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BENEFICIAL ARTHROPOD BEHAVIOR MEDIATED BY AIRBORNE SEMIOCHEMICALS

I. Flight Behavior and Influence of Preflight Handling of *Microplitis croceipes* (Cresson)[1]

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Abstract—Oriented responses of *Microplitis croceipes* (Cresson) to airborne odors of actively feeding *Heliothis zea* (Boddie) larvae were observed in a flight tunnel. The behavior of *M. croceipes* prior to and during sustained, oriented flights was videotaped and analyzed in detail. Preflight exposure of the parasitoid to feces and other components of the plant-host complex were found to be vital in effective flight behavior, while maturation of the parasitoid had little effect. The increased frequency of oriented flight that resulted from preflight exposure of a plant-host complex persisted for at least 24 hr.

Key Words—Hymenoptera, Braconidae, *Microplitis croceipes*, parasitoid behavior, habitat location, *Heliothis zea*, Lepidoptera, Noctuidae, flight tunnel, female flights, preflight handling.

SERIES PREFACE[5]

Improved methods for harnessing beneficial entomophagous arthopods are considered of priority importance as a means of providing effective, safe, and eco-

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nomically sound technology for combating the losses of agricultural products to insect pests. Current approaches for using these biological control agents include: (1) importing and establishing new species of beneficial parasitoids and predators from foreign countries; (2) encouraging maximum abundance and efficiency of these beneficial arthropods in crops or other target areas by providing food, shelter, attractive odors, etc.; and (3) mass production and release of beneficial arthropods into the target areas.

A central problem underlying all approaches for biological control is the inability to ensure retention and sustained activity of the biological agents in the target area. This problem exists in the case of many pests where the beneficial insects are naturally present in high numbers and/or technology for their mass production and release is available. A prerequisite for resolving this problem is a knowledge of factors and mechanisms governing the means by which they find and attack their hosts and prey.

Trail odors from the pest insects, cues from the host plants, and other biological communication mediators are vital to the host- or prey-finding process (Lewis et al., 1982a). A considerable amount of information has begun to accumulate regarding the role of these factors. For example, substantial progress has been made toward elucidating close-range foraging behavior of entomophages mediated by contact chemicals (Weseloh, 1981). However, major gaps in knowledge exist which seriously constrain employment of biological control (Greany et al., 1984). Of particular significance is the lack of information regarding the foraging behavior mediated by volatile semiochemicals. The work of Hagen et al. (1976) on the response of *Chrysopa carnea* Stephens to volatile tryptophan by-products from honeydew of aphids, the occurrence of volatile kairomones in prey searching by predatory mites (Sabelis and Van de Baan, 1983; Sabelis and Dicke, 1985), reports of Lewis et al. (1982b, 1985) and Noldus and van Lenteren (1985) regarding the use of moth sex pheromones by *Trichogramma* spp. to locate host eggs, and various reports regarding host plant and habitat preferences by entomophages (Altieri, 1983; Vinson, 1981) demonstrate the importance of airborne semiochemicals to host and prey selection by entomophages.

Volatile allelochemicals have been shown to be an important tool for beneficial arthropods in microhabitat selection and niche segregation (Vet, 1983, Vet and van Opzeeland, 1984; Vet et al., 1984) and distant prey selection (Dicke and Groeneveld, 1985). Yet, essentially no information is available about the behavioral mechanisms involved in entomophage responses to the volatile semiochemicals. Additionally, little is known about the chemical nature, role, or interactions of components from different sources (plants and host insects) that comprise the total semiochemical system. Only a few structures of volatile allelochemicals emitted from host plants or fermentation products of host-associated frass have been elucidated (Greany et al., 1977; Dicke et al., 1984; Elzen et al., 1984).

Natural-enemy foraging behavior, as mediated by volatile semiochemicals, is the subject of a set of collaborative studies planned by J.C. van Lenteren and coworkers at the Agricultural University, Wageningen, The Netherlands, and J.H. Tumlinson, W.J. Lewis and coworkers, ARS, USDA, Gainesville, Florida, and Tifton, Georgia, respectively. These investigations will be the subject of this series of reports. The studies are designed to involve both generalist and specialist entomophages and those attacking different stages of insect pests, especially the egg and larval stages. Also, the research will involve entomophages from the European and United States agricultural ecosystems. It is hoped that these joint investigations will provide an impetus for breaking through this central barrier to biological control.

INTRODUCTION

The primary constraint in deciphering long-range host-searching behavior of entomophagous arthopods has been the lack of effective bioassay techniques. Much research has been conducted with various olfactometers, but these systems confine the parasitoids, making flight and related behavioral expressions virtually impossible (Kennedy, 1977; Vet et al., 1983). Moreover, olfactometers are inappropriate for insects that only orient to odors after initiation of flight. Previous attempts to use greenhouses or flight tunnels for behavioral response studies of parasitoids to airborne semiochemicals also have generally failed because the insects typically exhibit disoriented behavior under captive conditions.

These characteristics have prohibited bioassays of oriented flight behavior and other responses of entomophages to airborne stimuli. Gross et al. (1975) demonstrated that preflight exposure to kairomones could be used to override the tendency of *Microplitis croceipes* (Cresson) to disperse. Preflight exposure of two *Trichogramma* spp. and *M. croceipes* to the respective kairomones from moth scales and larval feces of their common host *Heliothis zea* (Boddie) increased rates of parasitization. It was postulated that the higher parasitization resulted from more consistent orientation to the host. This paper reports behavioral responses of *M. croceipes* to airborne semiochemicals in a flight tunnel, describes their flight characteristics, and evaluates the influence of preflight handling.

METHODS AND MATERIALS

Insects. Larval *H. zea* were reared on artificial diet using the method of Burton (1969). The *M. croceipes* were obtained from the Insect Biology and Population Management Research Laboratory, Tifton, Georgia. Parasitoids of both sexes were allowed to emerge and mate in acrylic cages (30 × 30 × 17 cm) at 28°C, 50–70% relative humidity, and a 16-hr photophase (Lewis and Burton, 1970). Mating occurs as soon as both sexes are present (Bryan et al. 1969; Lewis and Burton, 1970). Age of females used for experiments varied between 1 and 5 days.

Odor Source. The following kind of target was prepared to approximate the composition of cues present in a natural plant–host complex. Larvae were placed on cowpea leaves in the late afternoon the day prior to experimentation to ensure that larval feces came from pea leaves only. A water-filled vial (9.5 cm height, 6 dm diam.) that contained a cowpea leaf with an eating and defecating *H. zea* larva (plant–host complex) was placed in the middle of the intake end of the test section of the flight tunnel.

Flight Tunnel. A variable-windspeed flight tunnel was constructed to study the flight behavior of parasitic Hymenoptera (Figure 1). The tunnel has a 50 × 50-cm cross-section and is an assembly of three sections: intake, test, and exhaust, which has a venturi shape. Air enters the intake made of 6-mm-thick plywood by passing through a standard dust filter. Inside is a honeycomb of plastic soda straws (197 mm length, 6 mm diam.) packed loosely in a regular linear pattern. Two sheets of aluminum window screen (7 × 7 mesh/cm^2) hold the straws in place.

The test section is 120 cm long with a glass top and cast acrylic walls and floor, all 6 mm thick. A black and white pattern on poster board is supported underneath the floor of the test section by a sheet of cast acrylic to provide a visual reference for flying insects. Nylon mosquito netting (13 × 13 mesh/cm^2) covers both open ends of the test section. One side wall is hinged at the top to allow access to the test section; foam weather-strip tape is used to seal this portal when closed. Aluminum angle braces (2 × 2 cm) reinforce the edge joints of glass and acrylic. In addition, these braces are mounted at the ends of each section as flanges to permit fastening tunnel sections together. Overhead

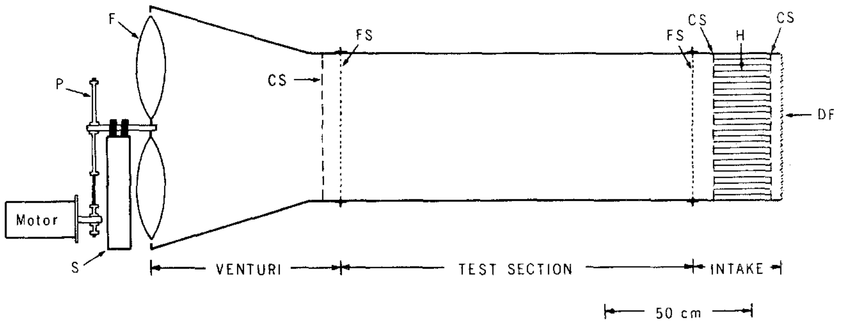

FIG. 1 Lateral section of the flight tunnel: H, honeycomb of 6-mm-diam. soda straws; CS, course screen (7 × 7 mesh/cm^2); FS, fine screen (13 × 13 mesh/cm^2); DF, dust filter; F, fan blade; and S, fan support.

lighting is provided by eight flood lamps arranged in two rows, or for video recording by two fluorescent bulbs. Light intensity in the flight tunnel varied between 3229 and 4090 lux for the flood lamps and between 2260 and 3014 lux for the fluorescent tubes.

The plywood venturi opens the cross-section from 50 × 50 cm to 80 × 80 cm over 55 cm, and internal baffles transform the section from a square to an octagon at the fan end. One sheet of aluminum window screen is mounted near the upwind end of the venturi. A three-blade aluminum fan, shrouded within 1 cm of the 76-cm-diam. blades, is powered by a variable-speed motor (model 4Z248A, 0.2 kW = $\frac{1}{4}$ hp, Dayton Electric Mfg. Co., Chicago, Illinois). The fan draws air through the intake into the test section. Screens and mosquito netting in the flight tunnel aid in achieving laminar air flow while the honeycomb removes some turbulence before air enters the test section.

Flight tunnel performance was assessed using both the smoke of a ventilation smoke tube (Mine Safety Appliance Co., Pittsburgh, Pennsylvania) and a hot-wire anemometer (model AB-27, Hastings Airmeter, Hastings Raydist, Hampton, Virginia). Air flow through the test section is approximately laminar as judged by viewing smoke plumes. Windspeed using the calibrated motor speed dial can be varied between 7 ± 1 cm/sec and 200 ± 3 cm/sec and was maintained at 23 ± 2 cm/sec, measured at the center of the flight tunnel during all experiments.

Flights were recorded on video tape from the center top with a RCA® TC 2055/C camera and a Panasonic® NV-8959 recorder. Tapes from sustained flights only were replayed in slow motion or frame by frame for analysis.

Preflight Handling. Standard exposure cups were prepared by placing one medium-sized cowpea leaf (ca. 3.45 cm^2) and one, third-instar *H. zea* larva in a 30-ml plastic cup in the late afternoon, allowing it to eat and defecate during the night. The next day, just prior to an experiment, each *M. croceipes* female was transferred with a clean 1-dram vial (6 cm height, 1.5 cm diam.) from the rearing cage and allowed to contact the material in the exposure cup during a 2-min period and sting the larva once. If a parasitoid could not find the host within 2 min, the larva was picked up carefully with forceps and presented to the female, which always resulted in a sting. If a female stung more than once, she was discarded. Immediately after this preflight exposure procedure, the parasitoid was transferred to the flight tunnel in a 1-dram vial. The vial was placed open end up at the downwind end of the flight tunnel, straight downwind from the target. Time from introduction into the tunnel until initiation of flight (latency), time from initiation of flight until landing on the target (navigation time), and type of flight were recorded for each female.

Observation time per individual was limited to a maximum of 10 min and stopped after a female landed on the target. Females that did not take off within 5 min were discarded. The number of no take-offs did not exceed 4% of the number of tested females for any experiment. For experiments in which a parasitoid was given a second flight trial immediately after landing, it was recaptured in the vial within 3 sec, before it could sting the larva. If oviposition could not be prevented, the parasitoid was excluded from the second trial and the target was replaced by one with an unparasitzed larva.

Flight Initiation and Orientation. The behavior of female *M. croceipes* exposed to the plant–host complex in the flight tunnel was observed. All females had the standard preflight exposure to a plant–host complex. Observations were restricted to the behavior expressed on the 1-dram release vial and in flight. During these observations, we defined a classification of discrete behavioral events. Relationships between these behavioral events during flights and on the 1-dram vial were described. Four categories of flight were used to evaluate the effect of different preflight exposures.

Next, characteristics of sustained flight toward a plant–host complex were determined and described. Video recordings were made only of the first flights of individuals, eight of which were used for the analysis.

Experiment 1. Flight responses to undamaged plants with and without associated infestation odors were observed. The responses of nine females provided with the standard preflight exposure were tested individually in three sequential trials. The female was recaptured after each trial and released at the introduction point for the next trial. The stimuli were given in the following order: (1) noninfested cowpea leaves, (2) noninfested cowpea leaves with the odor of a plant–host complex added, and (3) noninfested cowpea leaves again with infestation odors removed. The second treatment was achieved by placing the plant–host complex outside the flight tunnel, out of sight of the insects, at the intake end. In this way the insect perceived visual cues of the noninfested cowpea leaves in every trial, while it was exposed to additional olfactory cues of the plant–host complex only in the second.

Experiment 2. Five types of preflight exposure cups were used to determine the effect of preflight exposure to individual components of the plant–host complex on the initiation and characteristics of flight of *M. croceipes* females. In addition to the standard exposure cups containing a plant–host complex and empty control cups, these cups contained either an artificially damaged cowpea leaf, a larva, or larval feces. Leaves were artificially damaged by three pricks with a pair of forceps just prior to use so the damage would be fresh when presented to parasitoids. Larvae and larval feces were obtained from standard exposure cups and transferred to clean ones just prior to use. Latency, navigation time, and flight type were recorded for the first and second flights of each individual tested. Only females that landed on the target after the first flight were given a second trial. To determine whether maturation influenced the flight behavior of female *M. croceipes*, behavioral data of individuals with a preflight exposure to the larval feces or the plant–host complex were combined into three age groups.

Experiment 3. Retention of the effect of preflight exposure of a parasitoid to a plant–host complex was assessed. First flight responses were tested for females either 0 or 24 hr after their preflight exposure procedure.

Statistical Analysis. The number of females that exhibited a certain flight type after a given preflight exposure were analyzed with Chi-square test. Multiple comparisons of mean times spent to certain behavior were performed using the Duncan's multiple range test. A *t*-test was used when only two mean times needed to be compared. Latencies were transformed to log $(x + 1)$ to stabilize variance. The 0.05 level was set for the rejection of all null hypotheses.

RESULTS AND DISCUSSION

Description of Behavioral Responses. The behavior of female *M. croceipes* was observed from time of introduction into the flight tunnel and first contact with the wind until landing on the target. The typical sequence of the behavioral events observed during these studies was diagrammed (Figure 2). The left side of the diagram represents the preflight behavior performed on the 1-dram release vial while the right side shows the behavior performed by *M. croceipes* in flight.

Responses of the females to the odor sources were defined using the following classification of behavioral events.

1. Standing still: standing still on the substrate without moving antennae.
2. Standing still while drumming: standing still while drumming the substrate with antennae.
3. Standing still while preening: standing still while preening antennae, legs, or both sides of wings.
4. Standing still upwind oriented: standing still while sticking antennae in a vertical plane perpendicular to the wind direction.
5. Walking while drumming: Walking while drumming the substrate with antennae.
6. Initiation of flight pose: sitting on hind legs while making ''walking'' movements with front legs in the air and sometimes temporarily spreading wings (Figure 3A).
7. Initial zigzagging: making sideways excursions mainly in the horizontal plane and perpendicular to the wind direction soon after take off.
8. Straight flight: flying directly into the main wind direction.
9. Recurrent zigzagging: sideways excursions recurring after a period of straight flight. These excursions are similar to the initial zigzagging, except the movements are less restricted to the horizontal plane.

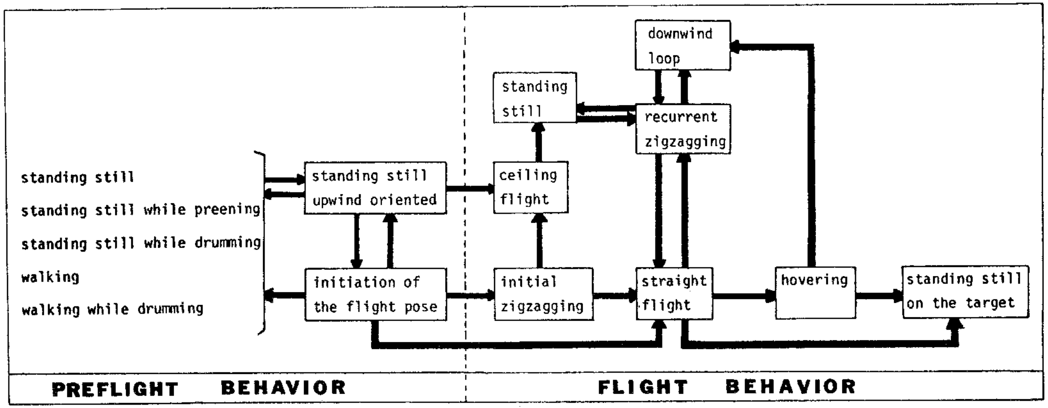

FIG. 2 Schematic representation of the behavioral events and transitions as exhibited by *Microplitis croceipes* responding to *Heliothis zea* larvae feeding on cowpeas.

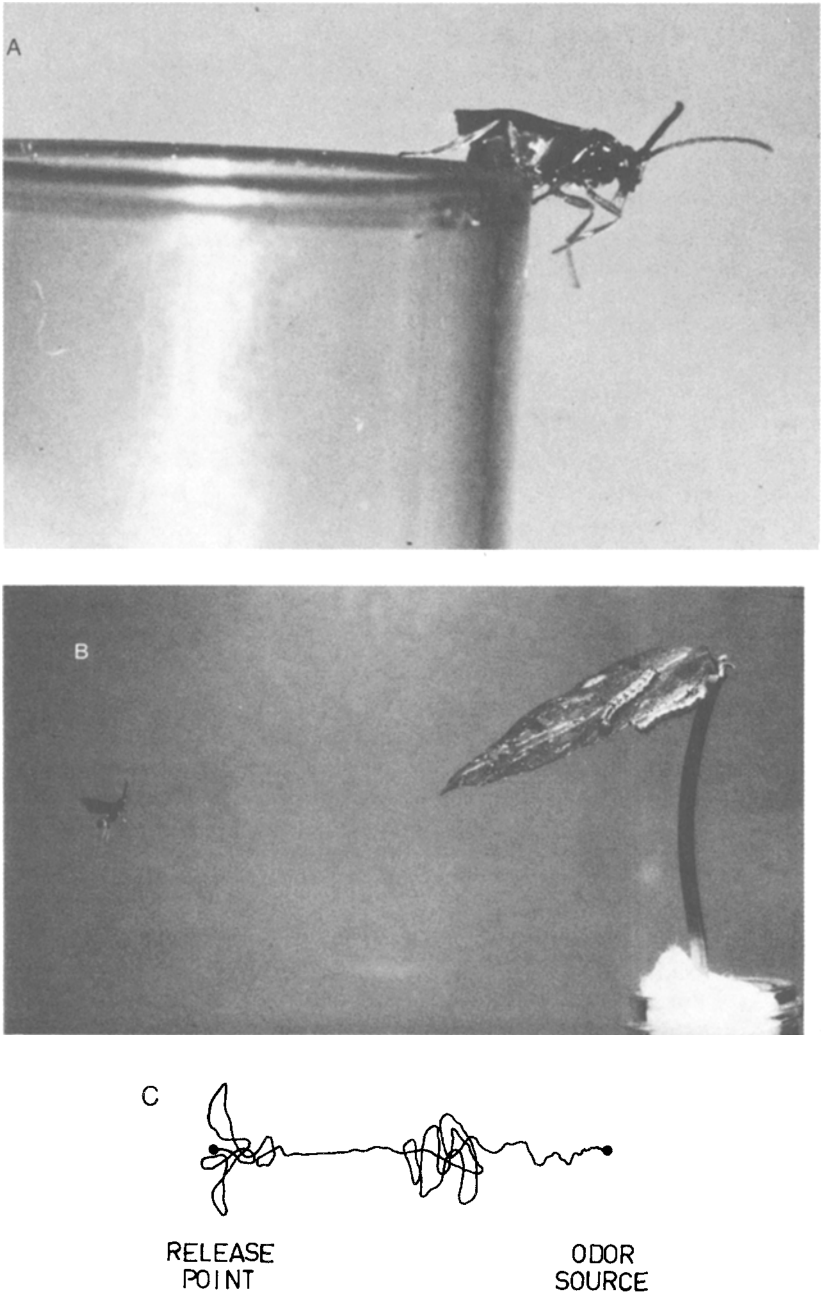

FIG. 3 Behavior of female *Microplitis croceipes* in the flight tunnel. (A) Initiation of the flight pose. (B) Hovering flight. (C) Top view of a sustained flight track.

10. Hovering: keeping stationary while flying at approximately 10 cm downwind from the target (Figure 3B).
11. Downwind loop: a looping maneuver, resulting in a return to an earlier downwind position.
12. Ceiling flight: a strong phototactic, ''nonplume-oriented'' response that results in the female being in contact with the ceiling of the flight-tunnel where she continues her wing beating.

Figure 2 represents the typical sequence of the described behaviors. Quantifications of these behaviors will be presented in subsequent reports of this series.

Preflight Behavior. Individuals that reached the open end of the 1-dram vial responded by taking an upwind oriented pose for several seconds. This action was followed either by walking, walking while drumming, or by the initiation of the flight pose. The process of intermittent upwind orientation and walking on the 1-dram vial was often repeated before flight was initiated

Flight Behavior. Four types of flight were distinguished; sustained flights, interrupted flights, temporary oriented flights, and non-target-oriented flights.

Sustained flights can involve all steps beginning with an initiation of the flight pose and ending with standing still on the target except a ceiling flight. A typical sustained flight (Figure 3C) begins with initial zigzagging, during which a little ''ground'' is lost and/or the parasitoid rises slightly above what is presumedly the odor plume. Subsequently there is a dramatic decrease in the extent of the sideways excursions followed by a straight approach toward the target. Sometimes this straight approach is interrupted by a brief recurrent zigzagging that occurred mostly at a 40-cm distance downwind from the target and consisted of sideways excursions smaller than the initial zigzagging. After resuming straight flight or continuing recurrent zigzagging, hovering takes place often at a 10-cm distance downwind from the target. From this point, quick darts were performed toward the target, ending with a landing on the target. Females allowed to fly a second time spent less time on both types of zigzagging.

Interrupted flights differ from sustained flights by the occurrence of a very wide recurrent zigzagging, which begins soon after the onset of straight flight, and sometimes include a downwind loop. These very wide zigzaggings lead to a landing on the sidewalls of the flight tunnel. Within a minute, an initiation of flight pose occurs again, after which flight continues first with a zigzagging and on to oriented flight.

Temporary oriented flights are the same as interrupted flights except that after the landing there is no further flight activity within 10 min.

Non-target-oriented flights include a ceiling flight that occurs immediately after release or after a brief initial zigzagging flight.

Experiment 1. No females had a sustained flight to undamaged plants only, compared to 89% that had one when the odor of a plant–host complex was added. Four of nine tested females flew beyond the cowpea leaves and landed on the screen at the upwind end of the flight tunnel when odor of a plant–host complex was provided behind the screen. Mean latencies of the first two trials (15.6 ± 2.8 sec ISE and 7.9 ± 3.7 sec ISE) were not significantly different. However, after response to the plant–host complex odors (second trial), there was a significantly longer mean latency in the third trial (102.0 ± 48.3 sec ± SE). Comparision of navigation times was not relevant, since sustained flights were only exhibited in second trials and not in the first and third.

Experiment 2. There are several factors in a plant–host complex that might contribute to the preflight experience of a female and hence influence the percentage of target-oriented responses in the flight tunnel. Contact with feces is known to elicit an intense searching response and initiate host-oriented flight (Lewis and Jones, 1971; Gross et al., 1975). When the female encounters a larva, she will generally oviposit. The deposition of an egg might have a different effect on searching behavior than contact with cues that give information about the presence of larvae. This experiment tested the respective effects of preflight exposures to individual components of a plant–host complex on subsequent flight behavior.

Exposures to larval feces or the full plant–host complex was the most effective preflight preparation, based on the percentage of resulting sustained flights. Preflight exposure to larval feces or a plant–host complex also significantly decreased the percentage of nontarget oriented flights (Figure 4). Stinging a larva without contacting other host-related cues did not increase the percentage of sustained flights as much as exposure to larval feces or a plant–host complex. After an *M. croceipes* female has parasitized a larva in a cup, there is usually a short postoviposition period (< 10 sec), during which the female does not search for other hosts. After this period, she continues walking and, upon encountering feces, she is reinforced to search. This reinforcement by feces apparently is important for the parasitoid to continue host searching. However, the effect of preflight exposure to a full plant–host complex reduced the percentage of non-target-oriented flights even more than feces, which suggests that the feces is not the only cue responsible for the behavioral change. Occurrences of temporary oriented flights did not differ significantly with the type of preflight exposure, whereas interrupted flights were observed only after preflight exposure to a larva, feces, or the plant–host complex.

Mean latencies of females exposed to feces or the plant–host complex were significantly longer than those of females exposed to either a damaged leaf, a larva, or nothing (control) (Table 1). This was mainly because females that were exposed to feces or the plant–host complex did not restrict themselves to an upwind orientation pose, they took off like the control group but searched

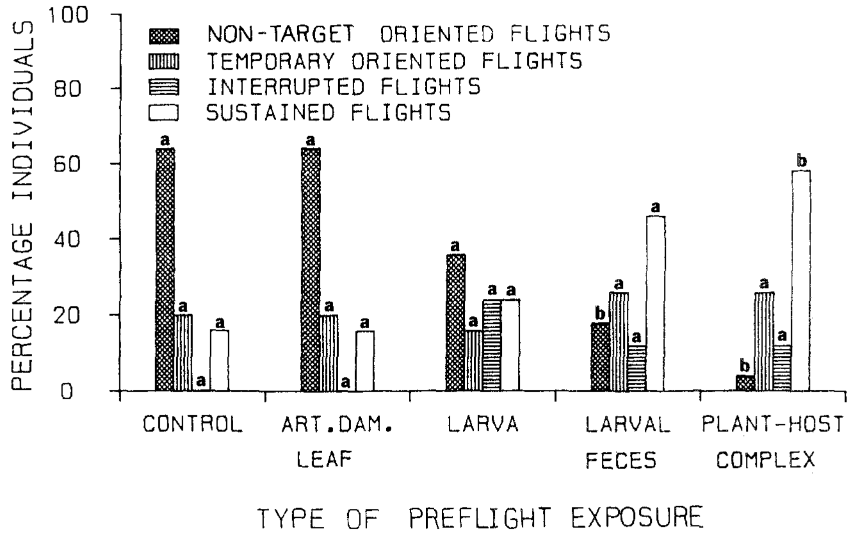

FIG. 4 Percentages of female *Microplitis croceipes* that exhibited various flight responses when provided the indicated preflight exposure. Bars within groups associated with different letters are significantly different (chi-square, $P < 0.05$).

TABLE 1. FLIGHT PERFORMANCE OF FEMALE *M. croceipes* PROVIDED VARIOUS PREFLIGHT EXPOSURES[a]

Preflight exposure	N	Sustained flights (%)	Flight characteristics: Mean latency ± SE (sec)	Flight characteristics: Mean navigation time (sec)
Control	25	16.0 a	30.6 ± 11.1 a	8.8 ± 3.2 a
Artificially damaged leaf	25	16.0 a	28.2 ± 11.7 a	10.0 ± 5.4 a
Larva	25	24.0 a	16.9 ± 3.4 a	11.0 ± 2.3 a
Larva feces	24	46.0 a	49.5 ± 12.7 b	15.9 ± 3.9 a
Plant-host complex	24	58.0 b	45.4 ± 11.8 b	12.3 ± 1.9 a

[a] Percentages (chi-square) and means (Duncan's multiple-range test) within columns not associated with the same letter differ significantly ($P < 0.05$).

the vial for a longer time. There were no significant differences in mean navigation times of females that had a sustained flight, which indicated that preflight exposure influences only the initiation of sustained flight.

If exposure to hosts and/or host-associated trail odors is vital for obtaining host-oriented responses of female *M. croceipes*, how do they find a host the first time under natural circumstances? Artificially reared parasitoids are separated from the host before they emerge and thus do not contact host cues before testing. In the field, however, *M. croceipes* emerge in close proximity to the host larva. Although these larvae are unsuitable for oviposition, they may give newly emerged female parasitoids basic information that increases their host selection efficiency significantly.

Second flights were more efficient than first flights. The percentage of sustained flights was significantly higher (74% vs. 52%) and the navigation times significantly shorter (2.6 ± 0.5 sec ± SE, $N = 31$ vs. 13.2 ± 2.1 sec ± SE, $N = 48$). Furthermore, second flights involved more straight flight than first ones. Little time was spent on initial zigzagging and only recurrent zigzagging was observed. Lewis (unpublished data) observed that *M. croceipes* females in field experiments repeatedly flew to visual structures associated with host cues, while Jones (personal communication) observed that *M. croceipes* males, when repeatedly responding to female sex pheromones, recognize visual structures in a flight tunnel. This might be a case of associative learning, and the results discussed above lend additional evidence in that direction.

Maturation did not influence the response of exposed females (Table 2). Data from females with a preflight exposure to larval feces and plant–host complex were pooled and rearranged in three age groups. There were no significant differences in the percentages of sustained flights among the three age groups.

TABLE 2. EFFECT OF AGE ON FLIGHT OF FEMALE *Microplitis croceipes*[a]

	Response per age class		
Flight characteristics	1–2 ($N = 29$)	3 ($N = 11$)	4–5 ($N = 8$)
Sustained flights (%)	68.9 a	45.5 a	75.0 a
Mean latency ± SE (sec)	50.9 ± 12.5 a	50.5 ± 15.9 a	30.9 ± 11.9 a
Mean navigation time ± SE (sec)	15.6 ± 2.8 a	8.6 ± 1.7 a	12.7 ± 2.7 a

[a] Observations of females with preflight exposure to larval feces and plant–host complex were pooled. Percentages (chi-square) and means (Duncan's multiple-range test) across columns not associated with the same letter differ significantly ($P < 0.05$).

TABLE 3. EFFECT OF 24-HR DELAY FROM PREFLIGHT EXPOSURE TO PLANT–HOST COMPLEX UNTIL TESTING ON RESPONSE OF FEMALE *Microplitis croceipes*[a]

	Preflight history and resultant response		
Flight characteristics	Control ($N = 15$)	Immediately after exposure ($N = 15$)	24 hr after exposure ($N = 15$)
Sustained flights (%)	20.0 a	73.0 b	66.7 b
Mean latency ± SE (sec)	7.6 ± 2.4 a	10.9 ± 2.3 a	10.8 ± 1.7 a
Mean navigation time ± SE (sec)	9.7 ± 1.8 a	10.1 ± 1.7 a	10.8 ± 1.7 a

[a] Percentage (chi-square) and means (Duncan's multiple-range test) across columns not associated with the same letter differ significantly ($P < 0.05$).

Experiment 3. Parasitoids exposed to a plant–host complex 24 hr before their first flight were more responsive than those that were not exposed (Table 3). This indicates that preflight exposure causes a prolonged behavioral change.

CONCLUSIONS

The results of this study demonstrate that oriented flight responses of *M. croceipes* to airborne host odors can be studied under laboratory flight tunnel conditions.

Characteristics of the behavioral responses prior to take off and during flight could be observed adequately. Thus, this system is efficient for elucidating behavioral mechanisms of parasitoids mediated by host-associated volatiles. Such experimental tests have not been developed previously for study of parasitoid host-searching behavior.

The nature of the flight responses of *M. croceipes* was strongly affected by preflight exposures to host-related materials. Prior exposure to host feces was the single most important experience for obtaining sustained flight responses to the target. However, preflight exposure to combinations of the plant–host complex was more effective than any of the single components. Influence of the preflight exposure on the flight characteristics lasts for at least 24 hr without any intermediate encounter to such materials. These findings demonstrate the importance of airborne, as well as contact, semiochemicals in the host-searching behavior of *M. croceipes* and show the value of the flight tunnel system as an approach for elucidating the mechanisms of the host-foraging behavior of *M. croceipes*.

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