-farnesene, Megoura viciae Buckton presents a mixture of three terpene hydrocarbons (α-pinene (11.8%), β-pinene (82.0%) and limonene (6.2%) with no (E)-β-farnesene, an effective kairomone for several coccinellid aphid predators (Francis et al., 2004a,b). Using a 4-way olfactometer, the syrphid predator response towards several aphid and host plant combinations was assessed. The different odour sources corresponded to potential situations met by E. balteatus in its natural habitats. The relation between prey suitability, volatile release and behavioural response of the predator were discussed in relation to the potential use of hoverflies in biological control of aphids.

MATERIALS AND METHODS

Plant and insect rearing

Broad beans (Vicia faba L.) were grown in 10 cm diameter plastic pots containing a 1 : 1 mixture of perlite : vermiculite in controlled environmental rooms at 20 ± 2°C temperature and 16 h daylight photoperiod. Acrystosiphon pismum (Harris), Megoura viciae Buckton and Aphis fabae Scopoli were taken from long-term cultures reared on bean plants in the laboratory for many years. Plants were inoculated at the 2–3 true leaf stage with one of the aphid species. Each combination of aphid and plant species was isolated in separate conditioned rooms at 20°C ± 2°C and 16L : 8D photoperiod. Mass rearing of Episyrphus balteatus Degeer had also been maintained for many years in the laboratory. Control syrphid larvae were reared in aerated plastic boxes and fed with M. viciae on V. faba. From hatching, larvae which were used in olfactometry assays were individually reared in 5 cm diameter Petri dishes. When they were 24 h old, hoverfly larvae were used to test the chemical cues.
Olfactometer setup

The 4-way olfactometer which was used to test the behavioural responses of hoverfly larvae towards several stimuli was similar to that described by Vet et al. (1983). Compressed air was circulated through active charcoal and a water bottle before entering the exposure chamber. Air left the latter through a hole in the chamber roof. Airflow in each of the four arms was adjusted with a flowmeter to 60 ml min⁻¹, thereby creating four equal distinct fields in the exposure chamber. Odour-emitting samples were placed in a 25 ml glass flask linked by plastic tube to one of the four olfactometer arms. The olfactometer system was placed in a controlled temperature room at 20 ± 2°C. Before the beginning of the olfactometer assays, the system was cleaned with pure ethanol and rinsed with distilled water.

Odour sources as chemical cues

Several stimuli were tested as odour sources:
(1) undamaged, unstressed whole aphids (*A. pisum*, *M. viciae* or *A. fabae*);
(2) crushed aphids representing a stressed aphid colony (one of the three previous species);
(3) a host-plant section (a part of a bean stem with some leaves);
(4) a host-plant section with aphids: i.e. a part of a bean stem with aphids, assumed to represent volatile production corresponding to aphids feeding on the plant;
(5) pure (E)-β-farnesene (2 µg) in hexane solution on Whatman paper.

Aphid and host-plant samples (250 mg of insects or plant) were placed in the glass flask, in the air stream of one of the four arms of the olfactometer. The three aphid species were selected for their volatile releases: *A. pisum* and *A. fabae* only released (E)-β-farnesene whilst a mixture of α- and β-pinene and limonene were emitted from *M. viciae* (Francis et al., 2004b).

Behavioural observations

Hoverfly larvae (24 h old, *n* = 20 per stimulus) were individually observed in the olfactometer. Each individual was placed at the centre of the exposure chamber and observed during 20 min. The olfactometer area was divided in one central area and four other ones related to the four odour sources. After insect introduction into the olfactometer, stay durations in each area were determined. Localisation in one of the four areas at the end of the observation duration was considered to be the final behavioural choice of the hoverfly larva. Every 5 observations, the position of the odour fields was changed. The exposure chamber was cleaned with pure ethanol and rinsed with distilled water after each assay.

Analysis of volatile releases from aphids and plants

Aphid and plant samples (250 mg) were crushed with a glass pestle in a glass tube adapted to the SPME method. Each aphid species was tested at least in duplicate. Crushed samples were first maintained for 30 min at 30.0 ± 0.2°C in glass tubes adapted to SPME. The volatile metabolites were sampled for 30 min with 100 µm PDMS (polydimethylsiloxane) SPME fibers from Supelco® and immediately analyzed by GC-MS on an Hewlett Packard HP5972 Mass spectrometer coupled with an HP5890 series II gas chromatograph. The following analytical conditions were used: split-splitless injection at 250°C, HP5-MS (5% phenyl-dimethylpolysiloxane) column (30 m × 0.25 mm, df = 1 µm). Samples were purged with He at 4 ml min⁻¹ for 11 min, and the temperature program was from 40°C (1 min hold) to 180°C at 6°C.min⁻¹ then to 280°C at 15°C.min⁻¹. MS spectra were obtained in the EL mode at 70eV (scanned mass range from 30 to 300 amu). The analytes were identified on the basis of their retention times and by interpretation of MS fragmentation patterns. Spectra were compared to those of the Wiley238.L spectral library. (E)-β-farnesene in hexane was analyzed by the same procedure.

Statistical analysis

Observed frequencies related to the final choice of *E. balteatus* to localise the prey were compared to the corresponding expected frequency (1 odour source and 3 controls) using a χ² test. Relative stay durations of hoverflies were compared by the contrast method using the residual mean square from ANOVA after arcsin√x transformation (Dagnelie, 1973).

RESULTS

Observation of times spent by the hoverflies in each of the four olfactometer arms confirm the attractive effect of the whole unstressed (Fig. 1) and crushed (Fig. 2) aphids...
reared on V. faba for E. balteatus larvae. Similar results were observed when testing separately the three aphid species in association with the host plant on the hoverfly larvae behaviour (Fig. 3). Plant leaves alone used as chemical cues elicited no significant attractive effect on times spent by the syrphid larvae in the olfactometer field relative to the odour source sample. Finally, the (E)-β-

Table 1. Responses of Episyrphus balteatus larvae towards crushed, unstressed whole aphids and plant-aphid associations as odour sources (n = 20). Pure (E)-β-farnesene (2 µg on Whatman paper) and plant alone (V. faba) were also used as chemical cues.

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Observed frequencies</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. viciae</td>
<td>whole insects</td>
<td>0.50</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>crushed</td>
<td>0.45</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>aphid + plant</td>
<td>0.50</td>
<td>8.40</td>
</tr>
<tr>
<td></td>
<td>whole insects</td>
<td>0.50</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>crushed</td>
<td>0.65</td>
<td>16.40</td>
</tr>
<tr>
<td></td>
<td>aphid + plant</td>
<td>0.55</td>
<td>10.00</td>
</tr>
<tr>
<td>A. pisum</td>
<td>whole insects</td>
<td>0.55</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>crushed</td>
<td>0.55</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>aphid + plant</td>
<td>0.75</td>
<td>26.80</td>
</tr>
<tr>
<td>A. fabae</td>
<td>whole insects</td>
<td>0.65</td>
<td>16.40</td>
</tr>
<tr>
<td></td>
<td>crushed</td>
<td>0.55</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td>aphid + plant</td>
<td>0.75</td>
<td>26.80</td>
</tr>
<tr>
<td>— (E)-β-farnesene</td>
<td></td>
<td>0.65</td>
<td>16.40</td>
</tr>
<tr>
<td>— Plant alone</td>
<td></td>
<td>0.15</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Observed frequencies relate to the final choice of E. balteatus larvae: the odour sources were compared to the corresponding expected frequency (1 odour-source arm and 3 control arms) using a $\chi^2$ test. *, **, *** indicate significant differences at P < 0.05, P < 0.01 and P < 0.001 respectively, and NS for not significantly different.
farnesene in hexane solution was highly significantly attractive to the hoverfly larvae. The prey localisation by the hoverfly larvae using crushed or unstressed whole aphids from three species reared on *V. faba* are presented in Table 1. Each of the three aphid species significantly attracted the predatory syrphid larvae when presented either as whole insects or crushed and the pure (E)-farnesene solution was also highly attractive. All of the three associations of *V. faba* leaves and *A. pisum, M. viciae* or *A. fabae* were similarly attractive for the *E. balteatus* larvae. Only the plant sample without aphids did not significantly attract the predatory larvae.

**DISCUSSIONS**

Aphid samples from all three tested species were found to be effective kairomone cues for *E. balteatus*; attraction therefore was not dependent on the terpene hydrocarbon.

**TABLE 2.** GC-MS identification and relative proportion (in %) of volatile molecules released from aphid and plant samples. (E)-β-farnesene (EBF) pure solution was also analysed.

<table>
<thead>
<tr>
<th>Identified molecules</th>
<th>α-pinene Rt 8.9</th>
<th>β-pinene Rt 9.6</th>
<th>Limonene Rt 9.9</th>
<th>(E)-β-farnesene Rt 17.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pisum</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100.0</td>
</tr>
<tr>
<td><em>A. fabae</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100.0</td>
</tr>
<tr>
<td><em>M. viciae</em></td>
<td>11.8</td>
<td>68.0</td>
<td>6.2</td>
<td>14.0</td>
</tr>
<tr>
<td>Host plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. faba</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Purified EBF</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Values indicating retention times (Rt) are also presented. (–) corresponds to “no molecules detected.”
volatile pattern released by prey. Indeed, the same attractive
behaviour of the hoverfly was observed for *A. pisum*
and *A. fabae*, both (E)-β-farnesene producers, and *M.
*vicie* (which releases a mixture of three terpenic hydro-
carbons). Our results demonstrate that there was a sys-
tematic response of this polyphagous aphid predator
towards volatiles released from several potential prey.

Both crashed and whole unstressed aphids were attrac-
tive for the predatory syrphid larvae, unlike many other
predator species. Several beneficials do not positively
respond to the emitted chemical cues when whole aphids
are used alone as odour sources. The parasitoid *Aphidius
ervi* Haliday (Braconidae) was not attracted by whole
*A. pisum* aphids (Du et al., 1996); *A. bipunctata* significantly
respond to only crushed *A. pisum* and *Myzus persicae*,
whatever host-plant species they were fed on (Francis et
al., 2004a). It was advanced that crushed aphids thus
released higher and sufficient levels of volatile substances
to be perceived by the predators. That means that the
chemical cues released by whole aphids were not suffi-
cient to allow the prey localisation by the aphidophagous
beneficials. To be an effective kairomone source, the
whole aphid colony must be more important than the one
used as samples in the olfactometry assays. Using another
prey species, Han & Chen (2002) showed that the tea
aphids send out odours attracting *C. septempunctata*
when the number of aphids was at least 2000. The latter
authors found that *C. septempunctata* was the most sensi-
tive species between three tested natural enemies. Here,
*E. balteatus* was found to be very sensitive to small
amounts of volatiles from aphids, since the syrphid larvae
responded positively to almost 500 whole aphids (corre-
sponding to 250 mg samples) as odour sources.

Until our results, when the aphid species used as odour
sources release molecules other than (E)-β-farnesene, an
absence of any infochemical effect has been observed for
aphidophagous insects. Indeed, other components from
aphids modulated the kairomonal effect toward the preda-
tory species. For example, Al Abassi et al. (2000) demon-
strated that the attractiveness of (E)-β-farnesene for *C.
septempunctata* decreased with increasing amounts of
β-caryophyllene. Isothiocyanate emissions (detected by
GC-MS) from *Brevicoryne brassicae* was considered to
be acting as a kairomone inhibitor, informing *A. bipunc-
tata*, the two spot ladybird, of prey unsuitability (Francis
et al., 2004a).

In the current study, the release of several terpenes from
*M. vicie* was not less attractive for *E. balteatus*
larvae than the two other aphid species, which emitted
only (E)-β-farnesene. *E. balteatus* is well known to be
adapted to a broad range of aphid preys in terms of devel-
opment and reproductive efficacy (Gilbert, 1986; Van-
haelen et al., 2002), and this species has been
demonstrated here to be less restrictive in its prey location
behaviour. We think therefore that *E. balteatus* could be
considered as more of a generalist, showing a higher
potential as an aphid biological control agent than other
aphidophagous predators such as ladybirds.

The tested plant-aphid associations were all attractive to
*E. balteatus*. These results are in partial accordance with
previous works on beneficials. A too low concentration of
volatiles liberated by the aphid and host plant samples has
often been considered to explain a lack of attraction for
predators such as ladybirds or parasitoids. The foraging
behaviour of the aphid parasitoid *Aphidius ervi* was
shown to be influenced by semiochemicals emitted by
aphid-infested plants only when a certain threshold of
infestation has been reached in terms of number of aphids
and hours of feeding activity (Guerrieri et al., 1999). A
volatile dose-dependent response of another predatory
beetle, *C. septempunctata* towards prey-host plant com-
plex has also been observed: significant differences
towards odour source and control in a Y olfactometer
were only observed when the seven-spot ladybird was
exposed to at least 30 aphid damaged shoots with 1200
tea aphids. Below this amount of volatile emitted from
biological sample, no significant attraction on the
predator was observed (Han & Chen, 2002). In the cur-
rent work, the presence of whole aphids of whatever spe-
cies, with the plant samples was sufficient to ensure
hoverfly orientation. *E. balteatus* was able to locate every
tested potential prey when associated with the host plant.

In conclusion, we have shown that, contrary to other
aphidophagous predators and parasitoids, *E. balteatus* not
only reacts to (E)-β-farnesene, but responds also to other
semiochemical cues from prey. Host-plant and aphid-
host-plant complexes represent effective infochemical
sources for syrphid larvae under our experimental obser-
vations. More than other aphidophagous beneficial spe-
cies, the systematic response of a polyphagous aphid
predator to several potential preys confirms the potential
of *E. balteatus* in aphid biological control.

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