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Enemy-induced dispersal in a parasitic wasp

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Abstract. Females of the parasitic wasp *Aphidius uzbekistanicus*, a specialist aphid primary parasitoid, react to the presence of their specialist hyperparasitoid enemy *Alloxysta victrix* by leaving the area. The wasps leave to increase their reproductive success, because only their offspring, but not the wasps themselves, are threatened by enemy attack. Furthermore, this dispersal is elicited through the action of volatile chemical cues produced by the hyperparasitoids. We provide evidence for 6-methyl-5-hepten-2-one being one of the volatiles eliciting dispersal. The tendency of the wasps to leave areas which are being colonized by their enemies reduces their efficiency as aphid antagonists.
Key words. Parasitic wasp; dispersal; semiochemical; escape behaviour; reproductive success; pheromone; 6-methyl-5-hepten-2-one.

The larvae of parasitic wasps which develop in aphids as obligate hosts are often killed by hyperparasitic wasps. In contrast, the adult parasitoids are not attacked by any such specialized natural enemies[1,2]. It has been suggested that for the parasitoid female, leaving a host-containing environment which develops a high hyperparasitoid density may be rewarding in terms of reproductive success, even though searching for hosts in a hyperparasitoid-free environment may be costly[3,4]. However, this implies that primary parasitoid females are able to detect hyperparasitoid presence. This hypothesis is tested here.

Materials and methods

Cultures of the cereal aphid *Sitobion avenae* (Homoptera, Aphididae), the primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera, Aphidiidae) and the hyperparasitoid *Alloxysta victrix* (Hymenoptera, Alloxystidae) were maintained in different rearing rooms at 20 ± 1.5 °C on oat (cv. Bojar) seedlings. In the Petri dish experiments with live animals as treatments, 10 unparasitized second and third instar *S. avenae* were placed on a piece of oat leaf in a 35 mm i.d. Petri dish together with an *A. victrix* mated or unmated female or male (except in the control) for 24 h. The hyperparasitoids were left in the dish or removed 15 min before insertion of a standardized 3-day-old *A. uzbekistanicus* female. Females were standardized as follows: they emerged singly in gelatine capsules and were held for 24 h in groups of 9–11 with a corresponding number of males to allow mating. On day 2, each female gained parasitization experience with 10 *S. avenae* for 5 min. Experiments lasted 5 min. All attacks, i.e. female touches body of host with tip of her abdomen, and flight attempts, i.e. female spreads wings and leaves substrate by flight, were recorded.

The Petri dish experiments with *A. victrix* extracts or 6-methyl-5-hepten-2-one (MHO) as treatments were performed in dishes similar to the above, which however contained only a 35×5 mm Whatman number 1 filter paper to which 1 µl extract, MHO-solution, or pure solvent (control) was applied. MHO was synthesized in the laboratory of W. Francke and was approximately 99.5% pure. One *A. uzbekistanicus* female standardized as above was allowed to walk for 5 min on the filter paper 20 sec after application. We recorded whether the female a) crossed over the treated area, b) responded by turning 90°, or c) flew away after contacting the treated area. *A. victrix* extracts were produced by blowing purified air for 2.25–4.5 h at 0.5 l/min over 7–17 less than 24 h old virgin *A. victrix* females into hexane, using a simplified dynamic solvent collection system[5]. These air entrainment extracts were concentrated under nitrogen to a concentration of 1 *A. victrix* $\cdot h^{-1} \cdot \mu l^{-1}$ and stored at −20 °C. MHO was diluted in hexane to a concentration of $0.005\ nl \cdot \mu l^{-1}$.

The presence of MHO in *A. victrix* extracts was detected using a VG 70–250 SE mass spectrometer (VG Analytical, Manchester) coupled to a gas chromatograph (GC; Hewlett Packard, Bad Homburg) equipped with a 50 m 0.25 i.d. fused silica FFAP capillary column (Machery & Nagel, Düren) which was operated under a temperature programme of 60–200 °C at a rate of $5\ °C \cdot min^{-1}$. Structure assignment of MHO was carried out by comparing both the GC retention time and the mass spectrum of the natural compound and the synthetic sample. Chemical ionization data were

obtained using a VG 70–250 mass spectrometer with ammonia as a reactant gas at 100 eV: m/z = 144 $(M + NH_3)^+$, 5%; 127 $(M + H)^+$, 60%; 109 $(M - 18 + H)^+$, 15%; 46, 100%.

To quantify the amounts of MHO produced by *A. victrix*, purified air was blown for 5 h at 0.5 l/h over groups of 86, 75, and 228 unmated females, mated females, and males, respectively. Volatiles were trapped on a 1.5 mg charcoal filter (Brechbühler, Schlieren) and eluted with 20 µl methylene chloride. MHO was identified as the only substance produced abundantly by all three groups of insects. In addition to MHO, some other substances produced by *A. victrix* occurred in the chromatograms, yielding minor peaks. However, the peak areas of MHO in the chromatograms contributed to >95% to the total peak area (solvent peak and peaks due to contamination not considered).

Three 1 µl samples of each extract were separated by GC on a 30 m 0.25 i.d. fused silica DB 5 capillary column (J&W Scientific, Folsom) with the same temperature programme as for the FFAP phase, and the average values of the MHO peak areas were evaluated. The amount of MHO produced by the animals was compared to peak areas obtained from dilutions of synthetic MHO.

Flight tunnel experiments were performed with *A. uzbekistanicus* females standardized as described, which were allowed to explore aphid-free plants in a cage. Actively searching females were caught and immediately flown individually in a 230 cm long × 95 cm high × 75 cm wide flight tunnel. At 50 cm distance upwind from the release point, two pots with 12 aphid-free oat plants (12 cm tall) were placed at 30 cm distance from each other. One pot stood in air which had passed over 200 mated 1–7-day-old *A. victrix* females kept in a 10 cm (i.d.) × 20 cm long plexiglas™ tube or over a 0.5 µl glass capillary filled with MHO. Both tube and capillary were placed upwind 5 cm away from the plants. The other pot stood in clean air which had passed over an identical empty tube or capillary. The flight of the released wasps was observed for 10 min at 21–23 °C, at a wind speed of 20–40 cm^{-1}, and a light of approximately 3000 lux. The release rate of MHO was 16 nl · 10 min^{-1}. We recorded whether the wasps flew a) away from the plants, b) to a plant without hyperparasitoids or MHO, or c) to a plant with *A. victrix* or MHO.

Results and discussion

When single females of *A. uzbekistanicus* are presented with a cohort of suitable aphid hosts in a Petri dish, they attack fewer hosts and perform a higher number of flight attempts when an *A. victrix* female is present in the dish (fig. 1). The presence of an *A. victrix* male does not significantly reduce the number of attacks, whereas the number of flight attempts is considerably increased (fig. 1). Similarly, although less markedly, the wasps

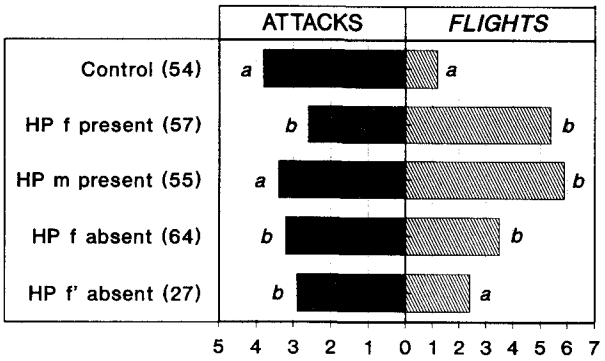

Figure 1. Response of experienced primary parasitoid *Aphidius uzbekistanicus* females to the simultaneous or previous presence of the hyperparasitoid *Alloxysta victrix* (HP) in a Petri dish. Bars represent the mean numbers of aphids (*Sitobion avenae*) attacked and mean numbers of flight attempts performed. Different letters indicate differences significant from the control, $p < 0.05$, Mann-Whitney test; f = unmated female, f' = mated female, m = male; (n replicates).

attack fewer hosts and fly more when confined with aphids in dishes which had contained an *A. victrix* female in the 24 h period before the experiment (fig. 1). The wasps' behaviour, however, does not differ significantly between dishes which previously contained a mated as opposed to an unmated *A. victrix* female (Mann-Whitney test, $p = 0.240$). It would appear that *A. victrix* males, and mated and unmated females, all induce dispersal in *A. uzbekistanicus* females. We conclude that the elicitor is a hyperparasitoid-derived behaviour-modifying substance, i.e. a semiochemical.

To investigate whether the semiochemical is volatile and therefore capable of eliciting a response at a distance, purified air was passed over a group of virgin *A. victrix* females and trapped in hexane using a simplified dynamic solvent system[5]. The concentrated extract showed activity when bioassayed (fig. 2). *A. uzbekistanicus* females responded by flying away or, less commonly, by

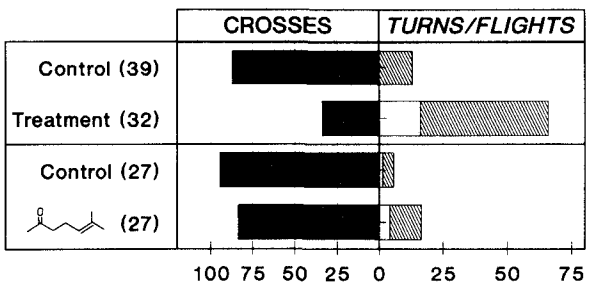

Figure 2. Response of primary parasitoid *Aphidius uzbekistanicus* females to the odour of the hyperparasitoid *Alloxysta victrix* (top) and to synthetic 6-methyl-5-hepten-2-one (MHO, bottom) in a Petri dish. Bars represent the mean percentages of females which crossed or responded by turning >90° (dotted) or flying away (cross-hatched) after contactng the treated area. Differences between controls and treatments are statistically significant, $p < 0.001$ (top) and $p = 0.003$ (bottom), Chi^2-contingency table; (n replicates).

changing their walking direction when coming into contact with the hyperparasitoid odour. In another experiment, we found that *A. uzbekistanicus* females performed a significantly higher number of flight attempts during 5 min when confined in a Petri dish with a hexane extract obtained from 1 virgin female *A. victrix* · h^{-1} · μl^{-1} (1 µl) as compared to the solvent control (mean = 5.6 and 2.6; n = 19 and 18, respectively; p = 0.048, Mann-Whitney test). We also tested the extracts for the possible presence of a female-derived sex pheromone: *A. victrix* males contacting extract-treated filter paper showed a highly sinuous searching behaviour and often exhibited wing-fanning. Thus, a sex pheromone was apparently present in the extracts.

The analysis of extracts from air passed over *A. victrix* males and mated or unmated females by combined gas chromatography/mass spectrometry revealed significant amounts of 6-methyl-5-hepten-2-one (MHO) in all three sample types. *A. victrix* males, and mated and unmated females, produced similar amounts of MHO ranging between 0.02 and 0.04 · 10^{-3} nl · h^{-1} per individual. The substance functions as a sex and spacing pheromone in *A. victrix*[6].

Synthetic MHO induced dispersal of *A. uzbekistanicus* females. In the Petri dish bioassay performed as with air extracts, *A. uzbekistanicus* females more frequently flew away or changed their walking direction when coming into contact with the spot on the filter paper where 0.005 nl MHO in 1 µl hexane had been applied (fig. 2). Given a) the high amounts of MHO produced by *A. victrix* and b) the similarity of the responses of *A. uzbekistanicus* females to *A. victrix* extracts and to MHO, we conclude that MHO is one of the substances, or the substance, which induces dispersal of the primary parasitoids. MHO did not perform as effectively as *A. victrix* odour (fig. 2). This could be because other substances produced by *A. victrix* also elicited dispersal, or because different quantities of MHO in the natural as compared to the synthetic sample caused differing responses. MHO also elicits defensive behaviour in a South American meliponid stingless bee, apparently because kleptoparasitic enemies are recognized through this substance[7].

As the air extracts and synthetic MHO proved to induce dispersal of *A. uzbekistanicus* females in the Petri dish, we tested whether the volatiles can also act over a longer distance. We assumed that in the field, adult primary parasitoids and adult hyperparasitoids rarely come as close to each other as in the Petri dish bioassay. We considered that if dispersal of primary parasitoids, as a response to hyperparasitoid odour, is moulded by natural selection, the odour will act at long range and cause long range dispersal. Although less costly[8,9], short range within-habitat dispersal would not be favourable, because dispersal will only increase reproductive success when the probability is high that the offspring will be deposited out of reach of hyperparasitoids.

To test this hypothesis, we examined the host plant location behaviour of *A. uzbekistanicus* females in a flight tunnel. It is well known that a number of parasitoids of herbivores are attracted by the odours produced by the host plant[10–13]. Wasps that were flown in the tunnel could respond as follows: a) by flying away from the airstream with plant and hyperparasitoid odour (e.g. up to the ceiling), b) flying 50 cm in the airstream to the plant without hyperparasitoids or c) flying 50 cm in the airstream to the plant with hyperparasitoids. The control consisted of plants without hyperparasitoids.

In accordance with the hypothesis, 52% of the wasps chose possibility a) when *A. victrix* odour was present in comparison to only 29% in the control (fig. 3). No significant preference was found for possibility b) vs. c). Apparently, a number of females took off for a longer flight when they detected hyperparasitoid odour at the release point, ignoring the possibility of searching nearby plants for hosts. Hosts on nearby plants are likely to be found by any hyperparasitoids present, especially since parasitized aphids are suitable hosts for alloxystid hyperparasitoids during the whole phase of larval development of the primary parasitoids[1,2].

A response similar to the presence of *A. victrix* odour was recorded in the wind tunnel when MHO was presented, with 92% of the wasps deciding to fly away in the presence of the semiochemical, in comparison to only 67% in the control (fig. 3). This is a further indication for the involvement of MHO in the dispersal-eliciting process. Since MHO functions as a spacing and sex pheromone in *A. victrix*[6], its 'maladaptive' production, which makes the presence of hyperparasitoids apparent to primary parasitoids, may be explained by the necessity to communicate.

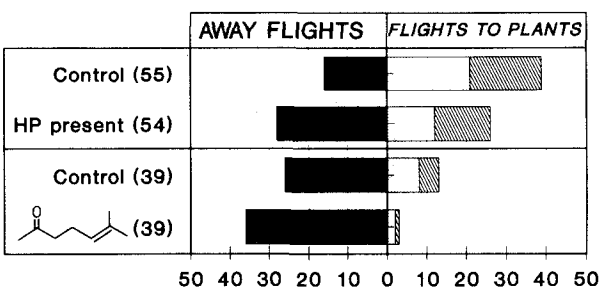

Figure 3. Response of primary parasitoid *Aphidius uzbekistanicus* females to odour of the hyperparasitoid *Alloxysta victrix* (top) and to synthetic 6-methyl-5-hepten-2-one (MHO, bottom) in a flight tunnel. Bars represent the numbers of females which flew away from plants or flew to a plant without hyperparasitoids or MHO (dotted) or to a plant with the hyperparasitoid or MHO (cross-hatched). In the controls, dotted and cross-hatched areas refer to the two pots with plants presented. Differences between control and treatment are statistically significant, p = 0.045 (top) and 0.016 (bottom), Chi^2-contingency table; (n replicates).

The dispersal of the primary parasitoids seems to be an offensive escape behaviour, rather than a purely defensive response to enemy attack. Inducible defenses coming into play after a prey animal has detected a predator, but before the predator has detected the prey, are common in vertebrates, e.g. in lizards[14], deer[15], and in aquatic invertebrates[16,17], but have only rarely been reported from terrestrial invertebrates[18]. A notable exception is the escape behaviour of moths and other insects which detect the echolocation signals of insectivorous bats[19]. However, enemy-induced dispersal of primary parasitic wasps is unique, because only the wasps' offspring, but not the wasps themselves, are threatened by enemy attack[1,2].

It has been suggested that a high dispersal rate can be an evolutionarily stable strategy, because migrating individuals benefit from colonising empty sites or sites where a competing allele predominates[8,20]. Dispersal induced by enemies of the offspring can be considered as an additional benefit for primary aphid parasitoids. In insects, this behaviour is likely not only to occur in aphid parasitoids. For instance, a winged parthenogenetic aphid immigrating into a particular habitat may take off again after detecting the presence of specialized enemies which are likely to attack its offspring. Another possible example would be a gravid butterfly female avoiding those habitats for oviposition which are colonized by egg parasitoids. Should these ideas prove true, then their implications for the population dynamics of invertebrate herbivores and carnivores are far-reaching. In addition, they open new and intriguing possibilities for manipulating populations of pest insects, for instance by treating crop plants with the odour of a herbivore's enemy. The dispersal observed here is likely to account for the low aphid controlling capacity of primary parasitoids at times when hyperparasitoid densities increase[3].

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