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Beneficial Arthropod Behavior Mediated by Airborne Semiochemicals. VIII. Learning of Host-Related Odors Induced by a Brief Contact Experience with Host By-Products in *Cotesia marginiventris* (Cresson), a Generalist Larval Parasitoid[1]

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Females of Cotesia marginiventris *(Cresson), a generalist larval parasitoid, were observed to respond to host related odors in a four-arm olfactometer. The females were significantly more responsive to the odors after a brief contact experience with host-damaged leaves contaminated with host by-products. During the experience, actual encounters with hosts were not required to improve subsequent responses to host-related odors. The response to odors of the plant–host complex with which parasitoids had experience was significantly higher than the response to odors of an alternative plant–host complex. This suggests that the experience effect is due, at least partly, to associative learning. We suspect that females of this generalist parasitoid, as was recently found for those of a specialist, recognize specific semiochemicals when they contact frass of suitable host larvae. The parasitoids, subsequently, associate the surrounding odors with the possible presence of hosts, and use these odors as cues in their search for more hosts. This could be an important component in the host-searching behavior of many parasitoids.*

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INTRODUCTION

In many species of insect parasitoids semiochemicals associated with the hosts and/or the host habitats play a major role in the host location process (Vinson, 1976, 1984). The optimal way for a parasitoid to take advantage of the available semiochemicals may vary in time. For those parasitoid species which can develop in more than one host species, the predominant host species at one time may be rare or absent at another. Also, the habitats that the hosts tend to occupy may vary greatly. To optimize host finding, parasitoids should be able to adapt to such changes. This would require the ability to adjust their response to certain semiochemicals.

Indeed, several studies on host habitat and host location have shown that host searching behavior can be modified by experience. Adult female experience with hosts and host-related substrates have been shown to increase further responses (Monteith, 1963; Arthur, 1966, 1971; Taylor, 1974; Vinson *et al.*, 1977; Sandlan, 1980; Vet, 1983, 1985; Vet and van Opzeeland, 1984, 1985; Wardle and Borden, 1985; Dmoch *et al.*, 1985). Learning and conditioning are the terms used most often to describe this effect.

Recent studies have shown that the flight response of *Microplitis croceipes* (Cresson) and *M. demolitor* (Cresson) to host-related odors in a flight tunnel increases significantly after the parasitoids have a brief contact experience with host by-products (Drost *et al.*, 1986; Eller *et al.*, 1988; Herard *et al.*, 1988). Lewis and Tumlinson (1988) have shown for the specialist *M. croceipes* that the females learn to respond to specific odors after they contact host frass in association with those odors. Preliminary observations on the host searching behavior of *Cotesia marginiventris* (Cresson) indicated that females of this larval parasitoid also perform better after a contact experience with hosts or host by-products. Unlike *M. croceipes, C. marginiventris* has a very broad host range of at least 20 different species (including *Spodoptera frugiperda, Trichoplusia ni*, and *Heliothis* spp.).

The function and the mechanisms behind the phenomenon of increased responsiveness after experience may differ between generalists and specialists. One possibility is that in generalists the perception of certain chemicals in the host by-products sensitizes the females and makes them enter a searching mode, resulting in a general increase in response. It is also possible that during the contact experience the females learn to respond to the specific odors emitted by the encountered plant–host complex and increase their response to those odors only. In order to test these two alternative hypotheses, we studied the effects of prebioassay contact experience on the response to host related odors by *C. marginiventris* in a four-arm olfactometer.

MATERIALS AND METHODS

Parasitoids. C. marginiventris (1985 Mississippi strain) were reared on fall armyworm larvae at the USDA-ARS, Insect Biology and Population Management Research Laboratory, Tifton, Georgia, according to the procedure described by Lewis and Burton (1970) for *M. croceipes.* The pupae were held in 25 × 25 × 25-cm, plexiglass cages, with one side made of fine-mesh nylon screen. The parasitoids were allowed to emerge in the cages and were held at 26°C, 50–60% RH, and a 15-h photophase. Only parasitoids that emerged on the same day were kept in the same cage. Males were removed after 2 days, allowing sufficient time for all females to be mated. All experiments were conducted with mated females that were 3 to 4 days old, 7–10 h after they experienced lights-on.

Hosts. The hosts used in the experiments were second-instar larvae of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), and of the cabbage looper (CL), *Trichoplusia ni* (Hübner). They were reared according to the method described by King and Leppla (1984). Initially, the larvae were fed on a laboratory-prepared pinto bean diet. Then, for about 18 h prior to testing, those larvae that were used as part of the odor source were allowed to feed on leaves. Thus, the FAW larvae were fed corn (*Zea mays* L.) leaves, and the CL larvae were fed cotton (*Gossypium hirsutum* L.) leaves. The leaves were obtained from plants that were 2–4 weeks old.

Olfactometer. Bioassays were performed in an airflow olfactometer similar to the one described in detail by Vet *et al.* (1983) with some modifications described by Eller *et al.* (1988). It consisted of an exposure chamber connected to four arms through which air flowed into the chamber. The air was pulled out through a center hole in the bottom of the chamber. By balancing the airflows (300 ml/min through each arm), four distinct flow fields were created in the exposure chamber. Each arm was connected to a flow meter, a water bubbler (to humidify the air), an odor chamber, and a catching jar. Materials tested as sources for semiochemicals were placed in the odor chambers. Parasitoids that walked up the arms were captured in the catching jars.

Female parasitoids were introduced through the vertical entry tube after temporarily disconnecting the extraction tube. While walking up the entry tube the test animal was exposed to a mixture of the four flows until it reached the chamber floor, where it moved freely, exploring the different flow fields. One wasp was introduced at a time and its behavior was observed during a 5-min period. If the test female showed a positive anemotactic response and walked up one of the airflows and out of the exposure chamber, it was recorded as a final choice if she did not return within 15 s. The temperature in the bioassay room was 28 ± 1°C at all times.

Odor Source. In all experiments, only a single odor source was used in one of the olfactometer arms. The three remaining arms served as controls, with only humidified air going through. The test odor source contained five second-instar larvae feeding on young leaves. Either FAW on corn leaves or CL on cotton leaves were used. The larvae were starved for 1 h before being introduced to the odor chamber with one of the already damaged and contaminated leaves and a fresh seedling. The larvae were allowed to eat for 1.5 h before wasps were tested in the olfactometer.

Experience. Females were provided with the following types of experiences prior to testing: (1) no experience with any hosts or host products (INEXPER); (2) one oviposition on a second-instar CL larva feeding on cotton (CLOVIP); (3) a 20-s contact experience with a cotton leaf damaged and contaminated by CL larvae (CLDAMAG); (4) one oviposition on a second-instar FAW larva feeding on corn (FAWOVIP); and (5) a 20-s contact experience with a corn leaf damaged and contaminated by FAW larvae (FAWDAMAG).

The leaves used in the oviposition and damage experiences were equally damaged by larvae and contaminated with larval by-products to ensure that oviposition was the only difference between these two types of experience.

The females were tested 30–60 s after they had their experience. Five females of each treatment were tested per day. The two odor sources were alternated between days. The arm containing the odor was switched systematically on different days. A total of 30 females of each treatment to each odor source was tested.

Data Recording. The behavior observed in the olfactometer was recorded with an Epson Geneva PX-8 portable computer. Response was measured in two ways: (1) the time spent in the quadrant of the introduction chamber containing the odor and (2) the number of final choices made for the arm through which the odor was entering the exposure chamber.

Statistical Analyses. The five treatments were compared with Duncan's new multiple range test after analysis of variance (ANOVA) (Steel and Torrie, 1960). The two response measures were analyzed using (1) the percentage of time spent in the odor field by each individual female and (2) for each treatment, the percentage of the five females tested daily that made a final choice. The percentages were transformed using the arcsin-square root transformation for statistical analysis.

To compare oviposition experience with damage experience, and FAW experience with CL experience, pooled means were analyzed by orthogonal comparison of the sums of squares (Chew, 1976). Significance levels were 0.05 in all tests.

RESULTS

Table I shows the observed responses for females that had different types of experiences prior to their introduction in the olfactometer. In most cases prior experience caused females to spend more time in the odor flow and to make more final choices for the odor arm. With some exceptions for the time spent in the odor quadrant, the increase in response was significantly less when the females experienced the alternative plant–host complex.

For a better comparison of the different types of experiences, the appropriate means in Table I were pooled and analyzed by orthogonal comparison of the sum of squares (Chew, 1976), resulting in the following.

Oviposition vs Damage Experience. A comparison of all females that had an oviposition experience with those that experienced only the host-damaged leaves showed that the increase in response did not require contact with a host. In response to the odors of CL on cotton and the odors of FAW on corn, females that had an oviposition experience and females with a damage experience showed no differences in the percentage of time spent in the odor quadrant ($F = 0.0186$, $F = 0.0047$) and the percentage of final choices for the odor arm ($F = 0.79$, $F = 0.05$) (Fig. 1a).

CL on Cotton vs FAW on Corn Experience. For each odor source, all females that experienced CL on cotton were compared with those that experienced FAW on corn (oviposition experience and damage experience lumped together). This resulted in highly significant differences for both the time spent in the odor quadrant ($F = 14.90$, $F = 9.70$) and the percentage of females that made a final choice for the odor arm ($F = 25.50$, $F = 12.71$). Figure 1b shows

Table I. Effect of Prebioassay Experience on the Response of *C. marginiventris* Females Exposed to Host-Related Odors in a Four-Arm Olfactometer[a]

		Odor			
		CL feeding on cotton		FAW feeding on corn	
Experience	*N*	% FC	% time in odor	% FC	% time in odor
INEXPER	30	20.0 a*	59.4 a	13.3 a	46.9 a
CLOVIP	30	73.3 c	85.6 b	43.3 b	69.9 b, c
CLDAMAG	30	70.0 c	87.2 b	36.7 b	62.0 a, b
FAWOVIP	30	46.7 b	67.1 a	60.0 c	79.2 c, d
FAWDAMAG	30	40.0 b	66.7 a	70.0 c	86.8 d

[a] Response is expressed as (1) percentage of females that made a final choice for the odor (% FC) and (2) percentage of the total time spent in the quadrant with the odor flow (% time in odor).

* Values followed by the same letter in each column are not significantly different [Duncan's new multiple range test after ANOVA with arcsin-square root transformation of percentages (Steel and Torrie, 1960)].

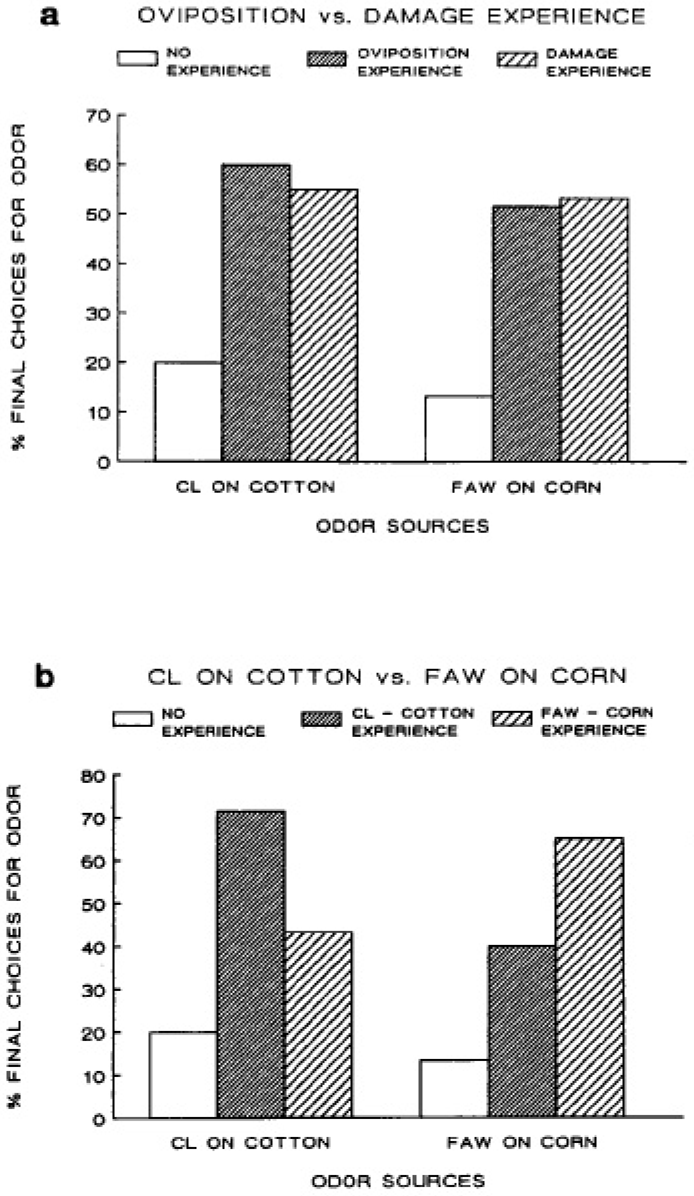

Fig. 1. Experience effect upon response by *C. marginiventris* females to odors of two plant–host complexes. (a) The responses of females (1) that had no experience with hosts or host products prior to a test, (2) that were allowed to oviposit in one larva (either CL or FAW) feeding on leaves, and (3) that contacted only leaves damaged by CL or FAW larvae prior to a test. (b) The responses of females (1) that had no experience with hosts or host by-products prior to a test, (2) that experienced CL on cotton (either oviposition or damage), and (3) that experienced FAW on corn (either oviposition or damage).

that the wasps responded much better when exposed to the odors of the plant–host complex with which they had their experience.

DISCUSSION

The response of *C. marginiventris* females to odors of host larvae feeding on leaves can be increased dramatically by allowing the females to contact host by-products prior to testing in an olfactometer. Intriguing is the fact that actual encounters with the hosts are not required to evoke this increase in response (Fig. 1a). Just a brief contact with larval by-products alone is sufficient to evoke an increase in response in the parasitoid females. This shows that the process is a powerful and important modifier of the insects' behavior.

It was also found that the parasitoids respond best to the odors of the plant–host complex that they experienced (Fig. 1b). Therefore, the results show that we are not merely dealing with a general increase in response but that the females learn to respond to specific odors that they encountered during the contact experience. We suspect that an associative learning process is involved, which is triggered when a parasitoid contacts one or more specific kairomones (unconditioned stimuli). The parasitoid then links the surrounding odors (conditioned stimuli) with the possible presence of host larvae. Subsequently, the wasps will use those odors as cues in the search for more hosts. A similar mechanism has been demonstrated for the parasitoid *M. croceipes* (Lewis and Tumlinson, 1988). *M. croceipes* is a specialist, attacking only *Heliothis* larvae, while *C. marginiventris* attacks many different species. Despite their different host ranges, both parasitoids seem to rely on a similar mechanism that controls their host searching behavior. The mechanism may be common among many parasitoids and may highly improve host-searching efficiency. Such a mechanism requires that specific semiochemicals (unconditioned stimuli) present in the by-products of suitable hosts are recognized by the parasitoids. The chemicals that serve as the unconditioned stimuli are likely to vary for different parasitoid species because of differences in host ranges.

The fact that experience with an alternative host still causes a substantial increase in response (Fig. 1b) suggests that associative learning is not the only process involved but that sensitization takes place also. Alternatively, however, some of the semiochemicals emitted by one plant–host complex might be the same or similar to those emitted by the other complex. Therefore, learning alone could still explain why experiencing one plant–host complex increases the response to semiochemicals emitted by another plant–host complex.

In the laboratory adult females seem to require a learning experience to make them highly sensitive to host odors. However, learning may also take place during or immediately following emergence, when the parasitoid contacts by-products of its own host on the cocoon and on the emergence site. The insects used in the experiments were reared on FAW larvae feeding on artificial diet. The information these parasitoids obtained during emergence might not be adequate to make them respond to the odors to which they were exposed during the experiments. This could explain the relatively poor response by inexperienced females. Females in the field may obtain enough information in an earlier stage to make them more responsive to the appropriate semiochemicals. In fact, Drost *et al.* (1988) and Herard *et al.* (1988) showed, for *M. croceipes* and *M. demolitor*, respectively, that inexperienced adult wasps reared from larvae feeding on cowpea leaves responded better than those reared on larvae feeding on artificial diet, when exposed to odors emitted by larvae feeding on cowpea. The effect of rearing on the response levels is likely to be of importance for *C. marginiventris* as well. However, considering the host range of this species and the many plant species these hosts might feed upon, modification of the response to semiochemicals after experience by an adult female should contribute to the female's host searching efficiency. Therefore, it is likely that adult females in the field, each time they contact host by-products, continue to increase and adjust their response to the associated airborne semiochemicals.

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