Influence of Physiological State and Experience on the Responsiveness of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) to Aphid Honeydew and to Host Plants

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The effect of physiological state (hunger) and experience on the responsiveness of the aphid parasitoid, Lysiphlebus testaceipes *(Cresson), to clean and honeydew-contaminated host plants was investigated in laboratory bioassays. Both fed and unfed parasitoids spent significantly longer examining honeydew-contaminated plants compared to uncontaminated controls, but the presence of honeydew did not influence attack latency (i.e., the speed with which naive parasitoids found and attacked hosts). Hunger, however, had a significant negative effect on attack latency, presumably as a result of a physiologically based shift from host- to food-location behavior in starved parasitoids. The parasitoid's response to clean plants was significantly increased as a result of classical conditioning procedures, whereas the response to honeydew-contaminated plants was not.*

INTRODUCTION

Aphid honeydew has been reported to act as a contact kairomone for a number of different aphidiid parasitoids. The presence of aphid honeydew on plants has been shown to increase the time spent searching by *Aphidius nigripes* Ashmead (Cloutier and Bauduin, 1990), *Ephedrus cerasicola* Stary (Hågvar and Hofsvang, 1989), *Diaeretiella rapae* McIntosh (Ayal, 1987), and *Aphidius rhopa-*

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losiphi De Stefani-Perez (Gardner and Dixon, 1985). In addition, arrestment of female parasitoids on filter-paper disks treated with water extracts of host honeydew has been reported for *A. nigripes* (Bouchard and Cloutier, 1984), *A. rhopalosiphi*, and *Ephedrus plagiator* (Nees) (Budenberg, 1990). Both of the latter studies demonstrated an increase in parasitoid response with increasing honeydew concentration (up to plateau levels) and a waning of the response with repeated exposure to the stimulus.

However, since honeydew is the principal food source of adult aphidiids (Stary, 1988), it could be argued that the reported arrestant effects may be at least partially due to food-seeking behavior rather than to host-seeking. The effect of physiological factors such as hunger on parasitoid responses to host-associated cues has been generally acknowledged as important but has been relatively little studied (Lewis *et al.*, 1990). Since honeydew may be both a food source and a host-location cue for aphidiid parasitoids, it provides an ideal system in which to examine how responsiveness to host-associated cues can vary in accordance with the parasitoid's dual requirements for food and hosts.

In addition to physiological state, the ability of individual parasitoids to modify their behavior as a result of experience has been identified as another major source of intraspecific variation in parasitoid foraging behavior (Lewis *et al.*, 1990). A large number of studies have now documented increases in parasitoid responses to olfactory cues following oviposition experience in association with particular odors (e.g., Drost *et al.*, 1986; Eller *et al.*, 1988; de Jong and Kaiser, 1991; Lewis and Tumlinson, 1988; McAuslane *et al.*, 1991; Vet, 1983; Vet and van Opzeeland, 1984). A few studies have also demonstrated an increase in parasitoid responsiveness to contact cues as a result of experience (e.g., Cardé and Lee, 1989; Dmoch *et al.*, 1985; Kester and Barbosa, 1991; Sheehan and Shelton, 1989; Strand and Vinson, 1982; Vet and Schoonman, 1988), although it has been suggested that behavioral plasticity (in the form of learning) may be more important with respect to long-range cues than to short-range or contact cues (Lewis *et al.*, 1990).

The effects of experience on aphidiid responses to host honeydew have been investigated in at least two species, but with differing results: while naive *A. nigripes* females showed a significantly higher response to honeydew than did females which had previously been exposed to hosts and/or honeydew (Bouchard and Cloutier, 1984), there were no significant differences between the responses of naive and those of experienced *A. rhopalosiphi* females (Budenberg, 1990). The principal objectives of the study reported here were, first, to clarify the role of hunger in the response of the aphidiid parasitoid *Lysiphlebus testaceipes* (Cresson) to host honeydew and, second, to determine whether or not previous foraging experiences could modify the parasitoid's response to either honeydew-contaminated or clean host plants.

MATERIALS AND METHODS

Insect Rearing

All insects were reared in controlled-environment rooms maintained at 25°C under a 16:8-h light:dark regime. Parasitoids were originally collected from *Aphis nerii* (Boyer de Fonscolombe) (the oleander aphid) on *Nerium oleander* (L.) but had been maintained on the bird-cherry oat aphid, *Rhopalosiphum padi* (L.), for at least 12 generations prior to testing. *R. padi* was cultured on wheat seedlings [*Triticum aestivum* L. (cultivar 'Klasic')].

Mummified aphids from the parasitoid stock culture were clipped from the plants and isolated in individual gelatin capsules (No. 0, Apothecary Products, Inc., Burnsville, MN) to await parasitoid emergence. Adult females were mated at emergence and isolated in small plastic vials for 4 to 6 h prior to testing. During this period they were denied access to both aphids and plant material but were kept supplied with dilute (approximately 20% v/v) honey solution (except in experiment 1, in which four groups of parasitoids were kept without food for 4–6 h before testing).

The Bioassay Technique

Standard bioassay units were prepared by planting wheat seeds in small (30-ml) plastic cups (Anchor Hocking Plastics Inc., St. Paul, MN). Approximately 15–20 seeds were sown per cup, and the resulting seedlings were infested with *R. padi* when approximately 1 cm tall. By the time the seedlings reached a height of 7–7.5 cm (approximately 3 days), most stems were fairly uniformly contaminated with honeydew. At this point, a single, centrally positioned seedling was selected for bioassay and the remaining plants were clipped off at soil level. All aphids and exuviae were carefully removed from the seedling with a small paint brush, and the area around the base of the plant was covered with crumpled tissue paper. A tightly fitting collar consisting of a 7.5-cm-diameter disk cut from green construction paper (Kroma No. 90, Pioneer Stationers, Inc., Los Angeles, CA) was placed around the base of the stem to provide a support for the upper portion of the bioassay unit; this consisted of a clear plastic vial (5 cm in diameter × 8 cm high) (Thornton Plastics, Salt Lake City, UT), which was inverted over the seedling to form a closed observation chamber. Clean control seedlings were prepared in the same way except that they were thoroughly washed with distilled water and allowed to dry before use. Individual parasitoids were transferred to the bioassay units via gelatin capsules and were allowed to walk onto the seedling as close to the midpoint of the plant as possible. The cover was then replaced over the seedling and the parasitoid's behavior observed for 8 min (experiment 1) or 10 min (experiments 2–4); the behavioral variables recorded are described for each individual experiment (see

below). All experiments commenced at 1300 (7 h after "lights on") and ran until 1800 (PST) (i.e., at a time when parasitoids could normally be observed foraging in the field).

Experiment 1: Influence of Parasitoid Nutritional Status and the Presence of Hosts on the Response of Naive *L. testaceipes* Females to Clean or Honeydew-Contaminated Wheat Seedlings

This experiment was conducted to test two hypotheses: first, that the parasitoid's response to honeydew is at least partially dependent on nutritional status and, second, that honeydew acts as a "contextual" cue, facilitating host recognition in naive parasitoids and resulting in more rapid host location and attack behavior.

To test these hypotheses, a 2 × 2 × 2 factorial experiment was conducted in a randomized complete block design (blocked over time) in which fed and unfed parasitoids were exposed to either honeydew-contaminated or clean wheat seedlings, with or without hosts (one second- or third-instar aphid per plant). In those treatments containing aphids, attempts were made to position the aphid as close to the midpoint of the seedling as possible, and parasitoids were released approximately one centimeter from the host. The behavior of each parasite was observed for 8 min and the following variables were recorded: (i) duration of first "visit" to the plant (i.e., the time between releasing the wasp onto the plant and the point at which she first left it), (ii) duration of subsequent visits, (iii) total number of visits, (iv) total time spent on the plant, (v) incidence of "probing" behavior (in which the parasite would attempt to strike at the surface of the plant with her ovipositor), and (vi) incidence of feeding behavior (in which the parasite lowered her mouthparts to the plant surface in a characteristic feeding posture). In those treatments which included aphids, two further variables were recorded: (i) attack latency (time between releasing the wasp onto the plant and the point at which she first attempted to oviposit) and (ii) total number of contacts between parasite and host before an attack was initiated. Any parasitoid which failed to initiate an attack within the 8-min bioassay period was discarded. Thirty parasites were tested per treatment and data were analyzed using the Statistical Analysis System (SAS Institute Inc., 1988). Prior to analysis of variance, all times were transformed using log(time + 1), while data on number of visits were transformed using square root (number + 0.5). Mean separations were conducted using Student–Newman–Keuls' test at the $\alpha = 0.05$ level. Probing and feeding behaviors were recorded as frequencies and analyzed as contingency tables using the chi-square test statistic.

Experiment 2: Effect of Experience on Parasitoid Responsiveness to Honeydew-Contaminated Plants

This experiment was conducted to determine whether or not the parasitoid's response to honeydew could be modified as a result of experience (for example, by learning to associate the presence of honeydew with the presence of hosts).

Bioassay units were prepared as above, and parasitoid responses to either clean or honeydew-contaminated seedlings were measured both before and after a series of conditioning treatments. In this and in all of the remaining experiments, the only behavioral variable recorded was the duration of the initial visit: bioassays were run for a maximum of 10 min but were terminated as soon as the parasitoid left the plant. The conditioning treatments involved exposing individual parasitoids to either (i) a honeydew-contaminated seedling with a single, feeding host aphid *in situ*; (ii) a single host aphid in a culture slide followed by a honeydew-contaminated seedling (to disrupt the predictive association between the two stimuli which is necessary for associative learning to occur); (iii) a honeydew-contaminated seedling without host aphids; (iv) a single host aphid (second-or third-instar *R. padi*) in a glass culture slide; or (v) no aphids or plants. The first conditioning trial was conducted approximately 45 min after the initial bioassay, and two further conditioning trials were conducted at hourly intervals thereafter. The final bioassay was conducted 1 h after the final conditioning trial. In those treatments involving plants, each conditioning trial lasted until the parasite left the plant (up to a maximum of 10 min), while those treatments in which a parasite was exposed to a host in a culture slide lasted 5 min. A small number of parasitoids failed to initiate an attack during the conditioning trials, and these were discarded. A total of 25 parasitoids was tested per treatment in a randomized complete block design (blocked over time). Bioassay times were transformed using log(time + 1) and analyzed by a factorial repeated-measures analysis of variance. Within-treatment tests for differences in bioassay times before and after conditioning were conducted using t tests (LSD) at the $\alpha = 0.05$ level (SAS Institute Inc., 1988).

Experiment 3: Effect of Level of Reward on Learned Responses to Honeydew-Contaminated and Clean Plants

Since the acquisition of learned responses in both vertebrates and invertebrates can be influenced by the amount, frequency, and/or quality of "reward" (Buchanan and Bitterman, 1988; Lowes and Bitterman, 1967), an experiment was conducted to determine whether quantity of reward affected the acquisition of a learned response to honeydew in *L. testaceipes*. Since aphidiids are solitary endoparasitoids and usually lay only one egg per host (Hågvar and Hofsvang, 1991), "quantity of reward" could in this case be manipulated by providing different numbers of hosts during the conditioning trials.

The effect of experience on the parasitoid's responsiveness to clean plants was also examined in this experiment, since it has been suggested that learning may be more apparent with stimuli which originally elicit little response than with stimuli which elicit much higher innate response levels (Vet *et al.*, 1990). The upward trend in responsiveness shown by parasitoids conditioned to honey-

dew-contaminated plants but tested on clean plants (experiment 2) suggested that this effect might occur in *L. testaceipes* if females were conditioned to associate clean plants with the presence of hosts. The experimental treatments were therefore set up as follows.

(A) Bioassay with honeydew-contaminated plants before and after three conditioning trials (repeated at hourly intervals) consisting of either

    (i)   exposure to a honeydew-contaminated seedling with a single, feeding host aphid *in situ* (terminated when the parasitoid left the plant or after 10 min);

    (ii) exposure to a honeydew-contaminated seedling infested with 20 host aphids (terminated when the parasitoid left the plant or after 10 min);

    (iii) exposure to a single host in a culture slide (for 5 min);

    (iv) exposure to 20 hosts in a culture slide (for 10 min), or

    (v) naive—no exposure to either hosts or plants between bioassays.

(B) Bioassay with clean plants before and after three conditioning trials consisting of essentially the same five treatments listed under A, except that clean plants were substituted for honeydew-contaminated plants. All other experimental conditions (age of parasitoids, bioassay conditions, data transformation and analysis, etc.) were as for experiment 2 except that 30 wasps were tested per treatment. As before, any parasitoid which failed to initiate attack behavior in any conditioning trial was discarded.

Experiment 4: Stability of the Conditioned Response to Clean Plants

Several definitions of learning make explicit reference to the fact that learned behavior has some degree of permanence (Miller, 1967; Kimble, 1961). An experiment was therefore conducted to determine whether or not the conditioned response to clean plants observed in experiment 3 would persist for at least 24 h after the end of the conditioning period. The experiment was not continued for a longer period since *L. testaceipes* from the laboratory colony lived on average only 3 days and laid most of their eggs in the first 2 days of adult life (Grasswitz, 1992).

In this experiment, the parasitoid's response to clean plants was assayed 1 h before and either 1 or 24 h after the following conditioning treatments: (i) exposure to a clean plant with a single, feeding host aphid *in situ*; (ii) exposure to a single host in a culture slide followed by exposure to a clean plant without hosts; (iii) exposure to a clean plant without hosts; (iv) exposure to a single host in a culture slide, or (v) naive—no exposure to either hosts or plants between bioassays. In those treatments involving plants, each conditioning trial lasted until the parasite left the plant (up to a maximum of 10 min), while those

treatments in which a parasite was exposed to a host in a culture slide lasted 5 min. Thirty parasitoids were tested per treatment, and data transformation and analysis were carried out as in experiment 2.

RESULTS

Experiment 1: Influence of Parasitoid Nutritional Status and the Presence of Hosts on the Response of Naive *L. testaceipes* Females to Clean or Honeydew-Contaminated Wheat Seedlings
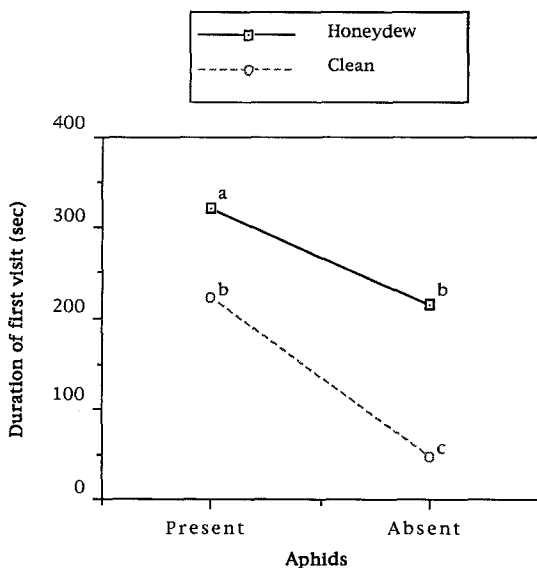
Duration of First Visit

Analysis of variance on the transformed data [log(time + 1)] showed significant main effects for honeydew ($F_{1,203}$ = 72.18, P < 0.01) and aphids ($F_{1,203}$ = 67.10, P < 0.01) and a significant honeydew × aphids interaction ($F_{1,203}$ = 25.05, P < 0.01). Since neither nutritional status nor any of the other interaction terms were significant, data from both fed and unfed parasitoids were pooled to produce a plot of the back-transformed means for the honeydew × aphids interaction (Fig. 1). In addition, because of the interaction effect, those treatments which did not include aphids were subsequently analyzed separately in order to clarify the relationship between nutritional status and the response to honeydew. The results are shown in Table I.

In the absence of aphids, initial visits were longer on honeydew-contaminated plants than on clean plants (Table I). They were also longer on both clean and honeydew-contaminated plants when aphids were present than when they were absent (Fig. 1).

Total Time on Plant

Data on the duration of first and subsequent visits were combined and analyzed as the total time spent on the bioassay plant. In this case, all three main effects were significant (nutritional status, $F_{1,203}$ = 8.65, P < 0.01; honeydew, $F_{1,203}$ = 46.67, P < 0.01; aphids, $F_{1,203}$ = 63.01, P < 0.01). Significant interactions were also obtained for honeydew × aphids ($F_{1,203}$ = 26.52, P < 0.01) and nutritional status × aphids ($F_{1,203}$ = 7.67, P < 0.01). The interaction effects are shown in Figs. 2 and 3. Again, because of these interaction terms, treatments without aphids were subsequently analyzed separately. The results (Table II) show that, in terms of the total time spent on the plant, honeydew did appear to have an arrestant effect over and above the effect due to hunger.
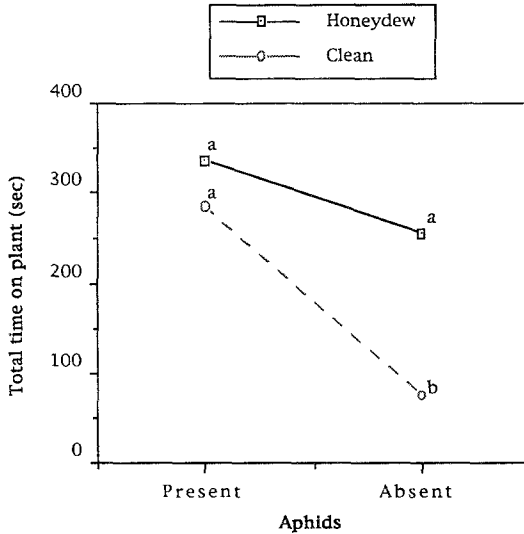
Fig. 1. Interaction between the effect of aphid presence and that of honeydew presence on the duration of the first visit to a host plant by *L. testaceipes* females (pooled data from both fed and unfed parasitoids). The means shown were back-transformed after analysis of variance on the transformed data [log(time + 1)]. Means sharing the same letter are not significantly different at the $\alpha = 0.05$ level (Student–Newman–Keuls' test).

Table I. Effects of Parasitoid Nutritional Status and the Presence or Absence of Honeydew on the Duration of the First Visit by *L. testaceipes* Females to Host Plants (Treatments Without Aphids Only)

Nutritional status	Plant treatment	Duration of 1st visit (s)[a]
Unfed	Honeydew	281.8 a
Fed	Honeydew	175.5 a
Unfed	Clean	50.6 b
Fed	Clean	42.8 b

[a] Back-transformed means (separated by Student–Newman–Keuls' test after analysis of variance on the transformed data); means followed by the same letter are not significantly different ($\alpha = 0.05$).
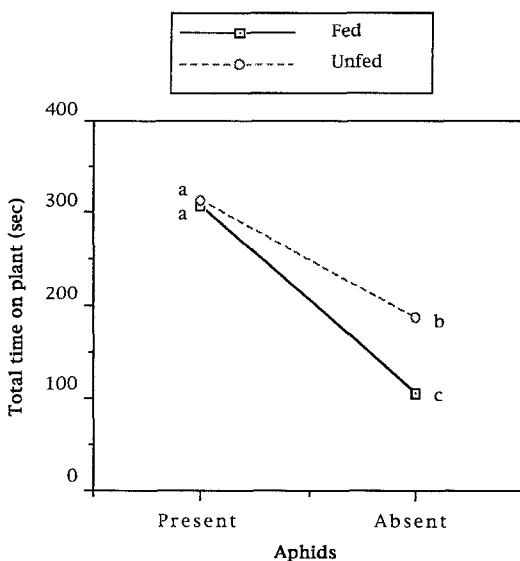
Fig. 2. Interaction between the effect of aphid presence and that of honeydew presence on the total time spent examining a host plant by *L. testaceipes* females during an 8-min bioassay. The means shown were back-transformed after analysis of variance on the transformed data [log(time + 1)]. Means sharing the same letter are not significantly different at the α = 0.05 level (Student–Newman–Keuls' test).

Total Number of Visits

Analysis of variance on the transformed data showed significant main effects of nutritional status [with unfed parasitoids making more visits ($F_{1,203} = 7.94$, $P < 0.01$)] and honeydew [with more visits being made to clean plants than to honeydew-contaminated plants ($F_{1,203} = 33.32$, $P < 0.01$)]; the interaction between nutritional status and aphids was also significant ($F_{1,203} = 6.52$, $P < 0.05$) and is shown in Fig. 4. The greatest mean number of visits was made by unfed parasitoids to clean plants without aphids (Table III). Unfed parasites tended to be more active than their fed counterparts but, when exposed to honeydew-contaminated plants, would spend a protracted time feeding (as reflected in the data for both duration of initial visit and total time on plant) (Tables I and II). When exposed to clean plants, however, the initial visit was terminated relatively rapidly (Table I), but the parasitoids would frequently reencounter and revisit the plant during the remainder of the bioassay period. Fed parasitoids, on the other hand, having once left the plant, tended to remain motionless on the sides or top of the chamber.
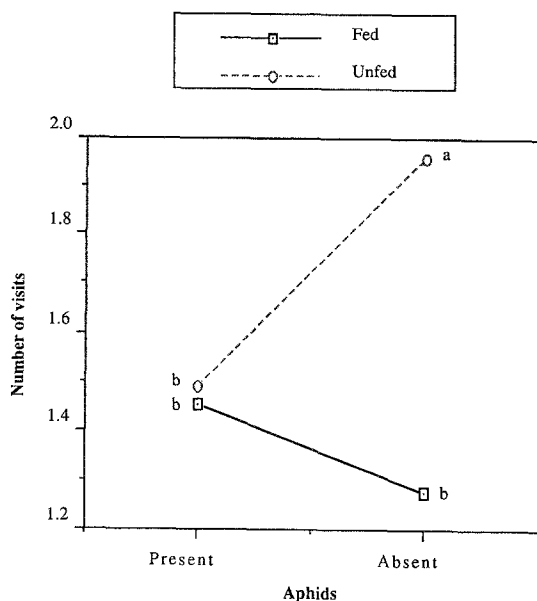
Fig. 3. Interaction between the effect of aphid presence and that of parasitoid nutritional status on the total time spent examining a host plant by *L. testaceipes* females during an 8-min bioassay. The means shown were back-transformed after analysis of variance on the transformed data [log(time + 1)]. Means sharing the same letter are not significantly different at the $\alpha = 0.05$ level (Student–Newman–Keuls' test).

Table II. Effects of Parasitoid Nutritional Status and the Presence or Absence of Honeydew on the Total Time Spent Examing Host Plants by *L. testaceipes* Females (Treatments Without Aphids Only)

Nutritional status	Plant treatment	Total time (s)[a]
Unfed	Honeydew	340.51 a
Fed	Honeydew	191.57 b
Unfed	Clean	101.82 c
Fed	Clean	57.14 d

[a] Back-transformed means (separated by Student–Newman–Keuls' test after analysis of variance on the transformed data); means followed by the same letter are not significantly different ($\alpha = 0.05$).

Fig. 4. Interaction between the effect of aphid presence and that of parasitoid nutritional status on the total number of visits made by *L. testaceipes* females to a host plant during an 8-min bioassay. The means shown were back-transformed after analysis of variance on the transformed data [log(time + 1)]. Means sharing the same letter are not significantly different at the $\alpha = 0.05$ level (Student–Newman–Keuls' test).

Table III. Effects of Honeydew, Parasitoid Nutritional Status, and Presence of Hosts on the Total Number of Visits Made to Individual Seedlings by *L. testaceipes* Females During the Course of an 8-min Bioassay

Plant treatment	Nutritional status	Aphids	Number of visits[a]
Clean	Unfed	Absent	2.698 a
Clean	Unfed	Present	1.818 b
Clean	Fed	Present	1.790 b
Clean	Fed	Absent	1.437 b
Honeydew	Unfed	Absent	1.319 b
Honeydew	Unfed	Present	1.180 b
Honeydew	Fed	Present	1.143 b
Honeydew	Fed	Absent	1.119 b

[a] Back-transformed means (separated by student–Newman–Keuls' test after analysis of variance on the transformed data); means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table IV. Influence of Parasitoid Nutritional Status on the Incidence of
Feeding Behavior Observed in *L. testaceipes* Females Exposed to
Honeydew-Contaminated or Clean Host Plants (Number in Parentheses
Are Row Percentages)

Plant treatment	Nutritional status	Feeding	Not feeding
Honeydew	Fed	11 (18.3%)	49 (81.7%)
Honeydew	Unfed	41 (68.3%)	19 (31.7%)
Clean	Fed	0 (0.0%)	60 (100%)
Clean	Unfed	3 (5.0%)	57 (95.0%)

Incidence of Probing Behavior

There was a significant association between the presence of honeydew and
the incidence of probing behavior [79% of parasitoids exposed to honeydew-
contaminated seedlings exhibited probing behavior compared to only 51% of
parasitoids exposed to clean seedlings ($\chi^2_1 = 23.485$, $P < 0.01$)]. There was
no significant association between nutritional status and probing behavior ($\chi^2_1
= 0.290$, $P > 0.5$).

Incidence of Feeding Behavior

Not surprisingly, feeding behavior was associated with both nutritional
status and the presence of honeydew (overall $\chi^2_3 = 99.514$, $P < 0.01$) (Table
IV).

Attack Behavior

For both attack latency and number of contacts, the only significant main
effect was nutritional status (latency, $F_{1,87} = 5.43$, $P < 0.05$; contacts, $F_{1,87}
= 6.30$, $P < 0.05$). Unfed parasitoids took significantly longer, and contacted
the aphids a significantly greater number of times before initiating an attack,
than did fed parasitoids (Table V).

Experiment 2: Effect of Experience on Parasitoid Responsiveness to Honeydew-Contaminated Plants

Significant main effects were obtained for test substrate (i.e., honeydew-
contaminated versus clean plants) ($F_{1,216} = 71.59$, $P < 0.01$) and time of testing
(i.e., before or after conditioning) ($F_{1,216} = 11.16$, $P < 0.01$). There was no
significant main effect due to type of conditioning treatment ($F_{4,216} = 1.94$, $P
> 0.05$), although there was a significant interaction between type of treatment
and time of testing ($F_{4,216} = 3.06$, $P < 0.05$). The results of experiment 2 are

Table V. Effect of Parasitoid Nutritional Status on Attack Latency and Number of Contacts Between Parasitoid and Host Before Initiation of an Attack[a]

Nutritional status	Mean attack latency (s)	Mean number of contacts
Unfed	28.76 a	1.99 a
Fed	15.92 b	1.38 b

[a] Back-transformed means (separated by Student–Newman–Keuls' test after analysis of variance on the transformed data); means followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

more easily understood by reference to Table VI, which shows that, with one exception, the mean response to either clean or honeydew-contaminated plants after any of the experimental treatments was less than the response obtained before conditioning. In only two cases, however, was this downward trend significant: in parasites which were both tested against honeydew-contaminated plants and subjected to repeated exposure to contaminated plants between bioassays (treatment iii) and in naive parasites which were tested against honeydew-contaminated plants but which were not subjected to any experimental manipulations between bioassays (treatment v). The only group which showed an

Table VI. Change in Response of *L. testaceipes* Females to Honeydew-Contaminated and Clean Plants Following Various Conditioning Treatments

Conditioning treatment	Test plants	Mean examination time (s)[a]	
		Before conditioning	After conditioning
Plant + aphid	Honeydew	172.9	165.2
Plant + aphid	Clean	66.9	108.0
Aphid followed by plant	Honeydew	206.1	129.1
Aphid followed by plant	Clean	59.0	39.1
Plant only	Honeydew	197.4	93.6*
Plant only	Clean	65.2	60.7
Aphid only	Honeydew	224.2	171.8
Aphid only	Clean	79.8	76.8
Naive	Honeydew	209.4	89.3*
Naive	Clean	83.0	45.5

[a] Back-transformed means after analysis of variance on the transformed data [log (time + 1)].
*Bioassay responses before and after conditioning are significantly different at the $\alpha = 0.05$ level (LSD).

Table VII. Effect of Quantity of Reward (Number of Hosts) on the Change in Response of *L. testaceipes* Females to Honeydew-Contaminated and Clean Plants Following Various Conditioning Treatments

Conditioning treatment	Test plants	Mean examination time (s)[a]	
		Before conditioning	After conditioning
Plant + single aphid	Honeydew	231.7	235.5
Plant + single aphid	Clean	80.8	156.9*
Plant + 20 aphids	Honeydew	256.1	355.8
Plant + 20 aphids	Clean	89.9	147.8
1 aphid only	Honeydew	217.7	196.1
1 aphid only	Clean	95.0	75.1
20 aphids only	Honeydew	290.5	190.7
20 aphids only	Clean	70.4	75.3
Naive	Honeydew	315.3	268.5
Naive	Clean	122.7	92.6

[a]Back-transformed means after analysis of variance on the transformed data [log (time + 1)].
*Bioassay responses before and after conditioning are significantly different at the $\alpha = 0.05$ level (LSD).

upward (though not significant) trend in responsiveness was that in which the parasites were conditioned to honeydew-contaminated plants with hosts but which were bioassayed against clean plants (treatment i).

Experiment 3: Effect of Level of Reward on Learned Responses to Honeydew-Contaminated and Clean Plants

Significant main effects were obtained for substrate ($F_{1,261} = 152.95$, $P < 0.01$) and type of treatment ($F_{4,261} = 3.59$, $P < 0.01$). There was also a significant interaction between type of treatment and time of testing ($F_{4,261} = 2.87$, $P < 0.05$). The bioassay results for each substrate/treatment combination are shown in Table VII. In none of the honeydew treatments were mean response levels after conditioning significantly different from response levels before conditioning, although parasitoids conditioned to honeydew-contaminated plants infested with 20 aphids did show an upward trend in responsiveness (in most cases, as in experiment 2, the trend was toward a reduction in response level after conditioning). Parasitoids conditioned to clean plants with a single host did, however, show a significantly higher response after training, and the same trend was observed in the group trained with clean plants infested with 20 hosts. In the latter case, however, the difference was not significant.

Table VIII. Change in Response of *L. testaceipes* Females to Clean Plants 1 or 24 h After Various Conditioning Treatments.

Conditioning treatment	Time of final bioassay (h after final conditioning trial)	Mean examination time (s)[a]	
		Before conditioning	After conditioning
Plant + aphid	1	75.5	82.9
Plant + aphid	24	55.0	116.4*
Aphid followed by plant	1	74.1	27.1*
Aphid followed by plant	24	64.2	44.4
Plant only	1	63.8	38.3
Plant only	24	75.4	53.6
Aphid only	1	65.4	44.1
Aphid only	24	78.0	76.1
Naive	1	69.5	75.6
Naive	24	55.8	55.2

[a]Back-transformed means after analysis of variance on the transformed data [log (time + 1)].
*Bioassay responses before and after conditioning are significantly different at the $\alpha = 0.05$ level (LSD).

Experiment 4: Stability of the Conditioned Response to Clean Plants

A significant main effect was obtained for time of testing (i.e., before or after conditioning) ($F_{1,261} = 5.09$, $P < 0.05$) but not for type of conditioning treatment ($F_{4,261} = 2.10$, $P > 0.05$). Significant interactions were obtained between type of treatment and time of testing ($F_{4,261} = 6.70$, $P < 0.01$) and between time of testing and period of delay between conditioning and the final bioassay (i.e., 1 or 24 h) ($F_{1,261} = 5.64$, $P < 0.05$). Parasites conditioned with aphid-infested plants showed an increase in mean response after conditioning, but the difference was significant only in females tested 24 h after conditioning (Table VIII). Parasites subjected to reverse conditioning (i.e., exposure to a host followed by exposure to a plant) showed a decline in response after conditioning, but the difference was significant only in parasites tested 1 h after conditioning.

DISCUSSION

The response of naive *L. testaceipes* females to host honeydew can be attributed partly to hunger and partly to host-seeking behavior. The principal kairomonal effect of honeydew appears to be to arrest foraging parasitoids on contaminated surfaces. Although there was a significant association between the presence of honeydew and the incidence of spontaneous (i.e., not host-directed)

probing behavior, there was no evidence to suggest that honeydew provides any kind of a contextual cue to facilitate host-finding and host-recognition in naive parasitoids. However, the shift in behavioral "priorities" from host-finding to food-seeking, which occurred as a result of several hours of starvation, was clearly seen in the increased time and number of contacts between parasites and hosts before attacks were initiated.

The results of the conditioning experiments were consistent in several respects with the predictions of Lewis *et al.* (1990), and Vet *et al.* (1990). The latter authors suggested that parasitoid responses to less preferred stimuli may be more influenced by learning than are responses to more preferred stimuli, and this does appear to be the case for the response of *L. testaceipes* to clean versus honeydew-contaminated plants. The response to the former could be significantly increased as a result of conditioning (experiments 3 and 4), whereas the response to the latter could not (experiments 2 and 3). These results are in agreement with those of Bouchard and Cloutier (1984) and Budenberg (1990), who found no increase in parasitoid response to honeydew as a result of experience. However, although the response to clean plants was more readily modified by experience, the effect produced was slight and variable: in experiment 4, parasites conditioned with aphid-infested plants and tested 24 h after conditioning showed a significant increase in response, whereas those tested 1 h after conditioning did not (in contrast to the results of experiment 3). This variability is consistent with the prediction of Vet *et al.* (1990), that weak responses will generally be more variable than strong ones, and with the observation of Lewis *et al.* (1990), that learned responses are less likely to develop in relation to short-range or contact cues than in relation to longer-range (primarily olfactory) cues. *Lysiphlebus testaceipes* is very short-lived as an adult, and, as a highly polyphagous parasite, must be able to rapidly locate diverse hosts on many different host plants. Learned responses to short-range or contact cues would be of little utility for such a species, and in fact *L. testaceipes* appears to learn long-range olfactory cues much more consistently (Grasswitz and Paine, 1993). It is possible, however, that either the number of conditioning trials in these experiments was too low or the intertrial intervals were too long for optimal acquisition of a learned response. In nature, a foraging parasitoid would be unlikely to encounter single aphids at such protracted intervals. Attempts to present a more natural situation by increasing the reward rate from 1 to 20 aphids did not, however, significantly increase the response either to clean plants or to honeydew-contaminated plants (experiment 3).

It is also possible that experience subtly altered the searching pattern of *L. testaceipes* in ways not revealed by the criterion of learning used here (i.e., a simple change in plant examination time). Ayal (1987) demonstrated that *D. rapae* uses a basic "predetermined" searching pattern when examining crucifer plants for hosts and that this basic pattern is modified in characteristic ways

according to the presence and spatial distribution of host honeydew. Similar changes in the fine-scale searching pattern of *L. testaceipes* as a result of experience may have been overlooked in these studies as a consequence of using too coarse a measure of learning. It seems likely, however, that the responsiveness of *L. testaceipes* to host honeydew is influenced to a greater extent by physiological state than by learning.

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