HOW CONTACT FORAGING EXPERIENCES AFFECT PREFERENCES FOR HOST-RELATED ODORS IN THE LARVAL PARASITOID Cotesia marginiventris (CRESSON) (HYMENOPTERA: BRACONIDAE)¹

TED C.J. TURLINGS, J.W.A. SCHEEMAKER,² L.E.M. VET,² J.H. TUMLINSON, and W.J. LEWIS³

Insect Attractants, Behavior, and Basic Biology Research Laboratory Agricultural Research Service, U.S. Department of Agriculture Gainesville, Florida 32604

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Abstract—Responses of individual females of the parasitoid Cotesia marginiventris to the odors of four different complexes of host larvae feeding on leaves were observed in a four-arm olfactometer. The plant–host complexes were composed of fall armyworm (FAW) larvae or cabbage looper (CL) larvae feeding on either corn or cotton seedlings. Prior to testing, each female was given a brief foraging experience on a plant–host complex and was then exposed to the odors of the same complex in the olfactometer. The experienced females responded to familiar odors in a dose-related manner, and these responses were virtually identical to all four complexes. Preferences for the odors of one of two plant–host complexes were tested in dual choice situations. Generally, FAW odors were preferred over CL odors and corn odors over cotton odors. A short foraging experience significantly affected the females’ odor preferences in favor of the odors released by the experienced complex. Additional experiments revealed that neither longer bouts of experience nor bouts that included ovipositions resulted in a stronger change in preference. Experience affected preference in combinations where only the host species was varied as well as in combinations where only the plant species was varied. The results, therefore, strongly indicate that both the plants and the hosts somehow are involved in the production and/or release of the semiochemicals that attract C. marginiventris.

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²Present address: Agricultural University of Wageningen, Department of Entomology, P.O. Box 8031, 6700 EH Wageningen, The Netherlands.
³Mailing address: P.O. Box 748, Tifton, Georgia 31793-0748.
INTRODUCTION

Females of many insect parasitoids rely on host and host-habitat related chemicals as cues in their search for hosts (for reviews see Vinson, 1976, 1981, 1984; Weseloh, 1981; van Alphen and Vet, 1986). Several studies have demonstrated that the response to these semiochemicals is flexible and can be influenced by learning (Thorpe and Jones, 1937; Monteith, 1963; Arthur, 1971; Taylor, 1974; Vinson et al., 1977; Sandlan, 1980; Strand and Vinson, 1982; Vet, 1983, 1988; Vet and van Opzeeland, 1984, 1985; Wardle and Borden, 1985; Dmoch et al., 1985; Drost et al., 1986; Lewis and Tumlinson, 1988). These studies show that experiences with hosts and/or their microhabitats, both by immature and mature stages, may influence an adult parasitoid’s response to semiochemicals. *Cotesia marginiventris* (Cresson), a solitary larval parasitoid of many Lepidoptera, shows a significant increase in response to host-related odors after only a brief contact experience with host damaged leaves contaminated with host by-products (Turlings et al., 1989). After females receive a contact experience with a particular plant–host complex, they respond significantly better when they are exposed to the odors of that plant–host complex than when they are exposed to the odors of an alternative plant–host complex (Turlings et al., 1989). This suggests that the experience effect is not merely the result of a general increase in response to semiochemicals, but that the insects actually learn to respond to the odors that they encounter during their experience.

This phenomenon of conditioning through experience has been suggested as a useful method for biological control programs in which parasitoids could be stimulated to respond to host-related odors prior to their release in a target area (e.g., Lewis and Nordlund, 1985). This would be particularly helpful if the wasps would not just increase their responses to the experienced odors, but would actually prefer these odors over those released by alternative plant–host complexes when given a choice.

Here we report on a study in which the effect of experience on odor preferences by *C. marginiventris* was tested. Shifts in preference in favor of an experienced odor were studied in situations where only the host species was varied and in situations where only the plant was varied. Thus, we obtained information on the specific roles played by both host larvae and plants in the production and release of semiochemicals essential for host-habitat location.
PARASITOID FORAGING EXPERIENCES AFFECT ODOR PREFERENCES

METHODS AND MATERIALS

Population of C. marginiventris. Parasitoids of the '85 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 the parasitoid Microplitis croceipes. Cocoons were collected a few days prior to emergence and shipped to Gainesville where they were kept in 25 × 25 × 25-cm Plexiglas cages, each with one side of fine mesh nylon screen. Parasitoids that emerged on the same day were kept in the same cage, and all cages were stored in cabinets at 26°C, 50–60% relative humidity, and a 15-hr photophase. Males were removed after two days, allowing sufficient time for all females to be mated. All experiments were conducted with 3- to 5-day-old mated females, 6–10 hr into the photophase.

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 procedure described by King and Leppla (1981).

Olfactometer. Individual females were exposed to host-related odors and observed in a four-arm olfactometer similar to the one described by Vet et al. (1983) with some modifications described by Eller et al. (1988). This device is designed so that four well-defined odor fields, each associated with one of the arms, are created in a square central arena. The fraction of an odor-containing flow that actually reached a parasitoid in the olfactometer could be controlled by diverting part of the flow before it entered the arena. The fraction that was split off was then replaced by clean humidified air. The total flow entering the central exposure chamber through each arm was kept at 300 ml/min.

Odor Sources. The odor sources consisted of five late second- or early third-instar larvae feeding on three seedlings. The larvae of either FAW or CL were put on the seedlings of either corn (Zea mays L.) or cotton (Gossypium hirsutum L.) 1.5 hr prior to the actual bioassays.

Data Recording. The behavior of the females in the olfactometer was recorded with the use of an Epson Geneva PX-8 portable computer. After a female was introduced into the olfactometer, the time it spent in each odor quadrant was recorded during a 5-min period. If the parasitoid walked into one of the arms and did not return within 15 sec, this was recorded as a final choice for that arm. The remaining time was added to the time spent in the quadrant of the final choice. For the dual choice tests, the odor quadrant in which a female spent the greatest amount of time was recorded as her odor field preference.
RESULTS

Dose-Response Experiments. Before actual odor preferences were tested, the responses to different odor doses were tested for each plant–host complex separately. Thus, dose–response curves could be generated to determine which concentrations of the different plant–host complexes evoke similar response levels. These concentrations were then used in the preference experiments to reduce the possibility that preferences were influenced by concentration differences.

The responses of female parasitoids were observed to the odors of FAW on corn, FAW on cotton, CL on corn, or CL on cotton. During each test the odor of one of these complexes was offered through one of the four flows, while the other flows contained humidified air and served as controls. For each of the four odor sources three concentrations were tested: 25, 50, and 100% of the original odor flow.

Just prior to being tested, a female was placed, for 20 sec, on a plant–host complex like the one used as the odor source. The parasitoid was prevented from actually encountering hosts. This type of experience significantly increases a female’s response to host-related odors (Turlings et al., 1989). The female was then introduced into the olfactometer. Odor sources were rotated to the next air flow after six females were tested for each of the three concentrations of a particular odor. A total of 24 females were tested for each concentration of all odor sources.

Figure 1 shows that the wasps responded in a dose-related manner to all four complexes. Regression analyses of the time spent in the odor quadrant (Figure 1b) and of the time it took the females to make a final choice (Figure 1c) show a significant increase in responsiveness to the odors with increasing odor dose. The total number of final choices made for the odor arms was also found to be significantly dose related, with the exception of CL on cotton (Figure 1a).

No significant differences were found between the same doses of the four odor sources. In all of the following preference tests, 50% doses were used.

Effect of Short Contact Experience on Odor Preference. The effect of a short-term contact experience on a female’s preference for the odors emitted by

Fig. 1. Responses of experienced C. marginiventris females to three doses of odors emitted by larvae feeding on leaves. Responses were measured as: (a) average percentage of the females that made a final choice for the odor arm; (b) average percentage of time that the females spend in the quadrant with the odor; (c) average time it took a female to make a final choice. The lines connect the average values, while the equations for the actual linear regressions are given with each graph.
different plant-host complexes was tested in a series of dual-choice experiments. A female wasp was allowed to contact, for 20 sec, a plant-host complex from which the larvae had been removed. Immediately following this contact experience, the wasp was transferred to the olfactometer. In the olfactometer, the wasp was exposed to the odors of two different plant-host complexes introduced through adjacent arms of the olfactometer; the two remaining arms carried humidified air only. One of the odors was from the complex that the female had just encountered, the other odor from a different complex. One combination of two odor sources was tested on a given day with 8–10 females that experienced one source and the same number of females that experienced the other source, before being introduced into the olfactometer. This was replicated six times for each combination.

The results, as summarized in Figure 2, show that the probability that a female chooses the odor of a particular complex is higher if she has had experience with that complex than if she has had experience with the other complex. The T test (SAS Institute, 1987) was used to make an overall comparison of the responses to the odors of complexes with which females had experience to those with which they had no experience. The differences were highly significant for both the number of final choices \((N = 6; \ T = 4.17; \ P = 0.009)\) and the odor field preference \((N = 6; \ T = 4.69; \ P = 0.005)\). However, for each individual combination, the differences in odor preferences were not always significant (Figure 2). For the numbers of final choices made for the odors, a significant difference was found only for the combinations FAW on corn versus CL on corn \((\chi^2 df_1 = 8.624; \ P = 0.003)\) (I, Figure 2c) and CL on corn versus CL on cotton \((\chi^2 df_1 = 3.907; \ P = 0.048)\) (IV, Figure 2c). Odor field preference was affected significantly for the combinations FAW on corn versus CL on corn \((\chi^2 df_1 = 6.596; \ P = 0.010)\) (I, Figure 2d) and FAW on corn versus CL on cotton \((\chi^2 df_1 = 4.318; \ P = 0.038)\) (V, figure 2d).

Pooling the combinations with different hosts feeding on the same plants (I and II in Figure 2) and the combinations with the same hosts feeding on different plants (III and IV) revealed an overall preference for FAW and for corn odors. The females divided their final choices more or less equally among FAW and CL (73:62), but the overall odor field preference deviated significantly from a 1:1 ratio \((116:76; \chi^2 df_1 = 3.802, \ P = 0.051)\). Plant preference in favor of corn was demonstrated with both the number of final choices \((98:45; \chi^2 df_1 = 9.778, \ P = 0.002)\) and the odor field preference \((131:68; \chi^2 df_1 = 9.285, \ P = 0.002)\).

Effects of experience were also analyzed with a loglinear model (SAS Institute, 1987) with five dependent variables: experienced host, experienced plant, alternative host, alternative plant, and preference (experienced or alternative
Fig. 2. Effects of experience on the preference of C. marginiventris females for host-related odors. (a) Diagram of the olfactometer with the odors of two different plant–host complexes entering the exposure chamber through adjacent arms. (b) List of the six plant–host complexes that were tested. (c) Summary of results using the percentage of females that made a final choice for a specific odor arm as the measure of response. Females that did not make a final choice were excluded. (d) Summary of results using the percentage of females that spend most of their time in a specific odor field (= odor field preference) as the measure of response. The few females that never entered one of the two odor fields were excluded. The roman numerals (I–VI) in (c) and (d) refer to the combinations listed in (b). In (c) and (d) the bars above the x-axis represent the females that choose odor A and below the x-axis the females that choose odor B. The actual numbers are shown in parentheses. For each combination, 48–50 females were tested of both experience types. The asterisks indicate significant differences in total numbers due to experience (chi-square; P < 0.05).

The number of females responding on a test day was used as one observation. There were 24 response levels with a total frequency (N) of 415 for the final choices and 585 for the odor field preference. Again, the overall effect of experience in favor of the experiences odor was highly significant [final choices (FC): $\chi^2 df_1 = 12.21, P = 0.0005$; odor field preference (OFP): $\chi^2 df_1 = 13.84, P = 0.0002$]. Significantly more females chose the experienced odor if they had experienced corn than if they had experienced cotton (FC: $\chi^2 df_1 = 15.82, P = 0.0001$; OFP: $\chi^2 df_1 = 12.55, P = 0.0004$). No such difference in experience effect was found for the two host species. The general preference...
for FAW odors was demonstrated by the fact that significantly more females would choose the alternative odor if the alternative host was FAW (FC: $\chi^2 df_1 = 10.32, P = 0.0013$; OFP: $\chi^2 df_1 = 19.85, P < 0.0001$). No such difference was found for the plant species. Note that in those cases where the wasps experienced the source with the least preferred host and plant (i.e., CL on cotton; combinations II, IV, and V) no preference for either odor source was observed.

The combination FAW on corn–CL on cotton was chosen for the following additional preference experiments. This combination contains all four components, and the results obtained for this combination allow room for measurable increases and decreases in the effect of experience.

A More Complete Experience. The previous experiments were conducted with females that had a 20-sec contact experience without ovipositions just prior to their introduction into the olfactometer. Further experiments were performed to determine whether a longer, more complete experience, which included ovipositions, would result in a stronger effect upon the odor preference by the parasitoids, and whether this effect of experience would be retained over time.

Females were experienced by placing them in a glass container (26 cm diam., 10 cm high) containing either 70 FAW larvae on 12 corn seedlings or 70 CL larvae on 12 cotton seedlings. The containers were then covered with a Plexiglas plate. All females made contact with the plants and frass, and parasitized more than one larva. Females were exposed to the plant–host complex until they left the plants and attempted to leave the container. The exposure time varied from 4 to 11 min.

The persistence of the experience effect over time was tested by giving one group of females their experience in the morning 3–4 hr before being tested in the olfactometer (group 1); a second group was given their experience just a few minutes prior to the bioassay (group 2).

Results for the two groups are presented in Figure 3. Again, the differences between females that experienced different complexes were slight but consistent. The preference for the odor of FAW on corn was less for the females that experienced CL on cotton. For the females of group 1, the difference in preference was only found to be significant for the number of final choices ($\chi^2 df_1 = 5.326; P = 0.021$). For group 2, a significant difference between females experienced on a different complex was only found in the odor field preference ($\chi^2 df_1 = 15; P = 0.001$).

Group 1 and group 2 females did not differ from each other in their response to the odors except for females experienced on FAW on corn. Group 2 females with a FAW on corn experience preferred FAW on corn odors significantly more than group 1 females that had experienced the same complex ($\chi^2 df_1 = 5.87; P = 0.015$). This difference was not observed in the number of final choices made by the two groups.

Experiences with Ovipositions versus Experiences without Ovipositions.
Fig. 3. Responses of *C. Marginiventris* females to the odors of either of two plant–host complexes after a complete contact experience including ovipositions as indicated in the figures. Group 1 had their experience 3–4 hr prior to a bioassay; group 2 had their experience just prior to a bioassay. The actual numbers are shown in parentheses. The asterisks indicate significant preference shifts.

Finally, treatments were tested simultaneously to reveal possible differences between experiences with ovipositions and experiences without ovipositions, which may so far have been hidden by interday variation. Two sets of females were given a complete experience as described above, 1 hr before the bioassays. The first set encountered larvae and could oviposit freely during the experience. The second set of females, however, was experienced on a complex where the larvae were removed so that only the contaminated and damaged leaves could be contacted.

The results were very similar to those found for the treatments discussed before (Figure 4). No differences were found between females that had a total experience including ovipositions and females that only contacted the damaged and contaminated leaves.

DISCUSSION

The dose–response tests revealed that the females’ responses increase with an increasing dose of the host-related odors. No significant differences were found in the attractiveness of the four different plant–host complexes when the
parasitoids were exposed to the odors in single choice tests. The pooled results of the preference experiments, however, indicate a preference for FAW odors over CL odors and an even stronger preference for corn odors over cotton odors. Since FAW seems to be *C. marginiventris*' most important host and FAW larvae are found predominantly feeding on corn, other legumes, and grasses (Ashley, 1986), an innate preference for the odors of FAW larvae and damaged corn is not surprising. Furthermore, CL appears to be a very poor host since initial rearing experiments show minimal emergence from this host (unpublished data; M.R. Strand, personal communication). On the other hand, since all test animals were reared on FAW larvae, the observed preference for FAW may also have been the result of conditioning of the parasitoids as immatures. However, since host larvae are routinely fed artificial diet, the rearing procedure could not account for the corn preference.

The results not only indicated an innate preference for FAW and corn odors, they also showed that the preferences were affected by contact experiences with the plant–host complexes. For all plant–host complex combinations, it was
found that a particular complex was chosen more often by females that had experienced that complex than by the females that had experienced the alternative complex. Although not always significantly, this experience effect did cause a change in preference in each individual combination. The overall effect was found to be highly significant.

The results are in agreement with earlier results of single choice experiments presented by Turlings et al. (1989). The increase in response to host-related odors after experience is greatest to the odors emitted by the plant–host complex that the females experienced. The learning process that must be involved is triggered by a brief contact with host by-products and does not require actual contact with the hosts. When females were given a longer experience period, including ovipositions, they did not appear to respond differently than females that had a 20-sec experience without ovipositions. The effect of experience on the preference for host-related odors lasted at least several hours and is therefore likely to be an important factor determining the host-searching behavior of these parasitoids in the field.

Significant differences in preference were found when females were offered odor source combinations where only the host species varied, where only the plant species varied, and where both the host and the plant varied. We can therefore conclude that the females are able to distinguish between different host species and between different plant species. Evidently, both host and plant are somehow involved in the emission of the semiochemicals that evoke a response in the parasitoid females, either by producing the essential volatiles or by affecting the volatiles released by another component of the complex. The parasitoids are therefore likely to respond to more than one compound, and the intensity of their response to each compound probably increases when it is encountered in association with a foraging experience. The results suggest that each plant–host complex releases its own blend of semiochemicals that is detected by C. marginiventris. After exposure to a particular complex, a female will subsequently be attracted to an odor blend that is most similar to the blend she perceived during her experience. Future research will have to reveal whether females of C. marginiventris and other parasitoid species distinguish between variations in specific semiochemical blends or whether they are able to differentiate between different compounds altogether.

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