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6-methyl-5-heptene-2-one, a putative sex and spacing pheromone of the aphid hyperparasitoid, *Alloxysta victrix* (Hymenoptera: Alloxystidae)

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Abstract. Hyperparasitoids are considered to reduce the effectiveness of primary parasitoids as biological control agents of aphids in a variety of crops. Recently it was shown that volatiles produced by the hyperparasitoid *Alloxysta victrix* (Westwood) elicit a dispersal in female primary parasitoids thereby potentially reducing primary parasitoid activity in the field at certain times. The active chemical was identified as 6-methyl-5-heptene-2-one (MHO) which is produced by both male and female *A. victrix*. The function of this chemical in the intraspecific communication of *A. victrix* was studied. Y-tube olfactometer experiments revealed corresponding effects of MHO and natural odour sources. Males were attracted to females especially when not mated. Females were repelled by females. These effects could partly be mimicked with pure MHO in the olfactometer. In Petri dish experiments the behaviour of *A. victrix* in the presence of MHO odour was video-analysed. As in the olfactometer, males were attracted to the odour and females repelled. We conclude that MHO is a sex pheromone which attracts *A. victrix* males and as spacing pheromone enables an even distribution of females in the field.

INTRODUCTION

Alloxysta victrix (Westwood) (Hymenoptera: Alloxystidae) is an obligate secondary parasitoid (hyperparasitoid) of aphids (Homoptera: Aphididae). The larvae develop endoparasitically in aphid primary parasitoids (Hymenoptera: Aphidiidae). *A. victrix* together with other hyperparasitoid species has a strong influence on the populations of primary parasitoids in cereal fields causing ineffective control of aphid populations by primary parasitoids (Höller et al., 1993).

The study of the behavioural interactions between a primary parasitoid, *Aphidius uzbekistanicus* (Luzhetski) (Hymenoptera: Aphidiidae) and its hyperparasitoid, *A. victrix*, led to the identification of the volatile substance 6-methyl-5-heptene-2-one (MHO). This compound is emitted in high doses by both male and female *A. victrix*. MHO showed to have some repellent influence on females of *A. uzbekistanicus* (Höller et al., submitted). It is not known why this substance is produced by the hyperparasitoid.

The aim was to study intraspecific functions of MHO in *A. victrix*. Therefore the attractive and repellent effects of MHO were related to the effects of natural odours produced by *A. victrix* males and females.

MATERIAL AND METHODS

Animals were reared in *A. uzbekistanicus* in *Sitobion avenae* (F.) on oat (cv. Bojar). Plant and insect cultures were held under constant conditions (20±1°C temperature, 16 : 8 h light : dark). Virgin hyperparasitoid females and naive males which had no access to the other sex were obtained by transferring aphid mummies from the *A. victrix* culture into gelatine capsules. After emergence, males and females were sexed and kept separately in groups of nine to eleven individuals. They were provided with 15% sucrose water solution until the experiment began. Mated animals emerged in common mass rearing units.

An all-glass Y-tube-olfactometer was used for following experiments. Air was blown at constant rate through both Y-arms. After having passed through activated charcoal filters, air flow was controlled with two flow meters to a rate of 150±20 ml·min^{-1}. Two water bottles served for constant humidification. Stimulus application was as follows. The natural odour sources were groups of ten *A. victrix* placed in one of the two arms. Females were either virgin or mated; males were either experienced or naive, i.e. had or had not access to females previously. The artificial odour sources were capillaries filled with MHO (5 µl) and fixed with wire frames inside one arm. Glass wool separated experimental animals from odour sources inside the olfactometer. Groups of ten *A. victrix* (either mated or not mated females or naive or experienced males) were released at the base of the olfactometer. After 15 minutes time, the distribution of animals in the arms was scored. Only animals which had passed the first trap of a side arm were counted as having decided. Animals still in the base of the olfactometer were considered as not having decided. Each animal was only used once in the experiments. 10 to 32 replicates were performed. To account for any side bias in the experimental installation the number of replicates with stimulus application on the right and the left arm was equal. Observed distributions were tested statistically with a chi-squared goodness-of-fit test against an expected even distribution.

In a different experimental set-up, the Video Tracking and Motion Analysis System (Fa. Noldus, The Netherlands) served for the analysis of reactions of experienced males and mated females to MHO. A drop (2 µl) of solution of MHO in pentane (1 : 1000) was applied to the centre zone (8 mm diameter) of a filter paper disk. Control experiments were conducted with pure pentane. After evaporation of the pentane (10 sec), an *A. victrix* male or female was transferred onto the filter paper and was covered with a glass Petri dish (38 mm diameter). For five minutes the movement of the animal was digitally recorded and was later computer-analysed for the following parameters.

1. Latency: the time from introduction of the animal into the Petri dish until it entered the centre zone on the filter paper where the MHO solution had been applied (in video images).
2. Visits: the number of visits to the centre zone.
3. %Time: the proportion of time spent inside the centre zone.
4. Distance: the average distance between the animal and the centre point of MHO application during the experiment (in cm).
5. Speed: the average speed of the animals' movements (in cm·sec^{-1}).
6. Travelled Distance: the total distance that the animal travelled inside the centre zone (in cm).

For each treatment 25 replicates were used. Arithmetic means of the parameters were statistically tested using the Mann-Whitney test, as the data were not normally distributed.

RESULTS

Both naive and experienced males were attracted to virgin and mated females. Experienced males were repelled by naive males. Females never showed significant attraction to males or other females. Mated females were even repelled by virgin females and naive males. Generally, experienced males and mated females showed less attraction to the odour sources than their naive or virgin counterparts (Table 1).

Results with synthetic MHO are not significantly different from random distribution. However, mated females tended to be slightly repelled by MHO whereas naive males tended to be attracted to MHO ($p = 0.052$) (Table 2).

In the Petri dish under control treatment, males and females differed significantly in their behaviour (Visits $p < 0.001$, %Time $p < 0.001$, Distance $p = 0.001$, Speed $p = 0.009$, Trav. Dist. $p < 0.001$). Both males and females changed their behaviour in the presence of MHO when compared to pure pentane. Males significantly increased their number of visits to the centre zone, the proportion of time spent and distance travelled inside the centre zone. They significantly decreased their average distance to the centre. Females increased their latency to the centre zone and decreased their average speed, both significantly. Female movement patterns displayed the inverse reaction of males to MHO, with all observed values changing in the opposite direction (Table 3).

TABLE 1. Reaction of *A. victrix* to natural odour sources in a Y-tube olfactometer. Chi-squared goodness-of-fit test used.

Odour source	Animals tested															
	Virgin females				Mated females				Naive males				Experienced males			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Virgin females	200	105	45	50	200	81	44	75	140	60	58	22	120	18	62	40
						–				+				+		
		p = 0.608				p = 0.005				p < 0.001				p = 0.030		
Mated females	260	139	70	51	200	152	21	27	100	40	46	14	100	13	57	30
										+				+		
		p = 0.085				p = 0.387				p < 0.001				p = 0.004		
Naive males	199	99	59	41	200	117	32	51	200	64	74	62	200	78	49	73
						–								–		
		p = 0.072				p = 0.038				p = 0.304				p = 0.030		
Experienced males	200	103	51	46	200	72	58	70	200	85	69	46	201	67	61	73
										+						
		p = 0.612				p = 0.289				p = 0.032				p = 0.300		

1 no. of animals tested
2 no. of animals without decision
3 no. of animals in baited arm
4 no. of animals in non-baited arm
+, – attraction, repellency

TABLE 2. Reaction of *A. victrix* to synthetic MHO (6-methyl-5-heptene-2-one) in a Y-tube olfactometer (see Table 1 for explanations).

Odour source	Animals tested															
	Virgin females				Mated females				Naive males				Experienced males			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
MHO	222	110	54	58	200	75	58	67	203	75	75	53	200	51	76	73
										+						
		p = 0.706				p = 0.421				p = 0.052				p = 0.806		

TABLE 3. Reaction of *A. victrix* in a Petri dish to synthetic MHO (1 : 1000 solution of MHO in pentane) applied onto filter paper (video and computer analysis) (c.z. = centre zone). Mann-Whitney test used.

Parameter	Mated females			Experienced males		
	Pentane	MHO	p	Pentane	MHO	p
Latency to c.z. (images)	22.24	44.27	0.004	61.82	37.71	0.655
Visits in c.z. (no.)	20.32	16.24	0.084	9.96	16.12	0.004
%Time in c.z.	8.36	6.23	0.352	4.44	6.31	0.001
Distance to c.z. (cm)	1.30	1.38	0.086	1.45	1.39	0.004
Speed ($cm \cdot sec^{-1}$)	0.68	0.60	0.018	0.52	0.62	0.099
Trav. Dist. in c.z. (cm)	16.69	12.92	0.079	7.52	12.83	0.008

CONCLUSIONS AND DISCUSSION

Results of the olfactometer experiments with natural odour sources are quite clear. Males are attracted to females, whereas females are repelled by conspecific odours. Keeping away from conspecific females is a strategy to reduce competition, to the benefit of the producer and receiver of the information. Olfactometer results with synthetic MHO are not so clear. Nevertheless, these results tend to show a similar effect of MHO on the orientation of male and female *A. victrix*. The naturally produced quantities of MHO by *A. victrix* are much lower than those applied in the olfactometer (about 20 million fold) (Höller et al., submitted). This could have caused overdose effects resulting in no response of the experimental animals (Schneider, 1980; Mustaparta, 1984). In addition to this, the Y-tube olfactometer does not seem suited to study repellency. The repellent odour reduces the activity of the experimental animals (see Petri dish experiments), and animals inside the base arm are therefore not inclined to move forward.

Results in the Petri dish experiment revealed more clearly the influence of MHO on the behaviour of *A. victrix*. Basically, the pattern was as follows: males showed attraction to and arrestment in the centre zone where the MHO solution had been applied, and they increased their activity. Females showed repellency by MHO. They reduced their activity and kept away from the MHO spot.

The characteristics of MHO as intraspecific behaviour-modifying chemical resemble that of a female derived sex pheromone for males, causing increased activity, attracting them from a distance and arresting them at close range. At the same time MHO seems to function like a spacing pheromone for females (Prokopy, 1981) as it induces avoiding reactions, at least at close range. At the moment it is not known whether there are any other synergistic or inhibiting chemicals, besides MHO, involved in the pheromonal communication in *A. victrix*. Interacting chemicals could be either of intraspecific origin (Swedborg & Jones, 1992) or of extratrophic origin (Dickens et al., 1991).

These results imply that *A. victrix* may disperse in response to its own population density. This may have yet unforeseen consequences for primary parasitoid populations in the field. It makes sense to further investigate chemical communication in hyperparasitoids because the ineffective biological control of aphids by primary parasitoids is at least partly caused by hyperparasitoid activity in the field. The manipulation of the hyperparasitoids is a promising possibility for the enhancement of natural aphid control. Manipulation of natural enemies in the frame of biological pest control can not only concentrate on beneficial animals, but has to include those factors that regulate their population dynamics.

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