

Differences in behavioral responses of *Sitobion avenae* (Hemiptera: Aphididae) to volatile compounds, following parasitism by *Aphidius ervi* (Hymenoptera: Braconidae)¹

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Abstract: In spite of the wealth of information about the role of semiochemicals in the behavior of non-parasitized aphids, the olfactory responses of parasitized aphids have yet not been studied. Although parasitoid-induced behavioral changes have been shown in aphids, the cues involved in those behaviors are not well known. We studied the behavior of *Sitobion avenae* (Fabricius), non-parasitized and parasitized by *Aphidius ervi* Haliday, toward volatiles of conspecifics and of the hyperparasitoid *Alloxysta victrix* (Westwood) in an olfactometer. At 120 hours after parasitization, the aphids were significantly attracted by females of the hyperparasitoid, while 160 hours after parasitization the aphids were significantly repelled by conspecifics. Non-parasitized aphids showed neither attraction nor repellence toward either hyperparasitoids or conspecifics. Our results show that volatiles are involved in behavioral differences associated with parasitization. The results are discussed in relation to evolutionary hypotheses to explain the behavioral modifications in parasitized aphids.

Keywords: *Sitobion avenae*, *Aphidius ervi*, *Alloxysta victrix*, host-parasitoid interactions, volatile semiochemicals, olfactometry.

Résumé: Malgré l'abondance d'information sur le rôle des composés sémiocchimiques dans le comportement des pucerons non parasités, les réponses olfactives des pucerons parasités n'ont pas encore été étudiées. Bien que des changements comportementaux induits par le parasitoïde aient été observés chez les pucerons, les signaux propres à ces comportements ne sont pas bien connus. Nous avons étudié les réponses de *Sitobion avenae* (Fabricius) non parasité et parasité par *Aphidius ervi* Haliday, à des composés volatiles issus d'individus de la même espèce et de l'hyperparasitoïde *Alloxysta victrix* (Westwood) dans un olfactomètre. Cent vingt heures suivant l'infestation, les pucerons étaient attirés par des femelles de l'hyperparasitoïde, tandis que cent soixante heures après, les pucerons étaient repoussés par l'odeur d'individus de la même espèce. Les pucerons non parasités ne montraient aucune attraction ou répulsion à l'endroit des hyperparasitoïdes ou des individus de la même espèce. Nos résultats montrent que les différences comportementales dues au parasitisme impliquent des composés volatiles. Les résultats sont discutés en rapport avec des hypothèses évolutives pour expliquer les modifications comportementales des pucerons parasités.

Mots-clés: *Sitobion avenae*, *Aphidius ervi*, *Alloxysta victrix*, interactions hôte-parasite, composés volatiles sémiocchimiques, olfactométrie.

Introduction

Volatile semiochemicals play an important role in aphid behavior, mediating events such as mating, host plant recognition, escape responses, aggregation and spacing (Picket, Wadhams & Woodcock, 1992; Pettersson, 1993; Pettersson *et al.*, 1995). Odors are also important cues for other trophic levels, as host-finding by primary endoparasitoids of aphids (Hymenoptera: Braconidae) is influenced by volatile compounds of aphids and their host plants (Wickremasinghe & van Emden, 1992; Powell & Wright, 1992; Reed *et al.*, 1995; Micha & Wyss, 1996). Furthermore, it has been suggested that volatile compounds may repel foraging parasitoids from areas with high hyperparasitoid density (Höller *et al.*, 1994), although the importance of this interaction under field conditions has been questioned (Völkl *et al.*, 1995).

There is evidence of parasitoid-induced behavioral changes in aphids, related to their microhabitat selection (McAllister & Roitberg, 1987; Brodeur & McNeil, 1990;

Müller, Völkl & Godfray, 1997), and of the influence of the physiological state of the parasitoid larva on the changes observed (Brodeur & McNeil, 1989). However, despite the wealth of information about the role of volatiles in the behavior of non-parasitized aphids, the olfactory responses of parasitized aphids have not been studied.

The aim of the present study was to test whether behavioral responses of the aphid *Sitobion avenae* (Fabricius) to odors are altered at different times following parasitization by the primary endoparasitoid *Aphidius ervi* Haliday. Sources of odor tested were: *i*) non-parasitized conspecifics on the host plant, and *ii*) virgin females of the endophagous hyperparasitoid *Alloxysta victrix* (Westwood), which oviposits inside a developing larva of *Aphidius spp.*, within the living parasitized aphid (Gutierrez & van den Bosch, 1970).

Material and methods

Stock cultures of *S. avenae* were kept on oats, *Avena sativa* Linnaeus (cv. Nehuen). Colonies of both the primary parasitoid *A. ervi* and the hyperparasitoid *A. victrix*, were

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established by collecting aphid mummies from untreated wheat fields (INIA-Chillán, Chile). All laboratory colonies were reared at $20 \pm 2^\circ\text{C}$, 16 hours/8 hours light-dark cycle.

Newly-emerged *A. ervi* females were mated and used the following day to parasitize second instar *S. avenae*. Each parasitoid female was used only once and allowed to parasitize a cohort of 3 to 4 aphids. To avoid pseudoreplication, only one experimental result was obtained from each cohort. Controls were aphids of the same age, manipulated in the same way, but not parasitized.

The olfactory responses of parasitized and non-parasitized aphids were tested in a Pettersson olfactometer (Pettersson, 1970). The air is allowed to enter the arena through four arm zones and drawn out through a hole above the central zone by a vacuum pump with a flow of 250 ml/minute. Two opposing arms are permeated with air coming from a stimulus container, while the other two arms contained control odor. The air is purified by an activated charcoal filter at the inlet of the two odor sources containers. A lamp (25W) was placed 50 cm above the olfactometer arena, which was surrounded by a cardboard cover, ensuring uniform lighting.

The following paired stimuli were compared: *i*) five *S. avenae* feeding on a 10-day-old seedling of *A. sativa* (cv. Nehuen) held in a 160 ml bottle, with an uninfested *A. sativa* seedling, and *ii*) one 48-hours-old virgin *A. victrix* female (placed inside a 5 ml glass tube 1 hour before experimentation), with an empty tube.

Four types of aphids were tested with each pair of stimuli: parasitized aphids, 120 ± 4 hours and 150 ± 4 hours after parasitization, and non-parasitized aphids of the same ages. The experiment with plants and aphids was also repeated testing aphids, 160 ± 4 hours after parasitization and compared with similar aged control aphids. These ages were selected based on the developmental stage of the *A. ervi* larva at 20°C (Sequeira & Mackauer, 1992). The larva reaches the passive feeding stage by the fifth day after oviposition and begins the destructive feeding phase, when it actively consumes the whole host, during the early seventh day. The parasitoid larva is a suitable host for *A. victrix* oviposition only between the late fourth to fifth day of development (Gutiérrez & van den Bosch, 1970; Sullivan, 1987), while experiments conducted with the aphid parasitoid *Aphidius nigripes* Ashmead showed that parasitized aphids moved away from the aphid colony during the seventh day after oviposition, *i.e.*, shortly before mummification (Brodeur & McNeil, 1989).

For all experiments the test aphid, previously starved for 1 hour, was placed in the central zone for a 5 minute adaptation period before observations were taken. Any aphid remaining stationary for 2 minutes was recorded as "non reacting" and another from the same "cohort" was used.

The cumulative time that an aphid spent in each arm was recorded over 15 minutes. A comparison of the time spent by the test aphids in the two treatment and two control arms was made using the Wilcoxon signed ranks test (Siegel & Castellan, 1988). Ten replicates of "reacting" aphids were performed for each experiment, with the experimental setup being changed each time to ensure fully independent repetitions.

Results

Non-parasitized aphids, regardless of age, did not show significant preferences between control or treatment zones containing conspecifics feeding on *A. sativa* (Table I). However, parasitized aphids exhibited different behavioral patterns, showing no choice at 120 and 150 hours after parasitization, but spending significantly less time in the zone permeated by the odor of conspecifics at 160 hours (Table I). Non-reacting aphids in all the treatments never exceeded 29% of the replications. The total time spent by parasitized aphids in the olfactometer arms was not significantly different between the 3 treatments (Kruskal-Wallis test, $KW = 1.3$, $df = 2$, $P = 0.52$).

Parasitized *S. avenae* was attracted by the odor of *A. victrix* 120 hours after parasitization, but not 30 hours later. Non-parasitized aphids did not show significant preferences between control or treatment zones with the odor of the hyperparasitoid (Table II). Non-reacting aphids in all the treatments never exceeded 23% of the replications.

Discussion

Two non-exclusive hypotheses have been suggested to account for the evolution of behavioral changes in hosts parasitized by insect parasitoids (Stamp, 1981; Schmid-Hempel & Müller, 1991; Müller & Schmid-Hempel, 1992; 1993; Poulin, 1992). One argues that changes are to the host's advantage: by increasing its probability of dying, the host also reduces the parasitoid's likelihood of successful development, thereby diminishing the probability of subsequent attack of its kin (Smith Trail, 1980). McAllister & Roitberg (1987) observed significant differences in the

TABLE I. Responses (time spent in different zones of the olfactometer in a 15-minute assay) of parasitized and non-parasitized *Sitobion avenae* to the odors of non-parasitized conspecifics on *Avena sativa* (*A.s.*). There were 10 replications. nr = non-reacting aphids

Test aphid	Stimulus	120 hours after oviposition			150 hours after oviposition			160 hours after oviposition		
		Mean ¹ (SE)	nr	P ²	Mean (SE)	nr	P	Mean (SE)	nr	P
Parasitized <i>S. avenae</i>	5 <i>S. avenae</i> on <i>A.s.</i>	5.76 (1.38)	3	0.88	4.12 (1.08)	4	0.14	1.76 (0.68)	3	0.01
	<i>A.s.</i>	6.01 (1.39)			7.73 (1.34)			6.40 (1.42)		
Non-parasitized <i>S. avenae</i>	5 <i>S. avenae</i> on <i>A.s.</i>	5.67 (0.81)	2	0.96	6.27 (0.97)	3	0.96	5.62 (0.74)	2	0.44
	<i>A.s.</i>	5.90 (0.94)			6.39 (1.06)			6.94 (0.72)		

¹Mean (SE) given in minutes.

²Wilcoxon signed ranks test.

TABLE II. Responses (time spent in different zones of the olfactometer in a 15 minutes assay) of parasitized and non-parasitized *Sitobion avenae* to the odors of a virgin female of the endohyperparasitoid *Alloxysta victrix*. There were 10 replications. nr = non reacting aphids

Test aphid	Stimulus	120 hours after oviposition			150 hours after oviposition		
		Mean ¹ (SE)	nr	P ²	Mean (SE)	nr	P
Parasitized <i>S. avenae</i>	<i>A. victrix</i>	8.59 (0.67)	3	0.03	4.89 (1.05)	3	0.24
	Blank	4.16 (0.78)			6.41 (0.66)		
Non-parasitized <i>S. avenae</i>	<i>A. victrix</i>	5.57 (0.83)	2	0.17	5.55 (0.70)	3	0.17
	Blank	7.88 (0.74)			7.78 (0.62)		

¹Mean (SE) given in minutes.

²Wilcoxon signed ranks test.

escape behaviors of parasitized and non-parasitized aphids, and interpreted their findings in terms of adaptive suicide by parasitized aphids. Moreover, the behavioral responses observed are influenced by the reproductive potential of the parasitized aphid, being stronger in aphids parasitized at early larval stages (which would not reproduce before mummifying) than in those parasitized at later stages (which would be able to produce some offspring before dying) (McAllister, Roitberg & Weldon, 1990).

The second hypothesis states that the parasitoid modifies the behavior of its host for its own benefit (Fritz, 1982). In aphids, support for this comes from the observation that parasitized aphids move away from the feeding site shortly before mummification, thereby reducing the risks of hyperparasitism and predation (Höller, 1991; Brodeur & McNeil, 1989; 1992). Furthermore, Brodeur & McNeil (1989) showed that induced microhabitat selection was related to the physiological state of the parasitoid. Thus, *Macrosiphum euphorbiae* (Thomas) containing non-diapausing larvae of *Aphidius nigripes* left the feeding site and generally mummified on the exposed upper surface of the leaves, which are less visited by hyperparasitoids. On the other hand, aphids containing diapausing parasitoid larvae showed changes in phototactic and thigmokinetic responses, which favoured the selection of concealed sites, thus potentially reducing mortality of overwintering *A. nigripes* prepupae (Brodeur & McNeil, 1990). In addition, evidence shows that in aphid species with well developed parasitoid defense mechanisms, or provided with ant-attendance, parasitized individuals remain and mummify within the colony, which represents the safest environment (Müller, Völkl & Godfray, 1997).

Our results showed that parasitized aphids at 160 hours after oviposition are significantly repelled by conspecifics, while a few hours before, at 120 and 150 hours after oviposition, they did not show any significant response to conspecific odors. This effect suggests that the movement of parasitized aphids away from the feeding site (the aphid colony) shortly before mummification (Brodeur & McNeil, 1989) could result from the parasitoid larva altering the aphid's response to volatiles from feeding aphids, or to non-volatile cues (e.g., phototaxis and thigmokinesis, Brodeur & McNeil, 1990). This behavior would increase the parasitoid's fitness, since parasitized aphids move far enough to increase the probability of successful parasitoid development (Brodeur & McNeil, 1992), but not enough to prevent

the adult parasitoids from subsequently finding the aphid colony upon emergence.

On the other hand, about 120 hours after oviposition (two days before mummification), at a time when they are susceptible to hyperparasitism, parasitized aphids move toward the odor of hyperparasitoid females. This would increase the probability of encounter with hyperparasitoids, thereby potentially increasing primary parasitoid mortality and decreasing the danger of parasitism for the host's conspecifics. The evolution of this behavior is based on the assumptions of kin selection theory (McAllister & Roitberg, 1987; McAllister, Roitberg & Weldon, 1990), i.e., it would only happen in an evolutionary scenario where phenotypes showing attraction to the hyperparasitoid achieve a higher fitness than phenotypes lacking it.

Furthermore, parasitized aphids at 120 and 150 hours after oviposition did not show any significant response to conspecific odors. Absence of a repellent response toward conspecifics at this time may be interpreted as a partial support to the adaptive suicide hypothesis. Thus, since hyperparasitoids are attracted to aphid colonies (Siri, 1993) and our results indicate that parasitized aphids would remain in the colony, the probability of encounter and hyperparasitization would be increased, given that these aphids are devoid of well developed defensive behaviors or ant attendance. This possibility seems more plausible given that hyperparasitoid *A. victrix* is only able to distinguish between parasitized and non-parasitized aphids after introduction of the ovipositor (Gutiérrez, 1970).

The present study provides the first evidence that volatiles play a role in behavioral differences between parasitized and non-parasitized aphids. Moreover, our findings suggest that both hypotheses concerning behavioral changes may be applicable, depending on the ontogenetic stage of the aphid-parasitoid complex. However, further work is needed to establish if these behavioral differences are actually related with fitness components of the species involved, and to identify the chemical compounds that may account for these interactions.

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