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Effect of innate preferences, conditioning and adult experience on the attraction of *Aphidius ervi* (Hymenoptera: Braconidae) toward plant volatiles

Luis C. RODRÍGUEZ[1], Eduardo FUENTES-CONTRERAS[2] and HERMANN M. NIEMEYER[1]

[1] Laboratorio de Química Ecológica, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile; email: niemeyer@abulafia.ciencias.uchile.cl

[2] Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca, Casilla 747, Talca, Chile

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Abstract. The aphid parasitoid *Aphidius ervi* was collected and subsequently reared on *Sitobion avenae* on wheat or *Acyrthosiphon pisum* on alfalfa. Parasitoids from both origins were exposed in an olfactometer to alfalfa or wheat volatiles after plant experience (wheat or alfalfa) or after oviposition experience (*S. avenae* on wheat or *A. pisum* on alfalfa). The results showed the importance of adult experience, conditioning and innate preferences on the responses of *A. ervi* toward volatiles and provided a mechanistic explanation to the high prevalence of *A. ervi* on aphids on cereals and legumes in central Chile.

INTRODUCTION

Aphid parasitoids may use a variety of chemical cues for habitat and host location (Mackauer et al., 1996; Poppy et al., 1997). Responses of aphid parasitoids to semiochemicals can be affected by genetic factors, as well as by conditioning during development and by adult experience, (*i.e.* associative learning; Du et al., 1996, 1997; Poppy et al., 1997; Guerrieri et al., 1997; Powell et al., 1998), and may play an important role in the spatial dynamics of parasitoid populations, thus influencing migration between crops during the season.

Aphidius ervi Haliday is a Palaearctic parasitoid associated with several Macrosiphinae aphids in its region of origin (Starý et al., 1993), although it seems to be highly specialised on pea aphids (*Acyrthosiphon pisum* Harris) feeding on legumes (Takada & Tada, 2000). *A. ervi* is known to be attracted by host-plant volatiles (Powell & Zhang, 1983) and by pea aphids feeding on *Vicia faba* (host-plant complex) (Du et al., 1996). This parasitoid was introduced as a biocontrol agent in Chile during three consecutive years (1976–1978). The mummies introduced corresponded to cereal aphid mummies collected in France by the European Parasite Laboratory, after a ban was imposed on parasitoids collected from pea aphids in Czech Republic, because those introductions had not survived the quarantine stage (Zúñiga et al. 1986, Zúñiga, 1990). Currently, *A. ervi* is successfully established in Chile on both legumes and cereal crops and has become more common in wheat than other specialist parasitoids of cereal aphids, an infrequent situation in other regions of the world (Cameron et al., 1984; Gerding et al., 1989; Starý, 1993; Starý et al., 1993). As suggested earlier by Starý (1993), the occurrence of the parasitoid in cereals and legumes may be due to the proximity with which these crops are grown. We tested this hypothesis by investigating the attraction of the parasitoid toward wheat or alfalfa mediated by host plant semiochemicals, and the effect of previous experience of the foraging parasitoid upon this attraction to the volatiles. Parasitoid behaviour was assessed using olfactometric assays, following exposure to host plants (wheat and alfalfa) and oviposition experience on *Sitobion avenae* Fabr. on wheat and *A. pisum* on alfalfa.

MATERIALS AND METHODS

Parasitoids

The stock cultures of the parasitoid were founded collecting mummies from alfalfa or wheat at INIA-La Platina near Santiago. Once emerged, 120 *A. ervi* from each crop were identified using the key of Botto & Hernandez (1989) and reared on both pea aphid (*A. pisum*) on alfalfa (*Medicago sativa* L. cv. California 55), and grain aphid (*S. avenae*) on wheat (*Triticum aestivum* L. cv. Paleta). Stock cultures were maintained in separate environmental chambers operating at 22 ± 1°C and 16L : 8D photoperiod.

Olfactometer

The olfactometer used in the experiments was originally described by Pettersson (1970). Two opposing arms of the olfactometer were connected by Teflon tubing to a glass belljar which contained alfalfa plants, while the other two arms were connected to a glass belljar containing wheat plants. Charcoal filters at the inlet of the belljars were used to eliminate external odours during the experiments. The airflow (250 ml/min) was obtained with a vacuum compressor pumping air out from the arena through a hole in its center.

Experiments

Potted plants were used as the stimulus source in the olfactometer experiments. They were grown in the laboratory under the same conditions as the insect stock cultures and were 14 ± 2 days-old. Different numbers of alfalfa and wheat plants were used in order to ensure similar leaf areas of the two sources of volatiles.

Mummies were obtained from stock cultures and transferred to Petri dishes, avoiding their exposure to plants or aphids.

TABLE 1. Olfactometric response of *Aphidius ervi* reared in *Acyrthosiphon pisum* on alfalfa when the origin of the cultures were mummies collected from alfalfa. Average time in each arm presented in minutes (min). Level of accepted significance (p < 0.05).

Treatment	Average time in alfalfa arm (min)	Average time in wheat arm (min)	Significance level (p)
Alfalfa plant	5.6 (± 2.4)	3.5 (± 1.3)	0.01
Oviposition on alfalfa plant	7.7 (± 3.0)	2.4 (± 2.8)	0.01
Wheat plant	4.8 (± 1.5)	6.5 (± 1.9)	0.06
Oviposition on wheat plant	3.8 (± 1.4)	6.1 (± 1.8)	0.02

Within 24 hours of emergence, parasitoids (5th–8th generation in the laboratory) were mated and females kept in Petri dishes with diluted honey. The female parasitoids were introduced in the olfactometer and the time spent in each arm during 15 minutes was recorded. Pseudoreplication was avoided using a single parasitoid in each replicate, testing the parasitoids only once, and changing the biological material, olfactometer and belljars in every repetition, as suggested by Ramírez et al. (2000). To avoid bias, the olfactometer was rotated periodically and lighting was provided from above. The comparison of the total time spent by the parasitoid in wheat vs. alfalfa arms was performed with the Wilcoxon's rank sum test.

Two-choice olfactometries between alfalfa and wheat were performed using the following treatments of adult experience: i) plant experience: female parasitoids were allowed to walk individually during 30 minutes on alfalfa or wheat plants of the same age and conditions as the potted plants used as source of volatiles, ii) oviposition experience on plants: female parasitoids were allowed to walk and to forage individually during 30 minutes on wheat or alfalfa plants infested 30 minutes before with 30 adult host aphids. Each treatment was replicated 13 times.

RESULTS

Experiments with *Aphidius ervi* collected from pea aphid on alfalfa

When the parasitoids were reared on the pea aphid-alfalfa system, both experience on the host-plant (alfalfa) and oviposition experience on pea aphids on alfalfa resulted in a significant parasitoid preference toward alfalfa volatiles in relation to wheat volatiles (Table 1). When parasitoids reared on pea aphid-alfalfa were subjected to experience on wheat plants a marginally non-significant preference toward wheat was observed, which became significant after oviposition experience on grain aphid on wheat (Table 1). On the other hand, when parasitoids collected from pea aphids on alfalfa were reared on grain aphid on wheat, they did not show significant preferences for wheat or alfalfa after plant experience either on wheat or alfalfa (Table 2). Nevertheless, oviposition experience produced significant attraction of the parasitoids towards the plant where they had oviposition experience (Table 2).

TABLE 2. Olfactometric response of *Aphidius ervi* reared in *Sitobion avenae* on wheat when the origin of the cultures were mummies collected from alfalfa. Average time in each arm presented in minutes (min). Level of accepted significance (p < 0.05).

Treatment	Average time in alfalfa arm (min)	Average time in wheat arm (min)	Significance level (p)
Alfalfa plant	5.3 (± 2.6)	4.1 (± 1.6)	0.31
Oviposition on alfalfa plant	6.8 (± 1.9)	3.9 (± 1.6)	0.01
Wheat plant	4.2 (± 1.8)	4.9 (± 0.7)	0.55
Oviposition on wheat plant	3.5 (± 2.2)	6.8 (± 3.5)	0.03

TABLE 3. Olfactometric response of *Aphidius ervi* reared in *Sitobion avenae* on wheat when the origin of the cultures were mummies collected from wheat. Average time in each arm presented in minutes (min). Level of accepted significance (p < 0.05).

Treatment	Average time in alfalfa arm (min)	Average time in wheat arm (min)	Significance level (p)
Alfalfa plant	3.9 (± 2.2)	4.5 (± 2.2)	0.91
Oviposition on alfalfa plant	4.0 (± 2.0)	4.0 (± 1.7)	0.88
Wheat plant	3.7 (± 2.0)	6.1 (± 3.0)	0.08
Oviposition on wheat plant	3.7 (± 1.5)	6.3 (± 1.9)	0.01

Experiments with *Aphidius ervi* originally collected from grain aphid on wheat

When the parasitoids collected on wheat were maintained in their original system, they did not show a preference between wheat or alfalfa volatiles following experience with alfalfa plants; nevertheless, a marginal non-significant preference toward wheat volatiles was observed after experience with wheat plants (Table 3). Oviposition experience on grain aphids on wheat produced a preference toward wheat volatiles in the olfactometer, but oviposition experience on pea aphids did not cause a significant attraction to either alfalfa or wheat volatiles (Table 3). When the parasitoids were reared on pea aphid on alfalfa and subjected to experience on undamaged alfalfa, there was a significant preference toward alfalfa volatiles. This attraction was enhanced by oviposition experience on pea aphids on alfalfa (Table 4). Experience on wheat was not enough to produce prefer-

TABLE 4. Olfactometric response of *Aphidius ervi* reared in *Acyrthosiphon pisum* on alfalfa when the origin of the cultures were mummies collected from wheat. Average time in each arm presented in minutes (min). Level of accepted significance (p < 0.05).

Treatment	Average time in alfalfa arm (min)	Average time in wheat arm (min)	Significance level (p)
Alfalfa plant	5.5 (± 1.3)	3.9 (± 1.4)	0.04
Oviposition on alfalfa plant	5.6 (± 1.7)	3.6 (± 1.1)	0.02
Wheat plant	4.1 (± 1.1)	5.0 (± 1.5)	0.17
Oviposition on wheat plant	3.6 (± 0.8)	5.3 (± 1.8)	0.03

ence toward volatiles of this cereal, but oviposition on grain aphids on wheat caused an attraction of the parasitoids toward wheat volatiles (Table 4).

DISCUSSION

The results with parasitoids collected on alfalfa and reared on alfalfa or wheat and subjected to oviposition experience on a host-plant complex, agreed with previous work reporting the importance of associative learning for *A. ervi* (Du et al., 1996, 1997; Powell et al.,1998), as well as for other aphid parasitoid species (Sheehan & Shelton, 1989; Grasswitz & Paine, 1993a, 1993b) and parasitoids of other insects (Vet & Dicke, 1992; Turlings, 1993). The significant preference toward alfalfa in the absence of oviposition experience in parasitoids collected and reared on alfalfa may result from an innate genetic preference and/or conditioning during parasitoid development, not observed in the same magnitude when rearing took place on grain aphid on wheat.

In the absence of oviposition experience, parasitoids collected from grain aphid on wheat and reared in this original system did not show a significant preference for wheat or alfalfa volatiles. However, when parasitoids were transferred for a few generations to the pea aphid-alfalfa system, they significantly preferred alfalfa volatiles even in absence of oviposition experience on that plant. Such induction of preference may be associated with preimaginal conditioning during parasitoid development inside the aphid host as described by Wickremasinghe & van Emden (1992) and van Emden et al. (1996) for *Aphidius rhopalosiphi*, and Storeck et al. (2000) for *Aphidius colemani* Viereck. Since in the absence of oviposition experience, parasitoids collected on alfalfa and transferred to wheat did not prefer alfalfa or wheat volatiles, and contrarily, parasitoids collected on wheat and transferred to alfalfa preferred alfalfa volatiles, it may be suggested that in the absence of oviposition experience, wheat does not induce preference as strongly as alfalfa does.

Both the potential innate preference for alfalfa volatiles in parasitoids collected on alfalfa, and the potential conditioning for alfalfa volatiles during development on pea aphid-alfalfa of parasitoids collected on wheat, may be expected to occur with the pea aphid from legumes rather than with grain aphid from cereals, because the pea aphid-legume interaction is known as the main host-plant system for *A. ervi* at its genetic diversity center (Starý et al., 1993). However, parasitoids collected on wheat and reared on grain aphid on wheat, even after oviposition experience on pea aphids on alfalfa, were not attracted to alfalfa volatiles. This result may be explained by the absence of an innate preference or preimaginal conditioning for alfalfa volatiles when development has occurred on grain aphid on wheat, or by the existence of a weak preimaginal conditioning for wheat which, although statistically non-significant *per se*, could be of such magnitude as to counteract the effect of oviposition experience on alfalfa.

The results suggests that early in the season, when cereals and legumes are not heavily attacked by aphids, the parasitoids which overwintered on alfalfa (a perennial crop) will show an innate attraction to alfalfa volatiles. Only after an increase in aphid populations on nearby cereal fields, will oviposition on grain aphid on wheat take place, and then further attraction to wheat may be induced by wheat volatiles. Once parasitoids are established on wheat, they should be able to forage on alfalfa and wheat since no innate preference or conditioning is caused by this cereal. There is no evidence to support the existence of a distinct "wheat" strain of parasitoids in Chile, with innate responses towards wheat volatiles.

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