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ROLE OF OLFACTION IN HOST FINDING BY APHID PARASITOID *Aphidius nigripes* (HYMENOPTERA: APHIDIIDAE)[1]

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Abstract—The olfactory responses of the parasitoid *Aphidius nigripes* Ashmead were investigated with a four-field airflow olfactometer. Female and male parasitoids did not respond to potato plants, the food plant of their host. However, females were strongly attracted by odors from a preferred host, *Macrosiphum euphorbiae* (Thomas), and also from less preferred hosts such as *Myzus persicae* (Sulzer) and *Aphis nasturtii* Kalt. Moreover, *A. nigripes* females responded positively to odors from a nonhost aphid, *Rhopalosiphum maidis* Fitch. The honeydew produced by these four aphid species was also very attractive to females. Males did not respond to aphids or honeydew but were highly attracted by odors from conspecific female parasitoids.

Key Words—Habitat selection, host selection, kairomones, sex pheromone, potato aphid, *Macrosiphum euphorbiae, Homoptera, Aphididae, Aphidius nigripes*, Hymenoptera, Aphidiidae, olfaction.

INTRODUCTION

Olfaction is an important mechanism involved in host-habitat location and host location by insect parasitoids (Vinson, 1981; Weseloh, 1981). Olfactometer studies have shown that aphid parasitoids can be attracted by odors produced by the food plant of their host, thus partly explaining the choice of a particular habitat by a searching parasitoid (Read et al., 1970; Schuster and Starks, 1974; Powell and Zhi-Li, 1983). The parasitoids used in these studies also responded to odors

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from their aphid hosts, although in all cases possible contamination of the aphids by traces of plant substances cannot be ruled out.

Aphidius nigripes Ashmead is the major primary parasitoid attacking the potato aphid, *Macrosiphum euphorbiae* (Thomas), on potatoes in northeastern North America, and its possible use as a biocontrol agent has been suggested (Shands et al., 1965; Cloutier et al., 1981). In order to evaluate more precisely the potential of *A. nigripes*, laboratory studies on the searching behavior of this parasitoid were initiated (Bauduin, 1982). Bouchard and Cloutier (1984) demonstrated that contact kairomone(s) present in aphid honeydew elicit intense local searching by *A. nigripes*. The present paper deals with olfactory stimuli which may be used in the field by *A. nigripes* parasitoids in their search for hosts. Binary choice tests using a four-field airflow olfactometer were conducted to measure the olfactory responses of *A. nigripes* to plants, aphids, aphid honeydew, and male and female conspecifics.

METHODS AND MATERIALS

Aphids and parasitoids were obtained and maintained as previously described by Bouchard and Cloutier (1984). Female parasitoids used in experiments were 3–4 days old, mated, and had previously been exposed to aphids except in tests involving odors from conspecifics where test females were 1–2 days old, virgin, and had never had access to aphids. All male parasitoids tested were 1–2 days old, unmated, and had not been exposed to aphids. The species used as test aphids were *M. euphorbiae, Myzus persicae* (Sulzer), *Aphis nasturtii* Kalt., and *Rhopalosiphum maidis* Fitch. The first three species were reared on potato seedlings (cv. Norland), and the corn leaf aphid was reared on barley seedlings (cv. Loyola).

The olfactometer used was virtually identical to that designed by Vet et al. (1983). The apparatus consisted of a four-arm star-shaped test chamber, each arm being connected to a series of two 50-ml glass flasks. The odor source material was placed in the flask closest to the chamber; the second flask contained distilled water to obtain uniform humidity in incoming air. A variable area flowmeter (Cole-Palmer®, max. flow 516 ml/min) was connected to each set of flasks to regulate the incoming airflow through each of the four ports of the chamber. All parts of the apparatus were connected using Tygon® tubing. The airflow was obtained with a vacuum compressor by pumping air out of the chamber through a 1-cm-diam. hole in the center of the floor, thus creating four distinct moving air fields in the chamber. Tests were performed at a flow rate of 300 ml/min through each arm.

Parasitoids to be tested were introduced individually in the chamber through the outflow port on the floor, and they were allowed a maximum of 5 min to make a final choice between the four air fields. A final choice was recorded when the parasitoid exited the chamber through one of the 0.5-cm-diam. ports of the chamber. Most parasitoids showed a strong anemotactic response and made a final choice within 5 min. The approximate boundaries of the four air fields were drawn on clear acetate placed on the top of the chamber, so before a final choice was made, the time spent by a parasitoid walking in each of the four fields was recorded with a Datamyte 800® event recorder. The presence of an observer near the apparatus did not seem to disturb the insects. Preliminary tests showed that many parasitoids sampled only two or three of the four odor fields before making a final choice. Therefore all experiments were designed as binary choice tests, i.e., parasitoids were given a simple choice between test material odors and control (blank) "odors." As suggested by Vet et al. (1983), we placed test materials in two opposite arms, the two other arms being the control ones. Each experiment was replicated twice with 20 parasitoids tested per replicate. After the first replicate, the whole apparatus was thoroughly washed with hot water and 95% ethanol. For the second replicate, the material tested as the source of odors was placed in the two arms which served as controls for the first replicate, thus counteracting any bias in the system. All experiments were performed at ca. 21°C, and the only source of light in the laboratory was a 60-W incandescent bulb placed 50 cm over the chamber.

The first experiment was conducted with all four arms connected to blank sources to determine if the distribution of parasitoid responses over the four fields of the chamber was random. In one experiment, the following plants were tested as sources of odors: potato seedlings (cv. Norland, 1.5 g fresh foliage and stem); mature, greenhouse-grown potato plant (cv. Norland, 1.5 g fresh foliage); and 4-leaf barley plants (cv. Loyola, 0.80 g fresh foliage). Possible interference with the normal odor of test plants owing to volatiles emanating from the cut edges was minimized by submerging the cut parts in vials filled with water and sealed with Parafilm®. In an experiment involving aphids, 300 individuals at different stages of development were used for each of the two test fields, except when *M. euphorbiae* (the largest of the four species) was the test aphid in which case 150 individuals were used. The honeydew used in another experiment was collected from each of the four aphid species tested as described by Bouchard and Cloutier (1984). Water solutions containing 0.26 mg dry honeydew/μl were obtained, and a volume of 150 μl was applied to a piece of absorbant cotton-wool used as the source. Similar cotton-wool pieces treated with distilled water served as control sources. The olfactory response of *A. nigripes* to conspecific males and females was also studied. In this experiment, 30 unmated, 1- to 2-day-old individuals which had never had access to aphids were used as source of odors for each test field.

RESULTS

The test using blank fields indicated that no detectable bias was present in the system since the number of final choices (11, 9, 9, and 11) and the mean time spent per field (0.38, 0.38, 0.30, and 0.25 min) did not differ significantly between the four fields ($P > 0.05$). In experiments involving test materials, a positive response in terms of a greater percentage of parasitoids choosing test fields over controls was always confirmed by a significantly longer time spent walking in test fields (Table 1).

Aphidius nigripes females did not show any response to either potato seedlings, mature potato leaves, or barley leaves (Table 1, tests 1, 3, 4). Males were tested only with potato seedlings to which they did not respond (Table 1, test 2). Female parasitoids showed significant attraction to all aphid species tested as well as honeydew obtained from them (Table 1, tests 5, 7–10, 12–14). Males did not respond to *M. euphorbiae* or to its honeydew (Table 1, tests 6, 11).

TABLE 1. RESULTS OF TESTING VARIOUS MATERIALS AS ODOR SOURCES TO *A. nigripes* IN A FOUR-FIELD AIRFLOW OLFACTOMETER

Test	Material tested	Sex tested[a]	% Choosing test fields	Time spent (min, $\bar{X}$ ± SEM)	
				Tested fields	Control fields
Plants					
1	Potato seedling	F	55	0.59 ± 0.11	0.53 ± 0.10
2	Potato seedling	M	58	0.52 ± 0.12	0.49 ± 0.09
3	Mature potato leaves	F	48	0.23 ± 0.04	0.25 ± 0.05
4	Barley leaves	F	52	0.17 ± 0.02	0.21 ± 0.03
Aphids					
5	*M. euphorbiae*	F	90*[b]	0.75 ± 0.13	0.32 ± 0.06**
6	*M. euphorbiae*	M	45	0.24 ± 0.04	0.24 ± 0.05
7	*M. persicae*	F	85*	0.31 ± 0.05	0.13 ± 0.02**
8	*A. nasturtii*	F	85*	0.30 ± 0.04	0.12 ± 0.02**
9	*R. maidis*	F	75*	0.44 ± 0.07	0.18 ± 0.04**
Honeydew from					
10	*M. euphorbiae*	F	82*	0.71 ± 0.11	0.42 ± 0.09**
11	*M. euphorbiae*	M	48	0.37 ± 0.07	0.36 ± 0.07
12	*M. persicae*	F	78*	0.36 ± 0.05	0.24 ± 0.04**
13	*A. nasturtii*	F	88*	0.34 ± 0.05	0.17 ± 0.03**
14	*R. maidis*	F	72*	0.43 ± 0.06	0.23 ± 0.04**
Conspecifics					
15	Females	F	48	0.47 ± 0.06	0.40 ± 0.08
16	Males	F	45	0.38 ± 0.07	0.36 ± 0.04
17	Females	M	88*	0.67 ± 0.10	0.17 ± 0.05**
18	Males	M	50	0.31 ± 0.04	0.30 ± 0.04

[a] 40 parasitoids/test.

[b] *$P < 0.05$ (χ^2 test with correction for continuity); **$P < 0.05$ (paired t test).

Females and males were not attracted or repulsed by parasitoids of the same sex (Table 1, tests 15, 18), indicating that within experiments the choices made by parasitoids tested consecutively were probably independent. Females did not respond to males but males were significantly attracted to females (Table 1, tests 16, 17).

DISCUSSION

The results clearly demonstrate the *A. nigripes* females can use olfactory cues to locate their hosts. Female parasitoids were strongly attracted by odors from aphids and from aphid honeydew. Such attraction should increase the parasitoid's searching efficiency in the field since females would orient towards plants or plant parts on which aphids are present. However, care must be exercised in extrapolating laboratory results to field conditions, as positive responses in an olfactometer do not imply longer-range attraction (Kennedy, 1965).

Other parasitoids have also been found to be attracted by aphids in an olfactometer. For example, Rotheray (1981) observed that parasitoids of syrphid fly larvae were attracted to aphids, thus permitting these parasitoids to locate aphid colonies, the most likely places to find the syrphid hosts. Read et al. (1970) showed that females of the cabbage aphid parasitoid, *Diaeretiella rapae* (McIntosh), responded to hosts freshly removed from collards, but not to aphids removed from host plants 24 hr prior to the test. The authors concluded that attraction to aphids may have resulted from traces of substances from the host plant being still present on test aphids. The attraction of the parasitoid *Lysiphlebus testaceipes* (Cresson) to its host, the greenbug *Schizaphis graminum* (Rondani) (Schuster and Starks, 1974), could be explained similarly. Powell and Zhi-Li (1983) found that males and females of the parasitoids *Aphidius uzbekistanicus* Luzhetski and *A. ervi* Haliday were attracted by odors from the food plants of their aphid hosts, but only females responded to the aphids, which suggests that aphids were attractive owing to their own specific odors. However, attraction to traces of plant substances remaining on aphids cannot be ruled out since the authors found that, at least in the case of *A. uzbekistanicus*, males and females may differ in their reaction to different plant substances. In the case of *A. nigripes*, traces of host plant substances cannot be implicated since parasitoids did not respond to the host plants of the aphids.

Surprisingly, females of our *A. nigripes* strain not only responded to a preferred host, *M. euphorbiae*, but also to the much less preferred hosts *M. persicae* and *A. nasturtii*, and even to a nonhost aphid, *R. maidis*. This suggests that females react nonspecifically to volatiles emanating from various aphid species. Assuming that such general attraction by aphid odors is used to locate hosts over relatively short distances by *A. nigripes*, then host species selection could still be achieved either through proper host habitat selection and/or contact recognition of potential hosts. Then a broad response to nonspecific aphid odors may still be highly adaptive in enabling searching parasitoids to quickly find aphid colonies dispersed among plants. Females of the two species of aphid parasitoids tested by Powell and Zhi-Li (1983) would not have to rely on host-habitat selection or host recognition to locate the appropriate hosts as they apparently respond only to suitable hosts.

In Petri dishes, *A. nigripes* females did not appear to detect the presence of aphid honeydew from a distance (Bouchard and Cloutier, 1984). However, the findings of the present study clearly demonstrate that females are attracted by honeydew odors carried by an airstream. Thus honeydew, which is a good indicator of the presence of aphids on plants, not only has a contact effect resulting in intensified searching by *A. nigripes* (Bouchard and Cloutier, 1984), but can also attract females from a distance by means of olfaction. Different honeydew constituents are probably responsible for its contact and olfactory effects. For example, the arrestant effect of honeydew on the aphid predator *Chrysopa carnea* Stephens is due to sucrose, whereas volatile breakdown products of tryptophan act as attractants (Hagen et al., 1976; Van Emden and Hagen, 1976). Volatiles would also be implicated in short-range attraction of larvae of the predator *Aphidoletes aphidimyza* (Rond.) to honeydew from its aphid prey (Wilbert 1974).

Female *A. nigripes* did not respond to potato or barley plants in our tests. In *D. rapae*, both sexes were attracted to collards as well as to dilute solutions of allyl isothiocyanate, a mustard oil present in Cruciferae (Read et al., 1970). Similarly *L. testaceipes* responded to odors from sorghum, the food plant of its greenbug host (Schuster and Starks, 1974). The parasitoids *A. uzbekistanicus* and *A. ervi* also showed selective responses to the food plants of their host aphids (Powell and Zhi-Li, 1983). In *A. nigripes*, the absence of reaction to plants might have resulted from rapid changes in the volatile mixture emanating from the plants following cutting (Visser and Nielsen, 1977). An alternative explanation is that *A. nigripes* may use other than olfactory means to locate the habitat of its host, such as visual cues for example. Even though this parasitoid is closely associated with potato fields in North America (Duncan and Couture, 1956; Shands et al., 1965), its host range includes aphids which do not use potatoes as host plant, including *Macrosiphum avenae* Fab. and *M. rosae* L. (Mackauer and Stary, 1967). Moreover, the commonly reported hosts *M. euphorbiae* and *M. persicae* are extremely polyphagous, using host plants not taxonomically restricted to the family level (Eastop, 1977). Thus it is not surprising to find that *A. nigripes* does not seem to use plant volatiles as a means of host-habitat selection, as shown for parasitoids exhibiting greater host and/or habitat specificity such as *D. rapae*. A similar strategy might be inefficient for *A. nigripes*, given the diversity of plant chemicals that it would have to recognize.

Aphidius nigripes males were not attracted by aphid odors, which indicates that they do not locate females through the intermediary of aphids. Powell and Zhi-Li (1983) reported similar results for *A. uzbekistanicus* and *A. ervi*. Male

A. nigripes did not respond to conspecific males, but were strongly attracted to females. This confirms the observations of Dumas and McNeil (unpublished) indicating the existence of a female sex pheromone in *A. nigripes*, as reported for other aphid parasitoids (Read et al., 1970; Powell and Zhi-Li, 1983). Female *A. nigripes* did not respond to males or females, which suggests that the presence of conspecifics would not interfere with the attractiveness of aphid colonies to searching females.

The present study does not prove long-range attraction of *A. nigripes* to host aphids, but it clearly shows that once in the habitat of potential hosts, the searching female has the capacity to select, via olfactory cues, areas where hosts are most likely to be available. The actual mechanisms used by this parasitoid in host-habitat selection remain to be elucidated, but they are unlikely to implicate olfaction based on volatiles produced by the food plant of the host.

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