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Discrimination of Previously Searched, Host-Free Sites by *Microplitis croceipes* (Hymenoptera: Braconidae)

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The ability of free-ranging parasitoids to discriminate between previously visited and unvisited sites containing host kairomone (caterpillar frass) but not hosts was tested. Females of Microplitis croceipes, *a host specialist and plant generalist larval parasitoid of* Helicoverpa (Heliothis) zea, *were allowed to fly freely in a simulated plant patch in a flight chamber. Wasps spent less time searching frass sites previously searched by themselves or by conspecifics than unsearched frass sites. In addition to chemical marking, spatial memory of visual cues was implicated as a mechanism for discriminating against self-visited, host-free sites.*

KEY WORDS: parasitoid; frass; site discrimination; foraging; olfactory; visual; *Microplitis croceipes; Helicoverpa zea.*

INTRODUCTION

Efficient foragers must be able to differentiate profitable from unprofitable foraging sites. For foraging primary parasitoids, depositing eggs in parasitized hosts is generally less profitable because those eggs typically have a low probability of survival (Bakker *et al.*, 1985). Many insect parasitoids discriminate parasitized from unparasitized hosts and thus avoid superparasitism (van Lenteren, 1981). Wäckers and Lewis (1992) showed that *Microplitis croceipes* females,

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the subject of the present study, avoid previously stung hosts and discriminate in flight between self-parasitized and conspecific-parasitized hosts.

Discrimination against previously visited sites ("site discrimination") has received less attention than discrimination against previously stung hosts ("host discrimination"). It is well-known that parasitoids are attracted to or arrested by host-produced kairomones (see review by Vinson, 1984). However, few studies have examined parasitoid searching behavior at sites that contain host kairomones but not hosts ("host-free sites"). Searching parasitoids must frequently encounter host-free sites in nature because mobile hosts often move away from feeding sites (Heinrich, 1979) and any host can be removed by predators. It should also be adaptive to recognize previously searched sites, if hosts are at all concealed, to avoid wasting time refinding them (Roitberg and Mangel, 1988).

Several authors have demonstrated that parasitoids can discriminate between unvisited host feeding sites and host-free sites searched previously by themselves (Price, 1970; Sugimoto *et al.*, 1986), by conspecifics (Salt, 1937; De Bach, 1944; Greany and Oatman, 1972; Waage, 1979; Galis and van Alphen, 1981; Sugimoto *et al.*, 1986), or by congenerics (Price, 1970) and that time spent searching a site increases with increasing concentration of host kairomone (Waage, 1979; Galis and van Alphen, 1981; Dicke *et al.*, 1985). To date, studies have examined only behavior of walking parasitoids searching for concealed hosts in small, artificial arenas where successful parasitism had occurred, and none has examined visual discrimination of host-free sites. van Giessen *et al.* (1992) showed that recent (within 10 min) oviposition experience affects the propensity of *M. croceipes* to fly to a previously visited point odor source (hexane frass extract on filter paper) in a flight chamber. They found that wasps were less likely to fly to a fresh odor source if they had oviposited at that site on a prior visit than if they had only contacted frass at that site on a prior visit.

In this paper we examine the role of both visual and olfactory cues in discriminating previously searched sites by *M. croceipes* females foraging freely in an experimental patch. Sites contained a natural host kairomone [frass of larval *Helicoverpa (Heliothis) zea*] but no host. Both frass and feeding damage are known to contain semiochemicals that act as strong attractants or arrestants for *M. croceipes* (Drost *et al.*, 1986; Lewis and Tumlinson, 1988; Eller *et al.*, 1988; W. Sheehan, unpublished data; P. McCall, unpublished data). We observed wasps searching in a patch of cotton plants in a flight chamber and recorded searching duration at frass sites. We used protocols developed by Wäckers and Lewis (1992) to answer the following questions: (1) Do wasps discriminate against previously visited frass sites in the absence of hosts ("self site discrimination")? (2) Do wasps discriminate against frass sites previously visited by conspecifics ("conspecific site discrimination")? and (3) Is site discrimination based on visual cues, olfactory cues, or both ("discrimination cues")?

MATERIALS AND METHODS

Microplitis croceipes were reared from diet-fed *H. zea* larvae as described by Lewis and Burton (1970). Parasitoids were kept, with honey and water, in Plexiglas cages (30 × 30 × 17 cm) at 28°C, 50–70% RH, and a 16:8 L:D photoperiod. Three-day-old, mated females, inexperienced with plants, hosts, or frass, were used in all experiments. Frass was collected before experiments from fifth-instar *H. zea* fed on cotton leaves. Cotton plants (*Gossypium hirsutum* var. McNair 235) used for both frass production and experiments were grown in plastic pots (10-cm diameter) in a greenhouse. Plants about 30 cm high (five to seven leaves) were used in experiments.

The flight chamber used was similar to, but larger than, that described by Drost *et al.* (1986). Air was pushed at 31 ± 2 cm/s through a chamber 2 m in length and 0.75 × 0.75 m in cross section. Temperature and RH were ambient (24–28°C and 30–70%, respectively).

A plant patch was created in the flight chamber by placing 8 or 11 plants in the test section. Foraging sites on plants were simulated by placing three pellets of frass (approx. 25 mg) on small (4 × 2.5 cm) squares of paper. The targets were pinned to leaves. In all experiments eight plants each had a single frass target placed on an upper leaf. In two experiments we included three additional plants, each with a target having frass and a third-instar larva pinned to the upper portion of the paper, to prolong wasp searching time.

Parasitoids were allowed to antennate three pellets of frass until they started walking away (usually 2–5 min) before being released in the flight chamber. Exposure to frass puts the parasitoid in a searching mode (Drost *et al.*, 1986; Lewis and Tumlinson, 1988).

Data Recording. Behavior observed in the flight chamber was recorded on a TRS 80 Model 100 portable computer, using The Observer software (Noldus Information Technology, Vadaring 51, 6702 EA Wageningen, The Netherlands). Responses measured and logged in real time included time spent searching on individual plants and targets, frequency of hovering downwind of targets, and ovipositions.

"Search time" by *M. croceipes* included all time spent on a plant between first landing and first departure. To avoid ambiguity in determining a single searching bout, return visits to the same plant were not counted until a different plant had been visited. Hovering was defined as relatively stationary flight within 2 cm of a target. A hover not followed by a landing was considered a "rejection." We recorded behavior until all plants had been visited or until the wasp left the plant patch for 2 min (usually by flying to the ceiling).

Self Site Discrimination. To test the hypothesis that wasps spend less time searching previously self-searched than unsearched frass sites, we observed individual wasps foraging in an eight-plant patch. After being exposed to frass, wasps were released individually into the flight chamber at the downwind end. In both this and the following experiment, only searches that occurred on plants after the first three consecutive plants encountered were considered for analysis, since initial searches were highly variable and often lengthy (2–15 min) as wasps gained experience searching. All targets were renewed before each of 10 wasps was tested. Search times were compared with a t test.

Conspecific Site Discrimination. The experimental design used to test the hypothesis that wasps discriminate against frass sites visited by conspecifics was similar to that used in the self site discrimination study. Here, however, two wasps were released simultaneously, and individual observers recorded the behavior of each wasp (the wasps almost never encountered each other). We also added 3 plants, each with frass plus a larva on a target, for a total of 11 plants, in an attempt to increase foraging time, and we recorded ovipositions. A total of 167 search times was recorded from 14 pairs of wasps.

The data were analyzed first by analysis of variance, using PROC GLM in the SAS statistical package (SAS Institute, 1985). We hypothesized that search times might vary as a function of patch time and oviposition history. We therefore modeled cumulative patch time, time since oviposition, and number of previous ovipositions as covariates with visitation category (self-visited, conspecific-visited, and unvisited) to check for bias and to reduce variance. For each covariate the analysis was centered at the mean. Since variances associated with treatment means were unequal, and transformation did not stabilize variances, final comparisons were made with t tests.

Discrimination Cues. To determine whether discrimination is based on vision or olfaction, we again used eight plants with frass-only sites and three plants with frass-plus-larva sites to increase total searching time. In this experiment a parasitoid was allowed to search half (four) of the frass-only targets, after which she was recaptured and two visited targets were switched randomly with two unvisited targets (plants were not moved) (cf. Wäckers and Lewis, 1992). This resulted in two unvisited targets on visited plants, two visited targets on unvisited plants, two unvisited targets on unvisited plants, and two visited targets on visited plants. We then rereleased the wasp ($N = 14$) in the patch and recorded behavior as above (except that we excluded cleaning and inactive behaviors from "searching time"). We predicted that greater reliance on olfactory than on visual information in discrimination would result in short search times on visited targets on unvisited plants (assuming that targets were visually uniform), and that greater reliance on visual information would result in shorter search times on visited plants, regardless of target. Data were analyzed by analysis of variance on log-transformed search times. Means were separated with Fisher's protected least significant difference test.

RESULTS

Self Site Discrimination. The 10 wasps tested made 38 first visits to frass targets and 38 repeat visits. Wasps discriminated against previously visited targets, since repeat visits were significantly shorter than first visits [11.5 ± 1.8 (SE) and 32.8 ± 4.4 s, respectively; $P < 0.0001$]. Previously visited targets were also rejected in flight (i.e., wasps hovered within 2 cm but did not land on targets) more often (27 times) than newly encountered targets (once). Seven of the 10 wasps rejected such targets at least once.

Conspecific Site Discrimination. Search times on previously unvisited targets (59.4 ± 8.8 s; $N = 66$) were longer than searches on conspecific-visited targets (19.2 ± 3.4 s; $N = 39$; $P < 0.0001$), and searches on conspecific-visited targets were longer than on self-visited targets (8.5 ± 1.4 s; $N = 62$; $P < 0.001$) (Fig. 1). Patch time was insignificant as a covariate with search time within treatments ($F = 0.12$; df = 3,161; $P > 0.95$), meaning that wasps did

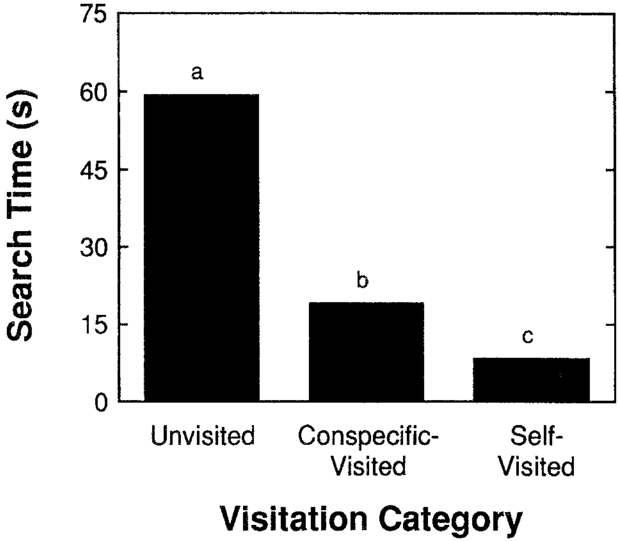

Fig. 1. Average search time by *M. croceipes* females at sites with frass from fifth-instar *H. zea* caterpillars. Sites had not been visited previously ("unvisited"), had recently been visited by a conspecific wasp ("Conspecific-visited"), or had recently been visited by the same or a conspecific wasp ("self-visited"). Means were separated with *t* tests (denoted by different letters above bars; $P < 0.05$) since transformation did not equalize variances.

not significantly reduce or increase site searching time the longer they foraged in the flight chamber. Likewise, search time was independent of time since oviposition ($F = 0.25$; df = 3,161; $P > 0.85$) and number of ovipositions ($F = 0.03$; df = 2,161; $P > 0.95$).

Discrimination Cues. Olfactory and, to a lesser extent, visual cues were used by *M. croceipes* females to discriminate between visited and unvisited sites (Fig. 2). Visited targets were searched less than unvisited targets both on unvisited plants (VU < UU; $P < 0.02$) and on visited plants (VV < UV; $P < 0.001$), thus suggesting responses to chemical marks. Visited targets were searched less on visited than on unvisited plants (VV < VU; $P < 0.01$), thus suggesting visual discrimination by parasitoids. There was also a nonsignificant trend for unvisited targets to be searched less on visited than on unvisited plants (UV < UU; $P < 0.07$). If rejections are included as null times, the latter difference is significant ($P < 0.05$). Wasps rejected visited targets on visited plants nine times, visited targets on unvisited plants once, and did not reject the other two target–plant combinations.

DISCUSSION

We have shown that free-flying wasps can discriminate between visited and unvisited sites in a simulated plant patch even in the absence of hosts. Such behavior should prevent wasps from wasting time and energy searching less profitable, already-searched sites (Price, 1970; Sugimoto *et al.*, 1986).

Odor marking appears to be involved in foraging site discrimination by *M. croceipes*. The reduction in time spent by conspecifics searching sites previously searched by other wasps (Fig. 1) strongly implies an olfactory mechanism, since wasps arriving later would not have seen the first wasps. Furthermore, the reduced search time of visited targets on unvisited plants (Fig. 2) also implicates olfactory cues. Odor marking has been shown to be used in host discrimination by *M. croceipes* (F. L. Wäckers, unpublished data); whether the same chemicals and means of detection are used in site discrimination remains to be tested.

Visual cues also appear to be used by *M. croceipes* in site discrimination, since wasps spent less time searching visited targets on visited than on unvisited plants (Fig. 2). We cannot entirely rule out chemical marking of the leaf surface beyond the target, but this cannot explain cases where wasps searched the target only during a first visit and that target was subsequently replaced with a fresh target ($N = 5$). In all such cases, searching time was significantly less compared with search time on unsearched targets on unsearched plants. This suggests that spatial memory of visual cues may be involved. Furthermore, probing (reflexing the abdomen toward the substrate while walking slowly), which may be the act of chemical marking, was restricted to frass placed on the paper targets. However, not all wasps probed, and those that did not still elicited a reduction in

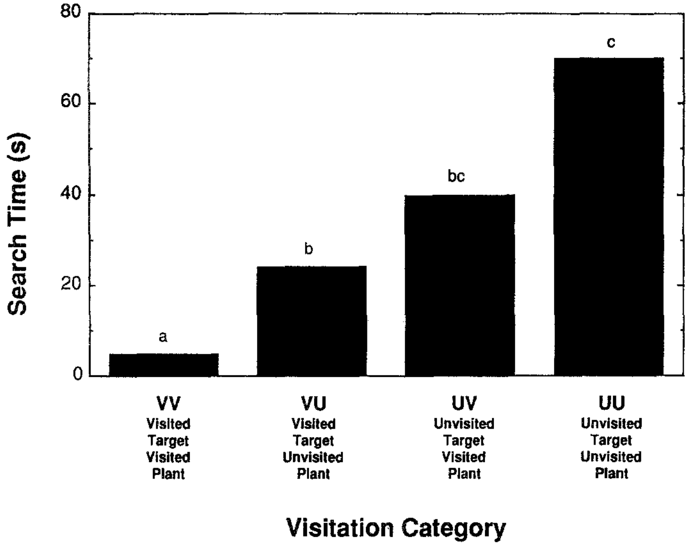

Fig. 2. Average search time by *M. croceipes* females on *H. zea* frass sites. Sites were visited and switched to an unvisited plant (VU; $N = 20$), unvisited and switched to an visited plant (UV; $N = 17$), visited and not switched (VV; $N = 20$), or unvisited and not switched (UU; $N = 9$). Bars with different letters above are significantly different at $P < 0.05$, based on an analysis of log-transformed values.

subsequent search time by conspecifics (suggesting chemical marking). Tarsal contact may be implicated, as in *Trichogramma* (Salt, 1937).

The role of vision has been examined in the context of learning different kinds of foraging sites (Arthur, 1967; Wardle and Borden, 1990), but visual aspects of discrimination have seldom been examined for parasitoid wasps other than *M. croceipes* (see also van Giessen *et al.*, 1992; Wäckers and Lewis, 1992). Sugimoto *et al.* (1986) dismissed a possible role for memory in site discrimination by *Dapsilarthra rufiventris*, a parasitoid of leaf miners, because wasps discriminated equally against self-visited and conspecific-visited sites. However, in this study wasps discriminated more against self-visited targets left in place than self-visited targets switched to an unvisited plant (VV and VU, respectively, in Fig. 2). One difference from the experiments of Sugimoto *et al.* (1986) is that they put wasps directly on leaves (previously infested), whereas we allowed wasps to forage freely among whole plants. Odor cues may well be of primary importance for many parasitoids at some levels of host finding, but other sensory modalities cannot be dismissed without testing in environments, such as flight chambers, where insects can move about freely.

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