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Role of Plant Volatiles in Host Location by the Specialist Parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera)

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A study was conducted to determine the primary source of volatile cues within the plant-host complex used by host-seeking free-flying female Microplitis croceipes *Cresson in flight tunnel bioassays. In single-source and two-choice tests, using wasps given an oviposition experience on either cotton* (Gossypium hirsutum) *or cowpea* (Vigna unguiculata) *seedlings damaged by corn earworm (CEW;* Helicoverpa zea *Boddie), the damaged seedlings were significantly more attractive than the CEW frass, which was in turn more attractive than the larvae themselves. In a series of two-choice wind-tunnel tests, the discriminatory ability of the wasps was examined, following various oviposition experiences. Significantly more wasps flew to plants with "old" damage than to plants with "fresh" damage, regardless of whether they had experience on fresh or old damage. In a comparison of plant species, wasps with only one experience on either host-damaged cotton or host-damaged cowpea were unable to distinguish between them, and showed no preference for either plant, whereas wasps with multiple experiences on a particular plant preferentially flew to that plant in the choice test. In comparing hosts with nonhosts, wasps successfully learned to distinguish CEW from beet armyworm (BAW;* Spodoptera exigua*) on cotton but were unable to distinguish CEW from either BAW or cabbage looper* (Trichoplusia ni) *on cowpea. The results show the important role played by plant volatiles in the location of hosts by* M. croceipes *and indicate the wasps' limitations in dis-*

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criminating among the various odors. The ecological advantages and disadvantages of this behavior are discussed.

KEY WORDS: Hymenoptera; Braconidae; *Microplitis croceipes*; cotton; cowpea; parasitoid; host location; kairomones; olfaction; induced plant responses; learning.

INTRODUCTION

The ability of parasitic Hymenoptera to locate hosts over long distances by using as cues the volatile chemicals associated with these hosts has been well documented (Weseloh, 1981; van Alphen and Vet, 1986; Nordlund *et al.*, 1988; Turlings *et al.*, 1992). *Microplitis croceipes* Cresson (Braconidae: Hymenoptera), a specialist larval endoparasitoid of the genera *Helicoverpa* and *Heliothis*, and other parasitoids can learn the odors associated with successful host location and will specifically orient to these odors during subsequent foraging flights (Lewis and Tumlinson, 1988; Vet and Groenewold, 1990; Turlings *et al.*, 1992). In nature, the range of plant volatiles in the environment of the wasp and its host could be enormous, with potentially as many variations as there are plant and herbivore species. Turlings *et al.* (1990) recently showed that the specific blend of volatiles released by corn seedlings varies with time following caterpillar damage and that certain of the released compounds occur only in response to caterpillar damage, and not to mechanical disruption alone. These induced compounds are also systemically produced by the plant, resulting in the whole plant releasing certain volatiles as a background to the blend released at the site of damage (Turlings and Tumlinson, 1992). Different species of plants, and different parts of the same plant, when combined with different species of herbivore, release different volatile blends, varying to greater or lesser degrees with each plant–host combination (McCall *et al.*, unpublished). Thus a foraging *M. croceipes* would be presented with a potentially bewildering variety of volatile blends originating from a range of plant–herbivore complexes in its environment, only some of which may be from a host. Furthermore, its hosts are highly mobile, are capable of feeding on many plant families, and can be found on different parts of an individual plant (Farrar and Bradley, 1985; Fitt, 1989). Without a well-developed mechanism, finding a host in such an environment using odor cues alone would be a formidable task. A general theory of host location by foraging parasitoids (Vet *et al.*, 1990) proposes that a wasp finds its first host by innate responses supplemented with information acquired immediately upon emergence during contact with puparium (Hérard *et al.*, 1988) and plant (Kester and Barbosa, 1991a, 1992) but that, during the first oviposition, it learns more accurately the odors associated with their host. Consequently, the

wasp adjusts and refines its response and, by preferentially orienting to these odors, may be more successful in future foraging expeditions.

How precise and accurate this ability is in *M. croceipes* is not yet known. It has been shown that the wasps can distinguish the odors from the frass of *H. zea* fed on cotton or cowpea (Eller *et al.*, 1988). Zanen and Cardé (1991) also noted some ability in distinguishing odor cues associated with hosts from cues associated with nonhosts. The purpose of the research presented here was to investigate the ability of *M. croceipes* in recognizing potential host sites in flight and how this might be improved upon by repeated learning experiences. This was achieved by determining the main source of cues from the plant–host complex (i.e., damaged plants, frass, and larvae) and by assessing the wasps' ability in differentiating the odors of various plant–host complexes. Comparisons were made between host larvae on different plant species, between fresh and old damage caused by hosts, and between hosts and nonhosts on the same plant species, using wasps given various learning experiences.

MATERIALS AND METHODS

Parasitoids

Microplitis croceipes were reared on *Helicoverpa zea* Boddie larvae at the USDA/ARS, Insect Biology and Population Research Laboratory, Tifton, Georgia, as described by Lewis and Burton (1970). Cocoons were collected 2–3 days before emergence, mailed to Gainesville, and kept in 25 × 25 × 25-cm fine mesh cages. Males were removed after 2 days and females were used for experiments at 2–4 days of age. Wasps were maintained at 26°C, 30–50% RH, and a 15-h photophase. Experiments were conducted at 6–8 h into the photophase.

Host/Nonhost Larvae

All of the larvae, both the host species, corn earworm (CEW; *H. zea*), and the nonhost species, beet armyworm (BAW; *Spodoptera exigua* Hübner) and cabbage looper (CL; *Trichoplusia ni* Hübner), were reared according to the method of King and Leppla (1984), using a pinto bean-based artificial diet. Third-instar larvae were used in all cases. The larvae were placed on the plants to be tested and allowed to feed for 20 h prior to bioassay (for "old" damage) or were starved overnight (to encourage immediate feeding) and placed on the plants 2–5 h before use (for "fresh" damage).

Plants

Bioassays were conducted using both cotton (*Gossypium hirsutum* L., var. McNair 235) and cowpea (*Vigna unguiculuta* L., var. Pink-eye, purple-hull), grown in a greenhouse in trays (35 × 50 cm) at 50 seedlings per tray. Plants

were used in experiments when 2–3 weeks old (four- to six-leaf stage). Odor sources were prepared by placing plants, cut at 1–2 cm above soil level and with the cut stems wrapped in wet cotton, in 1.5-L glass bowls, allowing larvae to feed for up to 5 h (fresh damage) or 20 h (old damage). Following damage, a quantity of larvae, leaves, and frass was removed for use as an odor source in the bioassay, and the remainder used to experience the wasps.

Flight Tunnel

All bioassays to odors were carried out in a Plexiglas wind tunnel, 60 × 60 cm in cross section and 2.4 m long, with an airflow of 0.2 m/s. During tests, the temperature in the room was maintained at 27–30°C, the humidity at 35–50% RH, and the light intensity at approximately 500 lux. Odors were released into the tunnel by blowing humidified air, at a rate of 500 ml/min, over an odor source held in a bell-shaped glass chamber, with its downwind end covered in nylon mesh (Turlings *et al.*, 1991a). This chamber was held 25 cm above the floor of the flight tunnel, and wasps were released from a glass vial, 80 cm downwind of this source in the odor plume, 25 cm above the floor. The details of this tunnel have already been described elsewhere (Eller *et al.*, 1988; Turlings *et al.*, 1991a).

Learning Experiences

For each female wasp, an experience on host larvae consisted of contact with and antennation of frass and leaf damage, followed immediately by a sting in a larva. During an experience on a nonhost larval species, wasps would walk over the damaged leaves but would normally not antennate nonhost frass or leaf damage and would rarely sting a nonhost. Each wasp was given an experience within 1 min before flying in an assay. Wasps given multiple experiences were given the prior experiences during a period of up to 4 h before the final one, and at least 1 h was allowed to elapse between each experience. In these cases the wasps were held in 0.5-L Styrofoam containers between each experience until flown. Care was taken in all cases to ensure that wasps did not repeatedly encounter the same material as either other wasps or they themselves had during earlier experiences, to prevent the wasps from learning to avoid previously visited and marked sites (Wäckers and Lewis, 1992). Wasps given no learning experience are referred to as naïve.

Bioassay Procedures

In all bioassays, each wasp was allowed three attempts to complete an oriented flight by landing on an odor source. The number of wasps that did not complete flights was also recorded. The relative positions of the odor sources

in the two-choice test were switched routinely for each pair. The numbers of completed flights from the single-source tests were compared by Duncan's new multiple-range test, following analysis of variance of angularly transformed percentages, and the results from the two-choice tests were compared using chi-square. In all tests, $P < 0.05$ was used to determine significance. The experiments were carried out as follows.

Importance of the Individual Components of the Plant–Host Complex. The attractiveness of the individual components of the plant–host complex (PHC) was compared by both single-source and two-choice tests. In the single-source tests, a total of 42 wasps was tested to each of the three components of the PHC (plants, host, or frass), on 7 different days, with three wasps tested to each of the three components for two different sets of components on each day. In the two-choice tests, four wasps were presented with a choice of a pair of components of the PHC, and all three possible combinations of pairs were tested twice on each day, on 5 separate days ($n = 40$ per pair of components). All of the wasps flown in both of these series of tests were experienced on the complete plant–host complex being tested. In both tests, the odor source being tested comprised either 1 water-washed caterpillar-damaged seedling leaf, with its cut stem wrapped in wet cotton, the frass from 3–4 damaged leaves on wet cotton, or 15 CEW larvae. These larvae were starved for 2 h prior to use to avoid frass production, and each was placed in a closed fine mesh wire tube, to prevent cannibalism. In the two-choice tests, both fresh and old damaged cotton and cowpea were tested, whereas in the single-source tests, only fresh damaged cotton and cowpea were used.

Comparing Fresh and Old Damaged Plants. The damaged plants were prepared as described above and offered in two-choice tests. Each odor source consisted of one leaf with the frass that had been produced during feeding on it and two CEW larvae. Equally damaged leaves and similar quantities of frass were used for each odor source in a pair, in all tests. In these tests, wasps were given either one or three ("multiple") experiences on either fresh or old damaged PHCs. A total of 50 wasps from each experience group was tested over 5 different days, with two different sets of odor pairs tested on each day.

Comparing Different Plant Species. The procedure for this experiment was as described in the previous section, using old damaged cotton and cowpea as the odor sources, with the experiment repeated on 4 different days.

Comparing Host- and Nonhost-Induced Plant Odors. Wasps were tested in a series of bioassays to a choice of odors from the PHC of CEW or BAW on cotton and cowpea and/or CL on cowpea. Old damaged plant–host complexes were used in all tests. Wasps were experienced on CEW (two experiences), nonhost (two experiences), CEW followed by nonhost (one experience on each), or nonhost followed by CEW (one experience on each) or were naïve. The purpose of the experiences on the nonhosts was to determine whether the wasps'

discriminatory ability would be enhanced by a "negative experience." A total of 40 wasps from each experience group was tested to each pair of odor sources, with two different sets of sources tested on each of 4 different days.

RESULTS

The Importance of the Individual Components of the Plant–Host Complex

All of the experiments investigating the relative importance of the individual components of the plant–host complex showed that the damaged plants were the most attractive, frass was less attractive, and the larvae themselves were relatively unattractive. This was found in all cases examined, regardless of whether fresh or old damaged plant–host complexes were tested. In the single-source tests, ANOVA showed a significant difference between treatments (cotton $F = 63.67$, 2, 8 df, $P < 0.0001$; cowpea $F = 88.8$, 2,8 df, $P < 0.0001$) (Fig. 1). The highest numbers of flights were to the plant odors (cotton, 86%; cowpea, 86%) and these were significantly different from the completion rates to frass in the cotton experiment (69%) but not in the case of cowpea (76%).

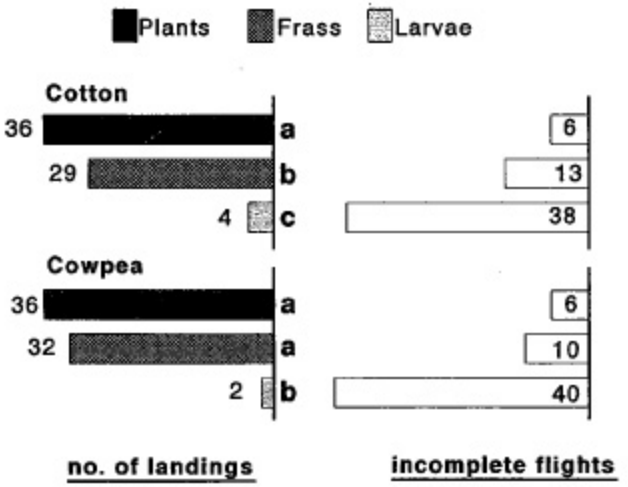

Fig. 1. Flight responses of female *M. croceipes* to the odors of individual components of the plant–host complexes of old damaged cotton and cowpea, in single-source flight-tunnel bioassays. The shaded bars indicate the numbers of wasps which landed on each odor source, and the open bars show those which did not land. Letters by each bar indicate significant differences in the attractiveness of each source (Duncan's multiple-range test, after analysis of variance, $P < 0.05$).

Very few wasps oriented to the odors from larvae (cotton, 9.5%; cowpea, 5%). In the two-choice tests (Fig. 2), the same ranking was even more clearly seen. Here, in a choice situation, plants were found to be significantly more attractive than frass in all cases tested. Wasps rarely landed on larvae when other odors were present.

Discrimination of Fresh and Old Plant Damage

Variation in learning experience did not appear to influence greatly the wasps' responses to fresh or old damage, since in all groups tested there was a tendency to orient to the odors from the plants with older damage, regardless

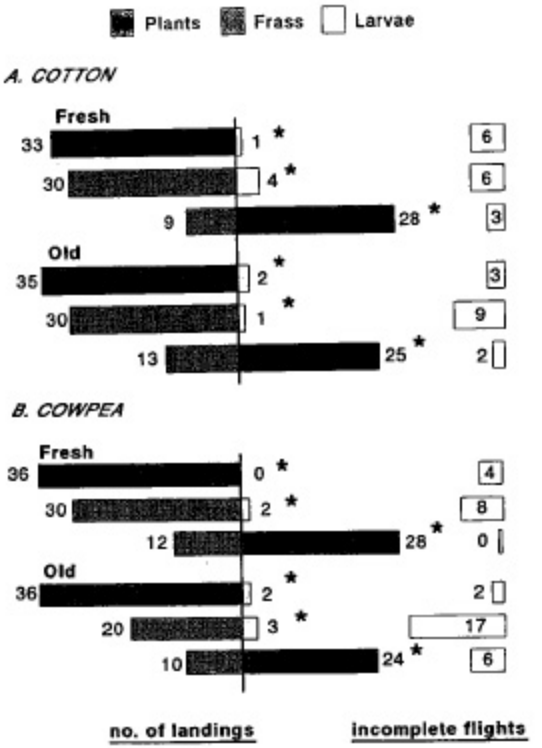

Fig. 2. Flight responses of *M. croceipes* to the odors of individual components of the plant–host complexes of fresh or old damaged cowpea (A) or cotton (B), in two-choice flight-tunnel bioassays. The shaded bars indicate the numbers landing on each odor source and the open bars the wasps which did not land, for each test. Asterisks indicate significant differences in preference within each pair of odors (χ^2, 1 df, $P < 0.05$, $n = 50$).

of experience (Fig. 3). Although the order of magnitude of this difference was similar in all groups, it was found to be significant only in the group with multiple old experiences. However, the overall trend of the total number of wasps was also significant.

Discrimination of Different Plant Species

Here, multiple experiences greatly increased the wasps ability to discriminate between the odors tested (Fig. 4). Wasps with single experiences on either cotton or cowpea were as likely to fly to either odor source, whereas wasps with multiple experiences showed a significant preference for the odor on which they were experienced.

Recognition of Host-Specific Plant Damage

Wasps showed no consistency in discriminating host-damaged plants from plants damaged by nonhosts (Fig. 5). Only one group, wasps experienced on CEW that were given a choice between BAW and CEW on cotton showed a significant preference for host-damage (Fig. 5.1, group A). In contrast, signif-

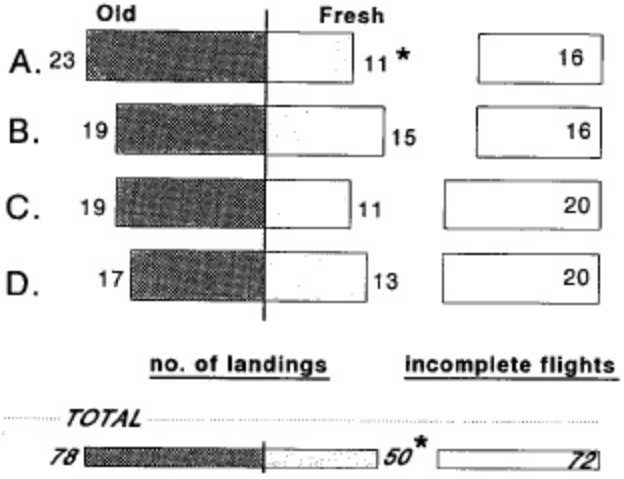

Fig. 3. Flight responses of *M. croceipes* to odors from the complete plant–host complexes of cotton, when given a choice between fresh and old damaged leaves. The shaded bars indicate the numbers landing on each odor source and the open bars the wasps which did not land, for each test. The letters indicate the four experience groups tested: A, three experiences on old damaged leaves; B, three experiences on fresh damaged leaves; C, one experience on old damaged leaves; and D, one experience on fresh damaged leaves. Asterisks indicate significant differences in preference within each pair of odors (χ^2, 1 df, $P < 0.05$, $n = 40$).

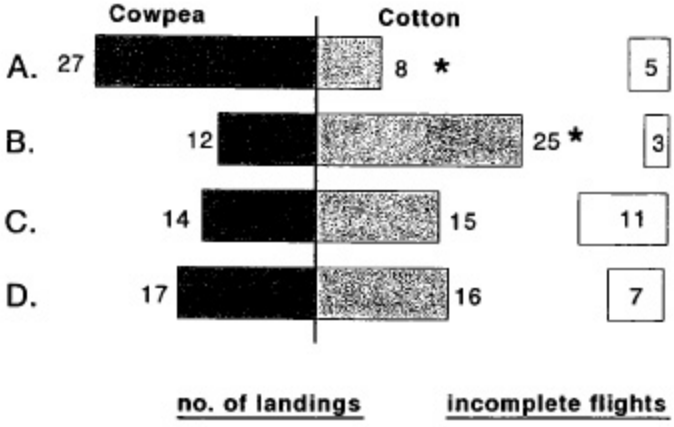

Fig. 4. Flight responses of *M. croceipes* to odors from the complete plant-host complexes of CEW on cotton or cowpea in a two-choice test. The shaded bars indicate the numbers landing on each odor source and the open bars the wasps which did not land, for each test. The letters indicate the four experience groups tested: A, three experiences on cowpea; B, three experiences on cotton; C, one experience on cowpea; and D, one experience on cotton. Asterisks indicate significant differences in preference within each pair of odors (χ^2, 1 df, $P < 0.05$, $n = 40$).

icantly more wasps with the same experience flew to nonhosts when given a choice between CEW and BAW on cowpea (Fig. 5.2, group B). It was noticed that despite efforts at equalizing the odor sources, CEW fed better than BAW on cotton, while the opposite was true on cowpea. Thus it seemed that the wasps may have been flying to the source emitting the most volatiles regardless of its origin. In comparing CL and CEW on cowpea, no preference was noted except where experience on CL was followed by experience on CEW (Fig. 5.3, group C; $\chi^2 = 3.9$, $P = 0.048$). This preference was not seen in wasps in similar experience groups in the other tests. Wasps given experience on nonhosts only (group B) did not show any increased flight responses over wasps with no experience (group E). Overall, the level of response was greater in wasps given experiences with CEW alone.

DISCUSSION

These results illustrate the importance of plant odors to foraging *M. croceipes* and concur with the role of plant odors in host location for other parasitoids (Nordlund *et al.*, 1988; Elzen *et al.*, 1983; Turlings *et al.*, 1990, 1991a). However, Eller *et al.* (1988), also working with *M. croceipes*, reported that of the two attractive components of the plant-host complex, plant odors and larval

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