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Flight behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in response to plant and host volatiles

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***Aphidius ervi*, wind tunnel, plant synomones, kairomones, leguminous crops**

Abstract. The flight behaviour of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) has been studied in a wind tunnel, in response to the following natural odour sources: broad bean plants infested with *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) (PHC, plant-host complex), damaged broad bean plants from which the aphids were removed (HDP, host damaged plants), aphids (H, host) and uninfested broad bean plants (P, plant). The most attractive odour sources were PHC and HDP, which both stimulated a similar high number of oriented straight flights. In contrast, H and P were much less attractive and did not seem to be important in the long range attraction of the parasitoids.

INTRODUCTION

Aphidius ervi Haliday is widely reported as an effective parasitoid of both the pea aphid, *Acyrthosiphon pisum* (Harris), and cereal aphids (Starý, 1978, 1981; Powell, 1982; Pennacchio & Tremblay, 1988; Pennacchio, 1990). Although several studies have been carried out on the ecology of this species, only scattered information is available about its host location, recognition and acceptance behaviour.

Host recognition and acceptance behaviour have been recently investigated and some sources of the chemical cues involved have been identified (Battaglia et al., 1993; Pennacchio et al., 1994).

Host habitat location and host location are behavioural steps controlled by a myriad of factors and the importance of chemical cues has been demonstrated for a number of parasitoid species (Vinson, 1985, 1991). For *A. ervi* both plant and host derived volatiles seem to be important in regulating its behavioural responses (Powell & Zhang, 1983; Starý et al., 1985; Pennacchio, 1988; Wickremasinghe & van Emden, 1992). However, all these studies used Y-tube olfactometers, which give an indication of the odour preference, but do not allow free flight in an air stream.

The purpose of this research is to describe the flight behaviour of *A. ervi* in a wind tunnel, in response to various natural odour sources.

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MATERIAL AND METHODS

The parasitoid *A. ervi* was reared on *A. pisum* colonies feeding on potted broad bean plants (*Vicia faba*). Aphid and parasitoid cultures were maintained in two separate environmental chambers at 20 ± 1°C, 75 ± 5% R. H. and a 18 hr L : 6 hr D photoperiod. Naive parasitoid adults used in all the experiments were derived from aphid mummies kept singly in glass vials (8 mm × 60 mm), plugged with cotton-wool and observed twice a day. The parasitoid adults were maintained under the same environmental conditions as reported above, and used within 24 hr from emergence and within 3–7 hr following the onset of a photophase.

The flight behaviour of *A. ervi* females in response to various volatile sources was studied in a wind tunnel similar to that described by Miller & Roelofs (1978). The flight chamber was a glass box 50 cm long and square-shaped in section (35 cm × 35 cm). The air flow was generated by an electrically operated fan which was controlled by a variable voltage regulator. Before entering the flight chamber the air was purified by passing through a filter (45 cm × 45 cm × 10 cm) of activated charcoal and the flow made laminar by 5 consecutive sheets of window screen (7 × 7 mesh/cm^2). The wind speed was monitored with a hot-wire anemometer (Alnor, mod. CGA-26) and in all the experiments adjusted to 20 cm·sec^{-1}. The exhaust air was vented to outside the room where the wind tunnel was located. Light intensity in the wind tunnel was 3,600 lux at the insect releasing point and provided by four U-shaped incandescent tubes placed on the top of the flight chamber. A black and white striped pattern for visual orientation was placed beneath the glass chamber floor. The wind tunnel was housed in a room mantained at 20 ± 1°C and 65 ± 5% RH.

Parasitoid females were tested individually by releasing them downwind in the odour plume, 20 cm away from the odour source. Each female was observed for 5 min. The releasing device consisted of an open glass vial (17 cm × 1 cm) on a plexiglass base so that the parasitoid emerged at a height of 17.5 cm from the floor.

The effect on *A. ervi* flight behaviour of the following four odour sources was studied: aphid infested broad bean plants (PHC, plant-host complex), previously infested broad bean plants from which the aphids were removed just prior to run the bioassay (HDP, host damaged plants), aphids (H, host), uninfested broad bean plants (P, plant). Plants (P) used were at two leaf stage, and not higher than 10 cm. PHC consisted of plants at the same growth stage and infested with ca 150 aphids of mixed ages. A group of ca 100 pea aphids (H) of mixed ages was kept in a glass vial (10 cm × 2.5 cm) closed at both ends with a window screen sheet, suspended at 17.5 cm from the floor and positioned so that the air flow was allowed to pass through the vial. The vial end facing the parasitoid releasing point was completed with a ring of green paper, to reduce the possible negative effects of the absence of plant-associated visual stimuli. Samples of 25 parasitoid females were considered and replicated 3 times for each experiment.

The behavioural data were recorded with the aid of a computer and event-recording software (The Observer, Noldus Information Technology, Wageningen, The Netherlands).

A χ^2 test was used to compare distributions of flight behaviours obtained in response to different odour sources.

RESULTS

The responses of *A. ervi* females were classified into one of the following behavioural events:

1. IN VIAL (IV): at least one antenna in vial.
2. OUT OF VIAL (OV): sudden flight outside the vial.
3. ANGLE STANCE (AS): body held at an approximate angle of 45° relative to substrate by standing on median and hind legs and with fore legs freely moving in the air.
4. WALKING (WA): walking and all the behaviours except flight and angle stance when outside of the vial.
5. CASTING (CA): flight in a horizontal plane without upwind movement.
6. ZIG ZAGGING (ZZ): upwind flight taking place from side to side in an horizontal plane.

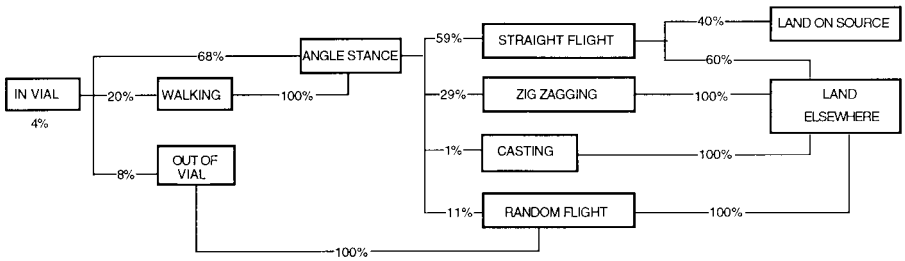

Fig. 1. Quantitative diagram of the flight behaviour shown by *Aphidius ervi* females in response to aphid infested plants (PHC) in a wind tunnel.

7. STRAIGHT FLIGHT (SF): direct upwind flight.

8. RANDOM FLIGHT (RF): any flight response except casting, zig zagging and straight flight.

9. LAND ON SOURCE (LO): landing on odour source.

10. LAND ELSEWHERE (LE): landing anywhere except on odour source.

The observed sequence of behavioural events recorded in response to PHC is reported in figure 1. Almost in all cases the pre-flight behaviour culminated in the angle stance position. Only in a negligible number of cases (4%) were females completely unreactive or flew away (8%) showing random flight behaviour. Upwind flights (SF + ZZ) were shown by 88% of the females and were considered to be oriented towards the odour source when the female landed on or not further than 5 cm from the odour source. Oriented flights in response to PHC made up 67% of the flights (Fig. 2) and the remaining nonoriented flights were made up of casting and random flight.

The percentage of oriented flights towards HDP was not significantly different from that registered for PHC (Fig. 2). In contrast, *A. pisum* alone (H) and noninfested broad bean plants (P) resulted in a significant ($P \leq 0.01$) reduction in oriented flights compared to PHC and HDP, with only 16% and 10% of oriented flights towards H and P, respectively (Fig. 2).

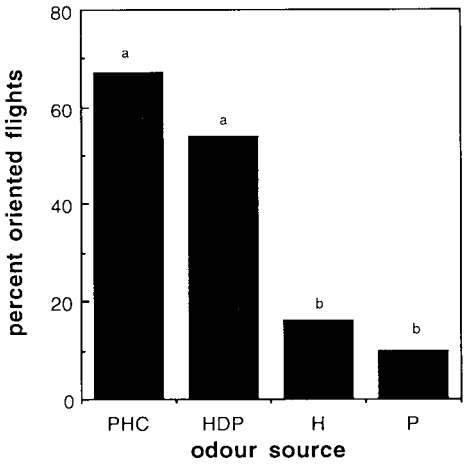

Fig. 2. Percentage of the flights by females of *Aphidius ervi* in a wind tunnel that were oriented towards the different odour sources (see the text for odour source abbreviations).

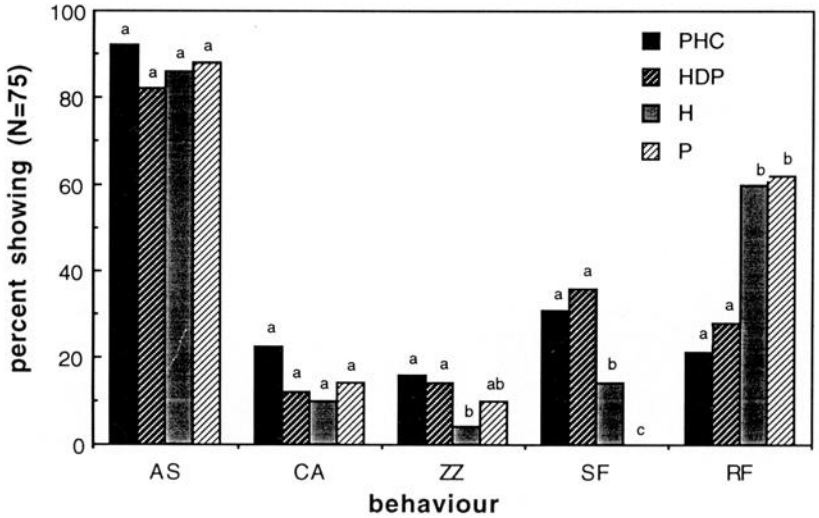

Fig. 3. Percentages of *Aphidius ervi* females showing different flight behaviours (see the text for behaviour abbreviations) when exposed to different odour sources in the wind tunnel.

The analysis of flight behaviour in response to the odour sources considered (Fig. 3) showed significant differences. The angle stance position was the most frequently observed pre-flight behaviour in all the experiments. Both PHC and HDP were significantly ($P \leq 0.01$) more active than H and P in stimulating straight flight responses (Fig. 3). Similar results were recorded in the case of zig zagging. However, straight fligths were comparatively more frequent ($P \leq 0.01$) than zig zagging both in the case of PHC and HDP (Fig. 3). The random flight responses significantly ($P \leq 0.01$) increased both for H and P, which were the odour sources associated with the lowest percentages of oriented flights (Fig. 3). Casting was observed to a similar extent in all cases (Fig. 3).

DISCUSSION AND CONCLUSIONS

In the process leading to successful parasitism the role of plant and host derived volatiles is of fundamental importance both in the location of the habitat and of the host (Vinson, 1985, 1991). The importance of these chemical cues in inducing behavioural responses of braconid aphid parasitoids has been demonstrated in a number of species (Read et al., 1970; Schuster & Starks, 1974; Powell & Zhang, 1983; Bouchard & Cloutier, 1985; Sheehan & Shelton, 1989; Wickremasinghe & van Emden, 1992). However, almost in all cases, with the exception of the study reported by Sheehan & Shelton (1989), only olfactometers have been used to assess the parasitoid response to a given odour source. The olfactometers used, even though variously designed, do not allow normal flight in an air stream. The present study describes for the first time, both qualitatively and quantitatively, the flight behaviour of an aphid parasitoid in response to plant and host derived odours.

A. ervi flew upwind towards some of the odour sources. The PHC and HDP were the most active in eliciting oriented flights and a substantial increase in straight flights towards the odour source. In contrast, both healthy plants and aphids stimulated a much lower flight activity. Similar results have also been reported for other braconid species parasitizing lepidopterous hosts (Eller et al., 1988; Keller, 1990). It is interesting to note

that both healthy plants and aphids were attractive to *A. ervi* females when tested in a Y-tube olfactometer (Powell & Zhang, 1983; Pennacchio, 1988). These contrasting results could have alternative explanations. However, it is important to appreciate that flight behaviour in a wind tunnel is not comparable with the behavioural responses observed in a Y-tube olfactometer, where flight is not possible.

Searching efficiency could be altered by continuously rearing the parasitoid in the laboratory, as quantitatively shown for other parasitoid species (Geden et al., 1992). Since the *A. ervi* population used was reared in the laboratory for a long time it is possible that its ability to use uninfested plant volatiles as a host habitat location cue is strongly reduced. For example, it is possible that the frequency of the genes controlling the reaction to uninfested plant synomones could be considerably reduced in a population always in contact with aphid infested plants. Alternatively, a strong behavioural modification induced by learning cannot be ruled out.

If we exclude these problems it is possible to interpret the results both in ecological and ethological terms. *A. ervi* consists of a complex of populations, each showing a given degree of host specificity (Powell & Zhang, 1983; Cameron et al., 1984; Pungerl, 1984; Němec & Starý, 1985, 1986; Tremblay & Pennacchio, 1988), which in some cases attain the status of distinct species (Pennacchio & Tremblay, 1987; Tremblay & Pennacchio, 1988; Unrhuh et al., 1989). Parasitoids, which are specialists both at herbivore and plant level, are expected to show strong and congenitally fixed responses to both kairomones and herbivore-induced synomones (Vet & Dicke, 1992). In the present study, host produced kairomones seem not to have been important in regulating *A. ervi* flight behaviour, but they do play a very important role over short distances, inducing responses that are strongly innate (Battaglia et al., 1993; Pennacchio et al., 1994). The use of herbivore-induced synomones by *A. ervi* females seems to be an efficient solution to the reliability-detectability problem as defined by Vet & Dicke (1992). *A. pisum* has an aggregated spatial distribution and a relatively small colony size (Pennacchio & Tremblay, 1986). Under such conditions it is more advantageous for the parasitoid to use host-induced synomones to locate its host. In-flight orientation to plant synomones not induced by aphid attack would increase parasitoid searching time and so reduce parasitoid fitness. However, plant synomones seem to be involved in some way in host recognition by the parasitoid, probably interacting with other cues at close range (Powell & Wright, 1992).

The study of *A. ervi* flight behaviour in a wind tunnel will allow the evaluation of the activity of various odour sources in the long range attraction of parasitoids to habitat and host. Furthermore, comparative studies on different host races or closely related species could give interesting insights into the ethological mechanisms involved in the splitting and adaptive radiation of natural populations.

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