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Olfactory Responses of *Anaphes iole* (Hymenoptera: Mymaridae) to Volatile Signals Derived from Host Habitats

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Anaphes iole Girault is a frequent parasitoid of Lygus spp. eggs in the United States, and has potential as a biological control agent against Lygus hesperus Knight in different crops. Feeding and oviposition by L. hesperus induce emission of plant volatiles, but studies to date do not address the role of plant volatiles in the host-searching behavior of A. iole. In this study, a four-arm olfactometer was used to test the responses of female parasitoids to odors emanating from cotton (Gossypium hirsutum L., Malvaceae) plants damaged by L. hesperus females, L. hesperus males, larvae of the nonhost Spodoptera exigua Hubner, or mechanically, or to odors from L. hesperus females alone. In addition, various plants damaged by L. hesperus females were evaluated in the olfactometer: cotton, alfalfa (Medicago sativa L., Fabaceae), common groundsel (Senecio vulgaris L., Asteraceae), annual ragweed (Ambrosia artemisifolia L., Asteraceae), and redroot pigweed (Amaranthus retroflexus L., Amaranthaceae). In all olfactometry bioassays, treatment odors were compared against three controls (humidified air). Results showed that A. iole females were consistently attracted to odors derived from different plant–L. hesperus complexes, while odors from plants subjected to nonhost (S. exigua) or mechanical damage and L. hesperus females alone were not attractive or only variably attractive. These findings suggest that while searching for hosts A. iole females use specific volatiles induced

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by L. hesperus feeding and oviposition to locate hosts inhabiting a wide variety of plants, including annual and perennial species from four plant families. It was suggested that future research should seek to identify the chemical elicitors involved in the release of plant volatiles attractive to A. iole females.

KEY WORDS: plant volatiles; host-habitat location; olfactometer; *Lygus hesperus*; egg parasitoids; biological control.

INTRODUCTION

Herbivore feeding induces emission of a blend of volatiles that are known, in many cases, to attract natural enemies (Turlings *et al.*, 1995; Dicke, 1999). To date, most studies on host-habitat location focus on larval parasitoids of lepidopteran pests. For example, females of the solitary endoparasitoid *Cotesia marginiventris* Cresson were strongly attracted to odors derived from maize seedlings damaged by *Spodoptera littoralis* Boisduval and *S. exigua* Hubner larvae (Turlings *et al.*, 1990a, 1991; Fritzsche-Hoballah *et al.*, 2002). In addition to feeding by herbivorous arthropods, egg deposition may also induce emission of volatiles attractive to egg parasitoids. Feeding and oviposition of the elm beetle, *Xanthogaleruca luteala* Muller, induces the field elm, *Ulmus minor* Miller, to emit volatiles attractive to the egg parasitoid *Oomyzus gallerucae* Fonscolombe (Meiners *et al.*, 2000; Meiners and Hilker, 2000). Similarly, odors from *Finns sylvestris* L. twigs laden with pine sawfly *Diprion pini* L. egg masses attract the egg parasitoid *Chrysonotomyia ruforum* Krausse (Hilker *et al.*, 2002). Finally, the egg parasitoid *Trissolcus basalts* Wollaston was attracted to odors derived from leaves of *Vicia faba* L. laden with eggs of *Nezara viridula* L. (Colazza *et al.*, 2001, 2004).

Anaphes iole Girault (Hymenoptera: Mymaridae) is the most common parasitoid of *Lygus* (Hemiptera: Miridae) eggs in the United States (Clancy and Pierce, 1966; Schuster, 1987). This parasitoid attacks *Lygus* eggs in a variety of crops, though egg parasitism rates vary with host plant species (Graham *et al.*, 1986). In field studies in Arizona, only *A. iole* was recovered from *Lygus* eggs in various plant species sampled, and rates of parasitism were in the range 0–100% (Jackson and Graham, 1983; Graham *et al.*, 1986). *Lygus hesperus* Knight is an important pest of different crops including cotton *Gossypium hirsutum* L. and alfalfa *Medicago sativa* L. in the western United States (Clancy, 1968; Jackson and Graham, 1983). It is polyphagous, feeding on numerous cultivated and uncultivated plant species (Scott, 1977). In early spring, *L. hesperus* populations are mainly found on flowering weeds and volunteer alfalfa; as these plants senesce or are harvested, *Lygus* migrate to nearby susceptible crops such as cotton

(Fye, 1980; Anderson and Schuster, 1983; Barlow *et al*., 1999). Thus, weedy host plants play important roles in the population dynamics *of Lygus* populations in crop fields, and proper weed management may contribute significantly to *Lygus* management.

Rodriguez-Saona *et al.* (2002) showed that adults of both genders and nymphs of *L. hesperus* induced emission of volatiles from both cotton and maize plants. Specifically, *L. hesperus* feeding *per se* was the prominent factor inducing emission of nonconstitutive volatiles by cotton plants. In addition, *L. hesperus* feeding induced emission of volatile blends similar to those induced by chewing caterpillars (Paré and Tumlinson, 1997a,b). Conti *et al.* (1996, 1997) demonstrated that chemicals derived from both host adults and eggs, and physical properties of protruding eggs are used as host recognition and acceptance cues by *A. iole.* However, no studies have addressed the influence of plant volatiles as cues used in host habitat location by this egg parasitoid. The goal of this study was to address the following questions via olfactometry bioassays: Are *A. iole* females attracted to odors emanating from its host's habitat, such as adult host odors or volatiles derived from plant–host complexes? Are *A. iole* females attracted to plant volatiles released following feeding by nonhost insects? And, are the responses of *A. iole* to plant volatiles affected by plant species?

MATERIALS AND METHODS

Insects

An *L. hesperus* colony was started from cultures obtained from USDA-ARS, Weslaco, Texas. *L. hesperus* were maintained on green beans (*Phaseolus vulgaris* L.) in the laboratory at 27 ± 1°C, 14L:10D photoperiod (photophase beginning at 8:00 h), and 50–70% RH (Beards and Leigh, 1960). Mated *L. hesperus* adults (10–15 days old) were starved for 4 h before placing them on plants for experiments (see below).

A. iole were obtained from laboratory colonies maintained on *L. hesperus* eggs at USDA-ARS, Mississippi State, Mississippi, and USDA-ARS, Weslaco, Texas. Parasitized *L. hesperus* eggs were held in Plexiglas cages (28 × 28 × 28 cm) under the environmental conditions noted above. Upon emergence, adult parasitoids were provided *ad lib* with food (honey:water, 1:1) and distilled water via absorbent matting. *A. iole* females used in experiments were <4 days old, and were assumed mated: *A. iole* is protandrous and females copulate soon after emergence. All experiments were conducted between 10:00 and 16:00 h.

Plants

Cotton *(Gossypium hirsutum* L., var. Deltapine 491, Malvaceae), common groundsel *(Senecio vulgaris* L., Asteraceae), alfalfa *(Medicago sativa* L., Fabaceae), annual ragweed *(Ambrosia artemisifolia* L., Asteraceae), and redroot pigweed *(Amaranthus retroflexus* L., Amaranthaceae) were planted from seeds in individual pots (13 cm diam.) in the greenhouse under natural light and Texas spring-summer conditions (~12–14 h daylight, 50–70% RH, and 25–35°C). Weed and cotton seed were obtained from commercial suppliers (Valley Seed Service, Fresno, California; Delta and Pine Land Company, Scott, Mississippi). All plants were about 3–4 weeks old (23–30 cm tall) when used, cotton plants had 4–6 fully expanded true leaves, and weeds were at preflowering stages.

Olfactometer

The responses of female parasitoids to odors were measured using a four-arm olfactometer similar to the one described by Vet *et al.* (1983), with modifications to accommodate the small size (~0.8 mm) of *A. iole.* The arena of the olfactometer was made of three parts: a 140 × 140 × 5 mm clear acrylic square (top), a 140 × 140 × 5 mm white acrylic square with a four-pointed star-shape cutout exposure chamber (90° arc, radius 80 mm), and a 200 × 200 mm white teflon square (base). The top was cemented to the exposure chamber, and two plastic clamps were used to hold it firmly to the base. Each olfactometer arm was divided into three regions: the "release" region at the center, the "visit" region nearest to the center, and the "selection" region nearest to the odor source (Fig. 1).

The airflow (25 ml/min/arm) inside the arena was equalized using one flowmeter at each arm and a terminal flowmeter (Riteflow®, Bel-Art Products, Pequannok, New Jersey) between the arena and the pump (Laboport®, KNF Neuberger, Inc., Trenton, New Jersey). Charcoal-filtered (Carbon Cap, Whatman®, Ann Arbor, Michigan) and humidified air was passed through treatment chambers that enclosed the odor sources inside a glass bell (15 cm diam × 43 cm tall), and tubing leading from each of the odors converged into the olfactometer arena. A white paper cover was placed ~1 m above the olfactometer to deflect direct overhead lighting from the arena and reduce biases due to other distractions. Light intensity in the olfactometer, measured with a light meter (Li-Cor LI-250, Lincoln, Nebraska), was 3.96 μmol photon/m^2/s. Behavioral data were visually recorded using a stopwatch. In all experiments, treatment odors were compared against three controls (humidified air). Individual *A. iole* females

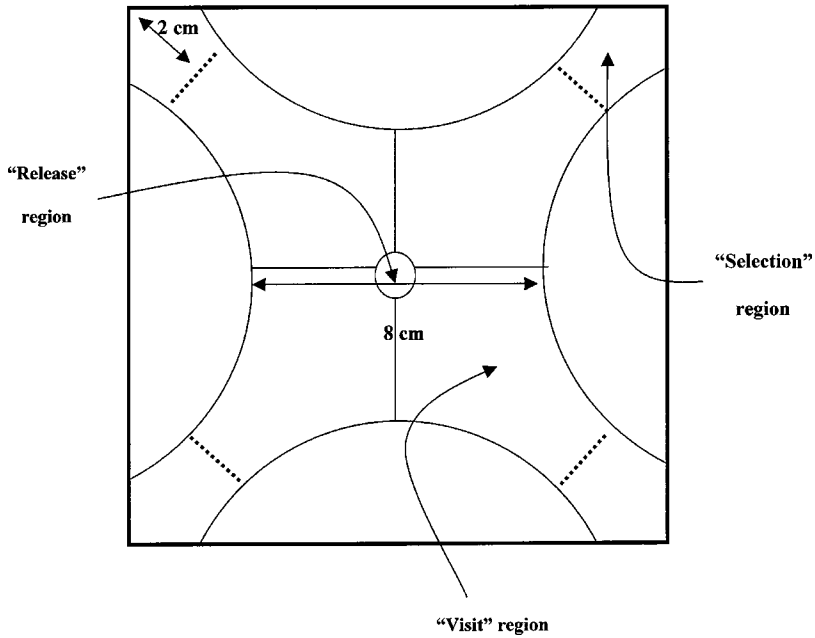

Fig. 1. Four-arm olfactometer arena (not to scale) used to measure behavioral responses of *Anaphes iole* toward odors emanating from *Lygus hesperus* habitats.

were used only once, and trials were conducted in a room maintained at $25 \pm 2°C$.

General Experimental Procedure

Individual *A. iole* females were transferred from a Petri dish and placed in the center of the olfactometer arena using a fine brush. Females were placed in the arena by carefully removing and then replacing the top of the arena; the arena was subsequently sealed and the vacuum pump was started. Female responses were observed for 5 min, and the times spent in each region and numbers of times the female entered the selection regions were recorded. Females not leaving the release region within 2 min of initiating a trial were eliminated and replaced. After testing four females, odor sources were rotated 90° clockwise, the entire arena was cleansed with 70% ethanol, and humidified air run through the entire apparatus for 5 min. Odor sources were replaced after testing eight females (~1.5 h), and a total of 16 females were tested per day.

Preliminary trials were conducted to compare responses of experienced and naïve wasps in olfactometry studies. Groups of mated *A. iole* females were preconditioned by exposing them to *L. hesperus*-infested plants (e.g., cotton when testing cotton, alfalfa when testing alfalfa, and so on), and allowing them to oviposit for 30–40 min prior to experiments: *L. hesperus*-infested plants were exposed to 10 *L. hesperus* females for 24 h at 27 ± 1°C, 14L:10D photoperiod, and 50–70% RH before trials. These trials revealed that *A. iole* females provided with such experience responded by spending more time in treatment odor fields in the olfactometer ($F = 4.27$; df $= 3, 104$; $P = 0.007$), while females denied such experience (i.e. naive) had weak to nil responses ($F = 1.16$; df $= 3, 120$; $P = 0.33$). Similarly, previous studies showed that female experience with hosts and host-related substrates enhances subsequent behavioral responses (Vinson *et al.,* 1977; Sandlan, 1980; Vet, 1983, 1985; Turlings *et al.*, 1989, 1990b; Fritzsche-Hoballah *et al.*, 2002). Thus, all experiments were conducted using "experienced" female parasitoids.

Respones *of A. iole* Females to Induced Cotton Volatiles and Adult Hosts

Five different experiments were conducted using the four-armed olfactometer. In the first four experiments, cotton plants received the following treatments 24 h prior to trials: (1) 10 *L. hesperus* females (feeding and oviposition damage); (2) 10 *L. hesperus* males (feeding damage only); (3) mechanical damage (scratching the underside surface of four leaves with a razor blade over an area of 0.5 cm^2); or, (4) four third instar larvae of *S. exigua* (Lepidoptera: Noctuidae). In all cases, cotton plants receiving treatments were maintained in cages under the environmental conditions described above, and *L. hesperus* adults and *S. exigua* larvae were removed from plants prior to experiments. The fifth experiment comprised treatment odors emanating from 10 *L. hesperus* females alone.

Responses of *A. iole* Females to Host Induced Plant Volatiles

Five different experiments using different plant species were conducted using the four-armed olfactometer described above. The following plant species were caged with 10 *L. hesperus* females 24 h prior to experiments: cotton, alfalfa, common groundsel, redroot pigweed, and annual ragweed. In all cases, plants with *L. hesperus* females were kept in cages maintained under the environmental conditions noted above, and *L. hesperus* adults were removed from plants prior to experiments.

Statistical Analyses

Kruskall–Wallis tests (Zar, 1999) were conducted using olfactometer arm (i.e., independent of assigned odor treatment) as the main factor to test for potential biases toward any arm. Subsequently for each experiment, the numbers of selections, and the total times spent in each odor field of the olfactometer were analyzed via Friedman's analysis of variance by ranks with odor treatments (treatment odor, and controls 1 through 3) as factors (Zar, 1999). Means were separated as warranted using Tukey's HSD tests on ranks. In addition, mean total times spent in treatment odor fields were compared among experiments using Kruskall–Wallis tests. A significance level of 5% was adopted for all statistical analyses.

RESULTS

Responses of *A. iole* Females to Induced Cotton Volatiles and Adult Hosts

Significant differences were not detected among total times spent in each olfactometer arm independent of assigned odor in all experiments (mechanical damage: $\chi^2 = 0.99$; df $= 3, 124$; $P = 0.80$; *Spodoptera* damage: $\chi^2 = 7.14$; df $= 3, 120$; $P = 0.07$; *L. hesperus* females alone: $\chi^2 = 7.20$; df $= 3, 104$; $P = 0.07$; *L. hesperus* male damage: $\chi^2 = 6.694$; df $= 3, 112$; $P = 0.082$; *L. hesperus* female damaged: $\chi^2 = 2.608$; df $= 3, 124$; $P = 0.456$). These results indicated that responses to individual olfactometer arms were unbiased.

A. iole females spent significantly more time in treatment odor fields (>100 s) compared to control odor fields (<55 s) in experiments involving cotton plants damaged either by *L. hesperus* females or males (females: $F = 5.44$; df $= 3, 124$; $P = 0.002$; males: $F = 7.54$; df $= 3, 112$; $P = 0.0001$) (Fig. 2a,b). In addition, females spent significantly more time (>80 s) in odors derived from *L. hesperus* females alone relative to controls (<50 s) ($F = 4.27$; df $= 3, 104$; $P = 0.007$) (Fig. 2c). In contrast, significant differences were not detected when plants were damaged mechanically or by *S. exigua* larvae (<83 s) compared to controls (>35 s) (mechanical: $F = 2.55$; df $= 3, 124$; $P = 0.060$; *S. exigua*: $F = 1.58$; df $= 3, 120$; $P = 0.197$) (Fig. 2d,e). Significant differences were not detected among experiments in the total times spent in treatment odor fields ($\chi^2 = 5.95$; df $= 4, 146$; $P = 0.203$).

A. iole females entered the selection regions of treatment odors more frequently than those of controls when plants were damaged either by

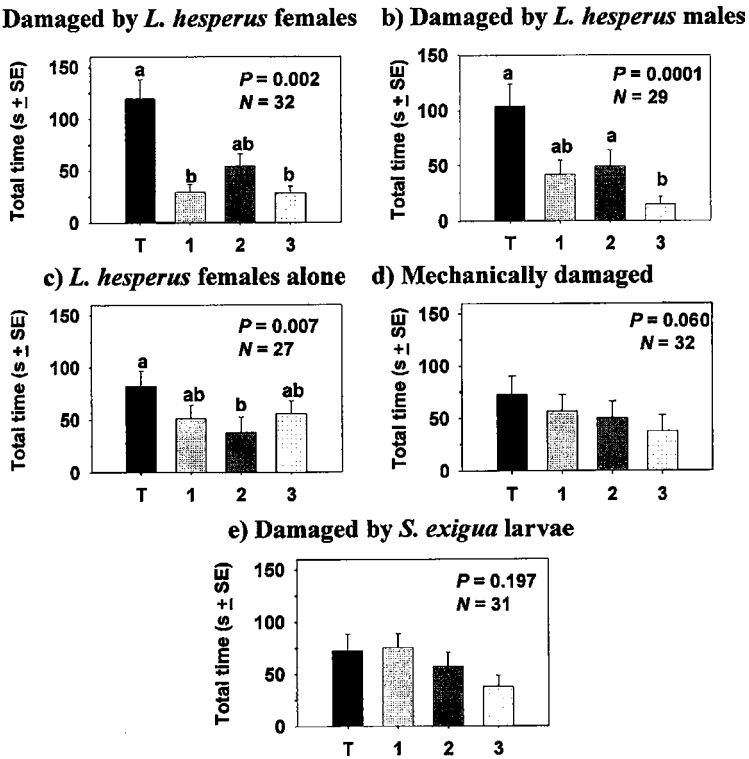

Fig. 2. Mean total time (s ± SE) *Anaphes iole* females spent in each odor field of a four-arm olfactometer. T = field with treatment odor; 1, 2, 3 = fields with control odors (air only). Treatment odors were (a) cotton plant damaged by *Lygus hesperus* females, (b) cotton plant damaged by *L. hesperus* males, (c) *L. hesperus* females alone, (d) cotton plant damaged mechanically, and (e) cotton plant damaged by *Spodoptera exigua* larvae. P values are inset and correspond to Friedman's one-way ANOVA by ranks ($P < 0.05$). Different letters above columns indicate significant differences, Tukey's HSD ($P < 0.05$).

L. hesperus females or males (females: $F = 3.47$; df $= 3, 124$; $P = 0.018$; males: $F = 5.04$; df $= 3, 112$; $P = 0.002$) (Fig. 3a,b). In contrast, odors derived from *L. hesperus* females alone did not result in significantly higher numbers of selections by *A. iole* females relative to control odor fields ($F = 1.10$; df $= 3, 104$; $P = 0.351$) (Fig. 3c). Similarly, differences were not detected in the numbers of selections among odor fields when plants were damaged mechanically or by *S. exigua* larvae (mechanical: $F = 1.99$; df $= 3, 124$; $P = 0.119$; *S. exigua*: $F = 0.63$; df $= 3, 120$; $P = 0.597$) (Fig. 3d,e).

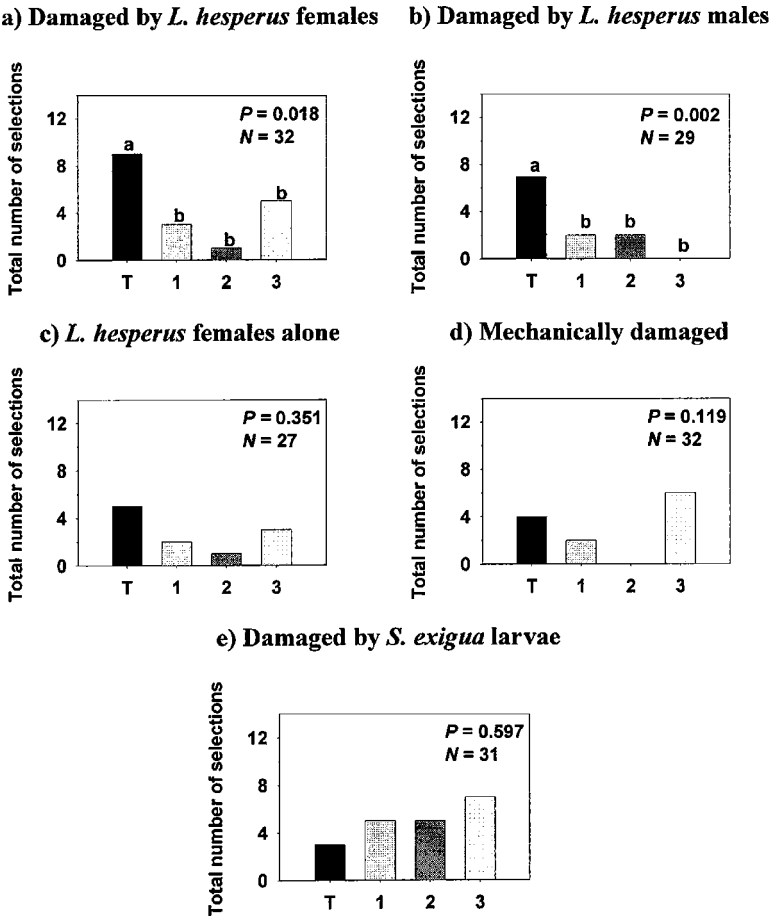

Fig. 3. Total number of times *Anaphes iole* females entered selection region of a four-arm olfactometer. T = field with treatment odor; 1, 2, 3 = fields with control odors (air only). Treatment odors were (a) cotton plant damaged by *Lygus hesperus* females, (b) cotton plant damaged by *L. hesperus* males, (c) *L. hesperus* females alone, (d) cotton plant damaged mechanically, and (e) cotton plant damaged by *Spodoptera exigua* larvae. P values are inset and correspond to Friedman's one-way ANOVA by ranks ($P < 0.05$). Different letters above columns indicate significant differences, Tukey's HSD ($P < 0.05$).

Responses of *A. iole* Females to Host Induced Plant Volatiles

Significant differences were not detected among total times spent in each olfactometer arm independent of the assigned odor in all experiments (cotton: $\chi^2 = 2.61$; df = 3, 124; $P = 0.46$; alfalfa: $\chi^2 = 1.12$; df = 3, 148;

$P = 0.34$; common groundsel: $\chi^2 = 5.33$; df $= 3, 140$; $P = 0.15$; redroot pigweed: $\chi^2 = 1.58$; df $= 3, 120$; $P = 0.56$; annual ragweed: $\chi^2 = 4.25$; df $= 3, 108$; $P = 0.23$). These results confirmed that responses to individual olfactometer arms were unbiased.

A. iole females spent significantly more time in treatment odor fields (>120 s) compared to control odor fields (<50 s) of the olfactometer in all experiments, with the exception of the experiment involving annual ragweed (cotton: $F = 5.44$; df $= 3, 124$; $P = 0.002$; alfalfa: $F = 4.08$; df $= 3, 148$; $P = 0.008$; common groundsel: $F = 7.08$; df $= 3, 140$; $P = 0.0002$; redroot pigweed: $F = 2.86$; df $= 3, 120$; $P = 0.040$; annual ragweed: $F = 1.21$; df $= 3, 108$; $P = 0.308$) (Fig. 4). However, significant

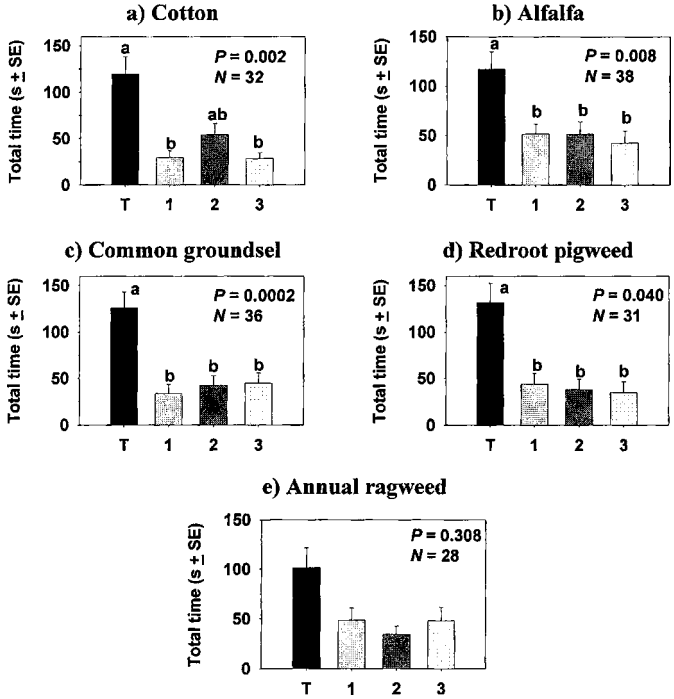

Fig. 4. Mean total time (s ± SE) *Anaphes iole* females spent in each odor field of a four-arm olfactometer using different host plants. T = field with treatment odor; 1, 2, 3 = fields with control odors (air only). Treatment odors consisted of plant species exposed to feeding and oviposition by *Lygus hesperus* females: (a) cotton, (b) alfalfa, (c) common groundsel, (d) redroot pigweed, (e) annual ragweed. P values are inset and correspond to Friedman's one-way ANOVA by ranks ($P < 0.05$). Different letters above columns indicate significant differences, Tukey's HSD ($P < 0.05$).

differences were not detected among experiments in mean total times spent in treatment odor fields ($\chi^2 = 1.427$; df = 4, 160; $P = 0.84$) (Fig. 4).

A. iole females selected treatment odor fields more frequently than control odors in all experiments (cotton: $F = 3.47$; df = 3, 124; $P = 0.020$; alfalfa: $F = 11.47$; df = 3, 148; $P < 0.0001$; common groundsel: $F = 5.79$; df = 3, 140; $P = 0.001$; redroot pigweed: $F = 4.46$; df = 3, 120; $P = 0.005$; annual ragweed: $F = 4.75$; df = 3, 108; $P = 0.003$) (Fig. 5).

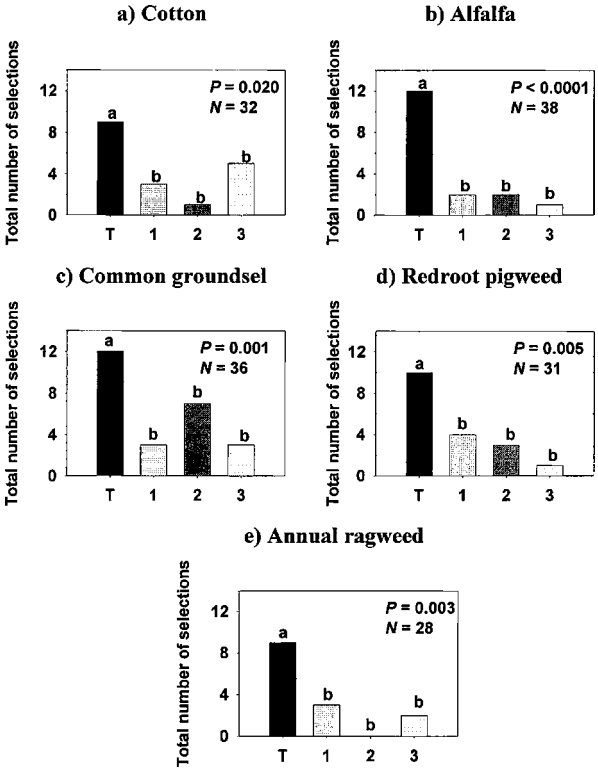

Fig. 5. Total number of times *Anaphes iole* females entered the selection region of a four-arm olfactometer using different host plants. T = field with treatment odor; 1, 2, 3 = fields with control odors (air only). Treatment odors consisted of plant species exposed to feeding and oviposition by *Lygus hesperus* females: (a) cotton, (b) alfalfa, (c) common groundsel, (d) redroot pigweed, (e) annual ragweed. P values are inset and correspond to Friedman's one-way ANOVA by ranks ($P < 0.05$). Different letters above columns indicate significant differences, Tukey's HSD ($P < 0.05$).

DISCUSSION

Herbivore-induced plant odors play major roles in the foraging behavior of natural enemies. This study provides the first experimental data demonstrating that the females of the egg parasitoid *A. iole* respond to volatiles released by different plant species following *L. hesperus* feeding and oviposition, whereas odors derived from cotton plants damaged by *S. exigua* larvae or mechanically had no apparent effect on their behavior. As discussed by Vet and Dicke (1992), the degree of specialization at a particular trophic level sets the degree of specificity of the information needed for successful foraging. In the case of *A. iole,* females attack mainly *Lygus* eggs that are embedded in the tissues of a variety of plant species (Scott, 1977; Jackson and Graham, 1983; Graham *et al.*, 1986). Results from our experiments suggest that this egg parasitoid may use volatile cues derived from plant–*Lygus* complexes to locate its host's habitat.

According to Rodriguez-Saona *et al.* (2002), *L. hesperus* feeding and oviposition induces emission of volatiles in cotton and maize, and most compounds were detected even after removing the insects from the plants. Moreover, the blend of volatiles induced by *L. hesperus* salivary gland extracts was similar to the blend obtained from plants treated with volicitin isolated from oral secretions of *S. exigua* larvae (Alborn *et al.*, 1997). The present study showed that *A. iole* females responded to odors derived from plants damaged by both genders of *L. hesperus*, whereas they did not respond to odors from plants damaged by *S. exigua* larvae. These results indicate that *A. iole* can discriminate among volatile blends released following feeding damage by different insect species. Several differences among volatile blends may account for this discrimination, e.g. differences in volatile concentrations and/or changes in the ratios of certain chemicals among treatment odors. For example, plants damaged by *L. hesperus* females cease to emit hexyl butyrate or (*E*)-2-hexenyl butyrate after insects are removed from plants (Rodriguez-Saona *et al.*, 2002; Rodriguez-Saona, personal communication). Moreover, cotton plants damaged by *L. hesperus* continue emitting volatiles stored in glands after insects are removed (Rodriguez-Saona *et al.*, 2002), while emissions of these volatiles by plants damaged by *S. exigua* larvae ceases shortly after insects are removed (Loughrin *et al.*, 1994; Rodriguez-Saona *et al.*, 2001). Though possible, it is unlikely that odors emanating from *L. hesperus* frass or other substances left on plants account for the responses of *A. iole* females in this study because a recent study showed that *A. iole* does not respond to frass of *Lygus lineolaris* Palisot de Beauvois (Beach *et al.*, 2003).

Since *L. hesperus* adults and eggs co-occur on plant hosts, *A. iole* females may use other indirect cues in addition to plant volatiles to find host

habitats. In this study, *A. iole* females spent more time relative to controls in treatment odors derived from *L. hesperus* females alone, though differences were not detected in the frequencies of selections. This inconsistent behavioral response may suggest that the set of cues emanating from *L. hesperus* alone is incomplete. In contrast, odors derived from host-plant complexes consistently elicited stronger behavioral responses (relative to controls) in *A. iole* females, pointing to the importance of plant volatiles in host habitat location by this egg parasitoid. Several egg parasitoid species respond to the sex pheromones of their hosts (e.g. Lewis *et al.*, 1982; Kaiser *et al.*, 1989; Noldus *et al.*, 1988, 1991; McGregor and Henderson, 1998; Fiaboe *et al.*, 2003). *L. hesperus* males and females are known to emit large amounts of hexyl butyrate and (*E*)-2-hexenyl butyrate (Aldrich *et al.*, 1988; Ho and Millar, 2002). In addition, calm and agitated *L. lineolaris* females released six major volatile compounds: (*E*)-2-hexenal, 1-hexanol, (*E*)-2-hexenol, hexyl butyrate, (*E*)-2-hexenyl butyrate, and (*E*)-2,4-oxohexenal (Wardle *et al.*, 2003). *A. iole* females may use cues from adult hosts to locate habitats where host oviposition is likely to occur, increasing the chances of successful parasitism.

Although volatile blends likely vary among the plant species tested in this study, *A. iole* females similarly responded to odors derived from a variety of plant-host complexes in the olfactometer. Overall, the results of this study suggest that a variety of plant-host complexes emit volatile blends that are broadly attractive to *A. iole* females. Specifically, *A. iole* females responded to volatiles induced by a polyphagous herbivore in both annual and perennial species from four plant families. In contrast, previous studies showed that volatile blends emitted by individual plant species under herbivore attack vary with herbivore species. For example, the specialist parasitoid *Toxoneuron (Cardiochiles) nigriceps* (Viereck) preferred odors emanating from cotton, tobacco, and maize infested with its host, *Heliothis virescens* Fabricius, compared to *Helicoverpa zea* Boddie (De Moraes *et al.*, 1998). Further studies should examine the volatile blends derived from the different plant-host complexes and identify the main compounds that are attractive to *A. iole* females.

In summary, *A. iole* females in the laboratory responded to odors derived from plants damaged by *L. hesperus* feeding and oviposition, but not from plants damaged mechanically or by *S. exigua* larvae. Given the polyphagous feeding habit of *L. hesperus*, *A. iole* females in the field face highly complex and variable environments, in which diverse plant–herbivore interactions likely result in emissions of different volatile blends coming from numerous potential host patches. The results of this study provide a starting point in the understanding of the importance of volatile cues in host habitat location by *A. iole.* Further studies are needed to elucidate

the intricate processes involved in the host searching behavior of this parasitoid, and determine the specific compounds used by *A. iole* females in locating their hosts and host habitats.

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