

**HOST HABITAT LOCATION MEDIATED BY OLFACTORY
STIMULI IN *ANAPHES IOLE* (HYMENOPTERA: MYMARIDAE),
AN EGG PARASITOID OF *LYGUS HESPERUS* (HEMIPTERA:
MIRIDAE)**

A Thesis

by

VERONICA MANRIQUE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Entomology

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ABSTRACT

Host Habitat Location Mediated by Olfactory Stimuli in *Anaphes iole* (Hymenoptera: Mymaridae), an Egg Parasitoid of *Lygus hesperus* (Hemiptera: Miridae).

(December 2003)

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Lygus hesperus is an important pest on different crops including cotton and alfalfa in the western U.S. *Anaphes iole* is a common parasitoid of *Lygus* spp. eggs in the U.S. and has potential as a biological control agent against *L. hesperus* in different crops. Its foraging behavior has been studied to a limited extent, but it is unknown whether *A. iole* females rely on plant volatiles to locate host habitats. *L. hesperus* feeding and oviposition are known to induce emission of plant volatiles in cotton and maize, but no studies have addressed the role of plant volatiles in the host searching behavior of *A. iole*. The objectives of this study were to evaluate the attraction of *A. iole* females toward volatiles derived from *L. hesperus* habitats and flight response of *A. iole* females toward cotton plants harboring *L. hesperus* eggs or treated with methyl jasmonate. Results from olfactometry bioassays showed that female wasps were attracted to odors emanating from different plant-*L. hesperus* complexes and from adult *L. hesperus*, while plants damaged by non-hosts or mechanically-damaged were not attractive. These findings suggested that *A. iole* females use specific plant volatiles released following *L. hesperus*

feeding and oviposition to locate host habitats. In addition, in flight chamber tests *A. iole* females discriminated between cotton plants with moderate (41 eggs) and high (98 eggs) levels of *L. hesperus* infestations relative to uninfested plants, but not between plants with low (7 eggs) infestations compared to uninfested plants. In larger scale experiments conducted in the greenhouse, female wasps responded to *L. hesperus*-infested plants but not to methyl jasmonate-treated plants under similar conditions. Overall, results from this study revealed that *A. iole* females employ volatile signals to locate its host's habitat and that they are attracted to plants damaged by *L. hesperus* feeding and oviposition. However, further research should seek to identify the chemical elicitors involved in the release of plant volatiles attractive to *A. iole* females.

To my mother, the woman I would like to become, for her unconditional love, passion
and dedication

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CHAPTER I

GENERAL INTRODUCTION

Lygus hesperus Knight (Hemiptera: Miridae) is an important pest of different crops including cotton *Gossypium hirsutum* L. and alfalfa *Medicago sativa* L. in the western U.S. (Clancy, 1968; Jackson and Graham, 1983). It is polyphagous, feeding on at least 117 non-crop plants and 25 cultivated plants (Scott, 1977). In early spring, *Lygus* 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). In Texas High Plains, *L. hesperus* is abundant on annual ragweed (*Ambrosia artemisifolia* L.), kochia (*Kochia scoporia* L.), and redroot pigweed (*Amaranthus retroflexus* L.), as well as alfalfa and canola crops (Armstrong, pers. comm.). According to Barlow et al. (1999), *L. hesperus* adults in northern California lived longer on and preferred shepherd's purse (*Capsella bursa-pastoris* L.) and common groundsel (*Senecio vulgaris* L.) relative to other common weeds and alfalfa. These studies highlighted the importance of weedy hosts as refugia for maintenance of *Lygus* populations in the vicinity of susceptible crops.

In cotton, *L. hesperus* feeding occurs primarily in fruiting structures (Jubb and Carruth, 1971; Wilson et al., 1984), and young squares and small bolls are preferred (Mauney and Henneberry, 1979; Leigh et al., 1988). In the western U.S., use of

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insecticides to control this pest causes severe outbreaks of some secondary pests (Walker and Smith, 1996). In addition, several studies showed that populations of *Lygus hesperus* have developed resistance to important insecticides (Zhu and Brindley, 1992, 1994). Management of *Lygus* spp. has been difficult mainly because of their high mobility, broad host range, cryptic damage, and poorly understood population dynamics (Ruberson and Williams, 2000). Thus, there is an increasing concern about the potential for *Lygus* spp. to cause severe losses.

Anaphes iole Girault (Hymenoptera: Mymaridae) is the most commonly encountered parasitoid of *Lygus* eggs in the U.S. (Clancy and Pierce, 1966; Schuster, 1987), and has potential as a biological control agent against *L. hesperus* in different crops (Ruberson and Williams, 2000). This parasitoid attacks *Lygus* eggs in a variety of crops, but its efficacy appears to vary with host plant species (Graham et al., 1986). Field studies in Arizona showed that only *A. iole* successfully parasitized *Lygus* eggs in various plant species sampled, and rates of parasitism ranged from 0-100% (Jackson and Graham, 1983; Graham et al., 1986). Weekly releases of *A. iole* in strawberry fields in California resulted in nearly 80% parasitism of *L. hesperus* eggs, and nymphs were suppressed by 43-64% (Norton et al., 1992; Udayagiri et al., 2000). In addition, *A. iole* attacks eggs of other Miridae and Membracidae, all of which are embedded in plant tissue (Stoner and Surber, 1969; Huber and Rajakulendran, 1988). Although *A. iole* successfully parasitized eggs of *Nabis americanoferus* Carayon and *N. alternatus* Parshley in the laboratory, parasitization of these predators by *A. iole* was not recorded in the field (Clancy and Pierce, 1966; Jackson and Graham, 1983).

In general, parasitoid host selection behavior can be divided into a series of steps and behaviors that result in oviposition in or on a host (Doutt, 1959; Vinson, 1998). Finding the host's habitat appears to be accomplished via olfactory (Van Alphen and Vet, 1986; Vinson, 1991) and/or visual stimuli (Vinson, 1985; Sheehan et al., 1993), both coming from the habitat. Within the habitat, the stimuli involved in host location are olfactory and gustatory (Hassell, 1968; Vinson, 1977a,b), and these cues stimulate female parasitoids to remain and continue searching in host-infested areas (Lewis et al., 1975a,b; Waage, 1979). Host recognition by female parasitoids includes mainly non-volatile chemical cues or physical characteristics such as shape, texture, or movement (Vinson, 1991).

Host location at a distance is usually accomplished via location of the host's habitat, and in particular by detection of volatile substances released by host plants (Tumlinson et al., 1992, 1999). Volatiles released from damaged host plants can be divided into i) constitutive compounds and ii) inducible (non-constitutive) compounds. Constitutive compounds are continuously present in the plant and are released from damaged leaves immediately after the onset of feeding damage, or after plants suffer mechanical damage (Turlings et al., 1990a; Röse et al., 1996). Inducible compounds are produced *de novo*, are released several hours after the onset of herbivore damage, and appear to be released specifically in response to herbivore damage (Turlings et al., 1990a; McCall et al., 1994; Loughrin et al., 1995; Paré and Tumlinson, 1997a; Röse et al., 1998; Rodriguez-Saona et al., 2002). Thus, damage by phytophagous insects increases the release of volatile substances by plants, and it has been demonstrated that

parasitoids are attracted preferentially to odors of host-infested plants rather than uninfested ones (Vet, 1985).

The responses to herbivore-induced plant volatiles have been extensively studied for the New World endoparasitoids *Cotesia marginiventris* Cresson and *Campoletis sonorensis* Cameron. In both cases, female parasitoids were strongly attracted toward odors emitted by caterpillar-damaged plants compared with mechanically-damaged plants (Turlings et al., 1990a; McAuslane et al., 1991). Phloem-feeding insects, such as Homoptera, also induce the release of plant volatiles that are attractive to natural enemies. For example, aphids induce production of plant volatiles that are attractive to aphid parasitoids (Mölck et al., 1999; Micha et al., 2000). In addition, several egg parasitoids are known to be attracted to odors from host products and/or host-plant complexes in olfactory studies (Meiners and Hilker, 1997; McGregor and Henderson, 1998; Hilker and Meiners, 2002; Hilker et al., 2002; Conti et al., 2003). According to Rodriguez-Saona et al. (2002), *L. hesperus* feeding induces emission of plant volatiles, and induction is caused by an elicitor found in the insect's salivary gland. However, it is unknown whether the egg parasitoid *A. iole* relies on plant- or host-derived volatiles to locate its host's habitat.

The quality of a host location cue depends on its reliable association with the presence of an herbivore, the detectability of the cue and the information content in the signal (Vet et al., 1995; De Moraes and Mescher, 1999). Cues arising directly from the herbivore may be highly reliable indicators of host presence, but are probably difficult to detect because herbivores are minor components of complex environments, and are more

important in short-range host location (Turlings et al., 1990a; Turlings and Tumlinson, 1992; Vet et al., 1995). In contrast, constitutive plant cues are highly detectable but, in general, are not reliable indicators of herbivore presence (Vet et al., 1995; De Moraes and Mescher, 1999). However, plant volatile compounds released in direct response to herbivore feeding (Dicke et al., 1990b; Turlings et al., 1990a, 1993b), are more reliable cues because they are linked to the presence of the host and are being released in large quantities increasing their detectability. Thus, herbivore-induced cues, e.g. synomones, provide a major solution to the reliability-detectability problem, and induced-plant compounds likely represent the most effective cues for long-range host detection by parasitoids (Vet and Dicke, 1992; De Moraes and Mescher, 1999).

Herbivory defense in many plants is induced through the octadecanoid pathway (Meyer et al., 1984; Karban and Baldwin, 1997), which is also involved in recruitment of natural enemies (Farmer, 1997). This pathway is induced by herbivory (Thaler et al., 1996) or by treating plants with jasmonic acid (McConn et al., 1997). In tomato plants, application of jasmonic acid resulted in decreased preference and abundance of common pests in the field, and increased levels of parasitism of caterpillar pests (Thaler et al., 1996; Thaler, 1999a,b). Moreover, the results of winter wheat field studies showed that cereal aphid numbers decreased with (*Z*)-jasmonate treatment (Birkett et al., 2000). According to Rodriguez-Saona et al. (2001), cotton plants treated with methyl jasmonate emitted volatiles that may serve as odor cues in the host-searching behavior of natural enemies. Therefore, the use of jasmonic acid, methyl jasmonate, or other elicitors, could

be an effective tool for pest management in agroecosystems by enhancing biological control via recruitment of increasing natural enemy populations into crops.

The long-term goal of this study was to contribute to developing *Lygus* spp. management strategies for cotton and other field crops based on attraction and retention of the egg parasitoid *A. iole* in the vicinity of crops. This goal could be accomplished by gaining a better understanding of the role of plant volatiles in host habitat location by *A. iole*. Specifically, the objectives of this study were to:

1. Evaluate the attraction of *A. iole* females toward volatiles derived from the host habitat in olfactometry studies, and;
2. Examine the flight response of *A. iole* females toward cotton plants exposed to *L. hesperus* feeding and oviposition or treated with methyl jasmonate in laboratory and greenhouse bioassays.

CHAPTER II

ATTRACTION OF *ANAPHES IOLE* TO VOLATILE SIGNALS DERIVED FROM HOST HABITATS

Introduction

A variety of physical and/or chemical stimuli from host eggs, substrate cues, host products, or host plants mediate host location and selection behavior of egg parasitoids (Vinson, 1985; Schmidt, 1991; Takasu and Nordlund, 2001). For a given host-parasitoid association, the assemblage of the different cues that leads to host selection has been defined as a “host unit” (Conti et al., 2000). In the case of egg parasitoids, the host unit may be represented by the characteristics of the host egg and immediate surroundings, represented by secretions of female hosts, traces left by prior host stages, and substances from the substrate itself or its interactions with the host (Conti et al., 2003). Eggs are sessile and probably release fewer cues than later developmental stages (Vinson, 1998), and egg parasitism is known to decline with time as eggs mature (Houseweart et al., 1982; Lopes and Parra, 1991). Therefore, some authors have suggested that egg parasitoids may face greater challenges in locating hosts in comparison with other parasitoids (Vinson, 1998).

Herbivore feeding induces emission of a blend of volatiles that are known, in some cases, to attract natural enemies (Turlings et al., 1995; Dicke, 1999). Most studies on host-habitat location have focused 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 lepidopteran larvae (Turlings et al., 1990a, 1991a; Fritzsche-Hoballah et al., 2002). According to McCall et al. (1993), plant odors were the most important volatile cues employed by *Microplitis croceipes* Cresson females to locate potential host sites. More recently, the role of aphid-induced production of plant volatiles in mediating the foraging behavior of aphid parasitoids was examined in a Y-tube olfactometer and wind tunnel studies (Reed et al., 1995; Grasswitz, 1998; Mölck et al., 1999, 2000; Micha et al., 2000). For example, *Diaeretiella rapae* M'Intosh females were attracted to odors emanating from a cabbage-*Brevicoryne brassicae* L. complex in a four-arm olfactometer (Reed et al., 1995). In addition to feeding by herbivorous arthropods, egg deposition may also induce plants to emit volatiles that are attractive to egg parasitoids. Feeding and oviposition of the elm beetle, *Xanthogaleruca luteala* Muller, induces the field elm, *Ulmus minor* Miller, to emit volatiles that are attractive to the egg parasitoid *Oomyzus gallerucae* Fonscolombe (Meiners et al., 2000; Meiners and Hilker, 2000). In addition, odors from twigs of *Pinus sylvestris* L. laden with egg masses of the pine sawfly *Diprion pini* L. attract the egg parasitoid *Chrysonotomyia ruforum* Krausse (Hilker et al., 2002). Thus, some egg parasitoids rely on volatiles derived from plant-host complexes to locate their host's habitat.

Parasitoid foraging strategies are hypothesized to be shaped by the degree of specialization of their host ranges and herbivore host plant ranges. In contrast to generalist parasitoids, specialists are expected to show more restricted preferences for their hosts and the plants they feed upon (Vet and Dicke, 1992). Thus, plant volatiles

induced by herbivore feeding may be a key to understanding successful host finding by specialist parasitoids. Herbivory-induced plant volatiles, acting as synomones, may be specific for the host feeding on the plant (Turlings et al., 1998; Dicke 1999), and are emitted systemically from undamaged portions of a plant (Turlings and Tumlinson, 1992; Röse et al., 1996; Rodriguez-Saona et al., 2002). For example, gas chromatography revealed consistent differences between the proportions of volatile compounds released by tobacco plants damaged by *Heliothis virescens* F. and those damaged by *Helicoverpa zea* Boddie (De Moraes et al., 1998). Moreover, the specialist parasitoid *Toxoneuron* (= *Cardiochiles*) *nigriceps* Viereck exploited those differences to distinguish infestation by its host, *H. virescens* from that by *H. zea*. In addition to leaf-eating larvae, phloem-feeding insects (Homoptera) also induce plant volatiles that attract natural enemies (Mölck et al., 1999; Micha et al., 2000). For example, *Aphelinus abdominalis* Dalman females employed specific volatile signals emitted by host-infested plants during long-range host location (Molck et al., 2000). Therefore, herbivores with different feeding habits may trigger the release of plant volatiles that are known to play important roles in parasitoid foraging behavior.

Lygus hesperus Knight is an important pest of several crops, including cotton and alfalfa in the U.S. (Clancy, 1968; Jackson and Graham, 1983). 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. *L. hesperus* feeding *per se* was the prominent factor inducing emission of non-constitutive 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).

Anaphes iole Girault is the most frequent parasitoid of *L. hesperus* eggs in the U.S. (Clancy and Pierce, 1966; Schuster, 1987). Its foraging behavior has been studied to a limited extent (Conti et al., 1996, 1997), and it is unknown whether *A. iole* females rely on plant volatiles to locate host habitats. The goal of this study was to address the following questions via olfactometry assays: Are *A. iole* females attracted to odors emanating from its host's habitat, such as adult hosts odors or volatiles derived from plant-host complexes? Do plants damaged by *L. hesperus* feeding release odors attractive to *A. iole* females? Are *A. iole* females attracted to plant volatiles released following feeding by non-host insects?

Materials and Methods

Insects

The *L. hesperus* colony was started from cultures obtained from USDA-ARS, Weslaco, Texas. *L. hesperus* were maintained on fresh green beans in the laboratory at $27 \pm 1^\circ\text{C}$, 10:14 (L:D) photoperiod and 50-70% RH (Beards and Leigh, 1960). Mated *L. hesperus* adults (10-15 days old) were starved for 4 h before caging them with plants for experiments (see below).

A. iole were obtained from a laboratory colony maintained on *L. hesperus* eggs at USDA-ARS, Mississippi State, Mississippi, and USDA-ARS, Weslaco, Texas.

Parasitized *L. hesperus* eggs were held in Plexiglass cages (28 × 28 × 28 cm) under the environmental conditions noted above. Upon emergence, adult parasitoids were provided

ad lib with distilled water via absorbent matting and food (honey:water 1:1). *A. iole* females used in the experiments were <4 days old, and were assumed mated (*A. iole* is protandrous and copulate soon after emergence). All experiments were conducted between 10:00 and 16:00 h.

Plants

Cotton (*Gossypium hirsutum* L. var. Deltapine 491), common groundsel (*Senecio vulgaris* L.), alfalfa (*Medicago sativa* L.), annual ragweed (*Ambrosia artemisiifolia* L.), and redroot pigweed (*Amaranthus retroflexus* L.) were planted from seeds in individual pots (13 cm diam.) in the greenhouse under natural light and Texas spring-summer conditions (16:8 (L:D) photoperiod, 50-70% RH, and 25-35°C). Weed seeds were obtained from commercial suppliers (Valley Seed Service, Fresno, California), and cotton seeds from 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 pre-flowering 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 3 parts: a 140 × 140 × 5 mm clear acrylic square (top), a 140 × 140 × 5 mm white acrylic square containing the four-pointed star-shape of the exposure chamber (90° arc, radius 80 mm), and a 200 × 200 white teflon square (base). The top was cemented to the exposure chamber, and 2 plastic clamps were used to hold it firmly to the base. Each

olfactometer arm was divided into three regions: the “visit” region nearest to the center, the “selection” region nearest to the odor source, and the “release” region at the center (Figure 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, USA) between the arena and the pump (Laboport®, KNF Neuberger, Inc., Trenton, New Jersey, USA). Charcoal-filtered (Carbon Cap, WhatmanR, Clifton, New Jersey, USA) and humidified air was passed through treatment chambers that enclosed the odor sources inside a glass bell, and tubing leading from each of the odors converged into the olfactometer arena. To determine the flow fields inside the exposure chamber, ammonium chloride (NH₄Cl) smoke tests were conducted as described by Vet et al. (1983). A white cover was placed above the olfactometer to provide a uniform light in 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 of 3.96 $\mu\text{mol photon/m}^2/\text{s}$. In all cases, treatment odors were compared against three controls (humidified air) in the olfactometer. Individual female wasps were used only once, and trials were conducted in a room maintained at $25 \pm 2^\circ\text{C}$.

Individual *A. iole* females were transferred from a petri dish and placed in the center of the olfactometer arena using a fine brush. Before introducing the wasp, the top of the arena was carefully opened and after placing individual females in the center, the arena lid was properly closed and the vacuum pump was turned on. Female responses were observed for 5 min., and the time spent in each selection, visit and release regions were recorded for the duration of the trial. If the wasps did not move from the center of

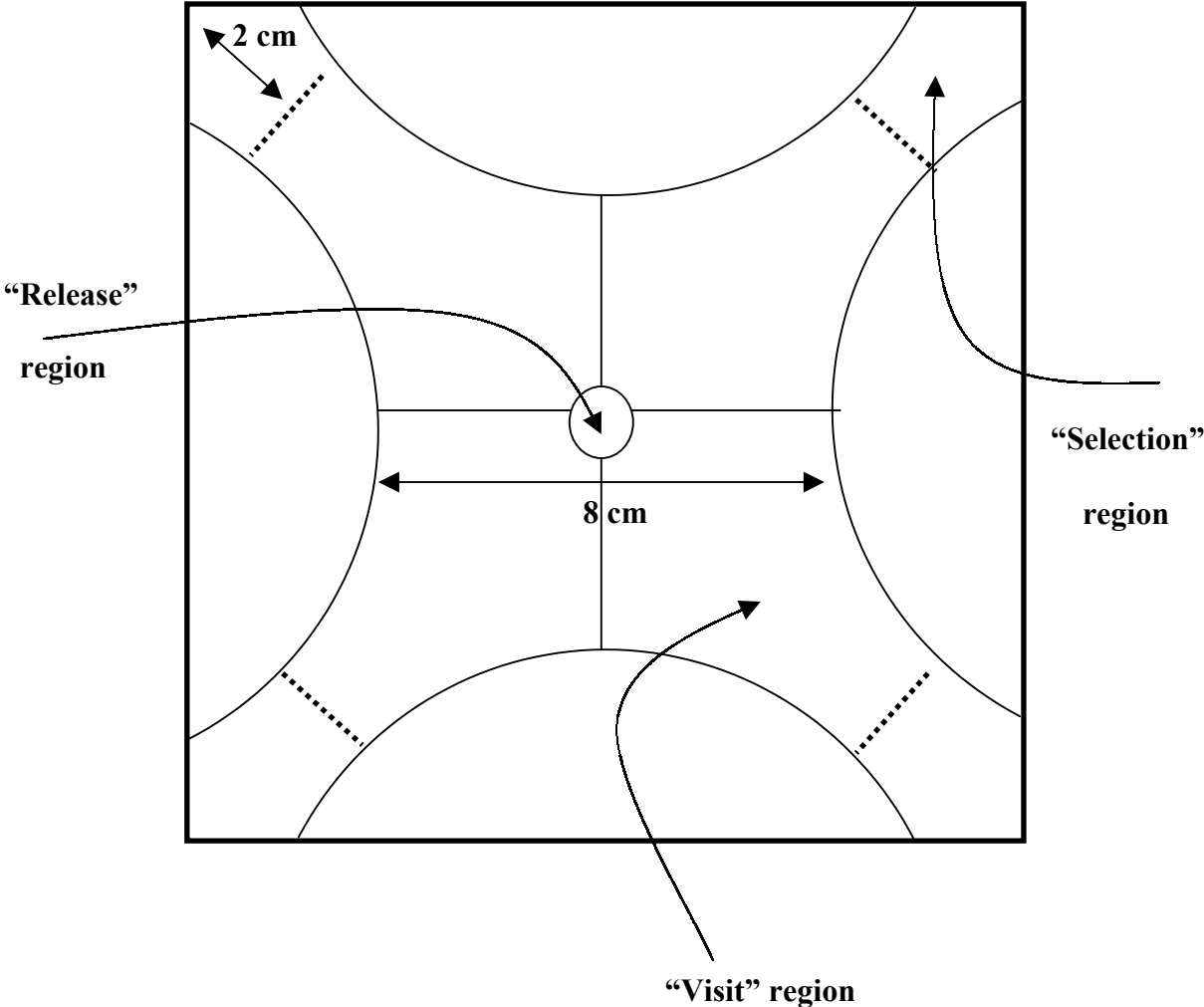


Figure 1. Four-arm olfactometer arena used to measure behavioral responses of *Anaphes iole* toward odors emanating from host habitat.

the arena for 2 minutes, they were eliminated and replaced. After testing 4 females, odor sources were rotated 90° clockwise, and the entire system was cleansed with 70% ethanol. Odor sources were replaced after testing 8 females (~1.5 h), and a total of 16 females were tested per day.

Adult Experience

Preliminary trials were conducted to compare responses of experienced and naïve wasps in olfactory studies. Groups of mated *A. iole* females were preconditioned by exposing them to host-infested plants and allowing them to oviposit for 30-40 min prior to experiments. Data from these trials revealed that *A. iole* females provided with such experience responded strongly to treatment odors in the olfactometer ($F = 4.27$, $df = 3, 104$, $P = 0.007$), while wasps denied such access (naïve) had weak to nil responses ($F = 1.16$, $df = 3, 120$, $P = 0.33$). Similarly, other studies have shown that female experience with hosts and host-related substrates enhances further responses (Vinson et al., 1977; Sandlan, 1980; Vet, 1983, 1985; Turlings et al., 1989, 1990b; Fritzsche-Hoballah et al., 2002). Thus, all the experiments in the olfactometer were conducted using “experienced” female parasitoids.

Attraction of A. iole Females Toward Induced Cotton Plants 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), (3) mechanical damage (scratching the underside surface of 4

leaves with a razor blade over an area of 0.5 cm²), (4) four 3rd instar larvae of *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae). In all cases, cotton plants receiving treatments were maintained in cages in an environmental chamber as described above, and *L. hesperus* adults and *S. exigua* larvae were removed from plants prior to the experiments. The fifth experiment comprised treatment odors emanating from 10 *L. hesperus* females alone.

Attraction of A. iole Females Toward Plant-Host Complexes

Five different experiments using different plant species were conducted using the four-armed olfactometer as 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 in an environmental chamber as previously described, and *L. hesperus* adults were removed from the plants prior to the 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. For each experiment, the number of selections, and the total time spent in each odor field of the olfactometer were analyzed via Friedman's one-way analysis of variance (ANOVA) by ranks with odor treatments (treatment odor, and controls 1-3) as factors (Zar, 1999). Means were separated as warranted using Tukey's HSD tests. In addition, total times spent in treatment odor fields were compared among experiments 1-

5 using a Kruskal-Wallis test. For all statistical analyses, a significance level of 0.05% was adopted.

Voucher Specimens

Specimens of *L. hesperus* and *A. iole* were deposited in the insect collection at Texas A&M University (voucher number 641), College Station, Texas, USA.

Results

Attraction of A. iole Females Toward Induced Cotton Plants and Adult Hosts

Significant differences were not detected among the total times spent in each arm of the olfactometer independent of the assigned odor in all the experiments conducted (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, $F = 5.75$, $df = 3, 124$, $P = 0.002$; males, $F = 6.83$, $df = 3, 112$, $P < 0.001$) (Figure 2). In addition, female wasps spent significantly more time (> 80 s) in odors derived from *L. hesperus* females alone in comparison to controls (< 50 s) ($F = 4.27$, $df = 3, 104$, $P = 0.007$) (Figure 2). Significant differences were not detected when plants were damaged mechanically (> 70 s) or by *S. exigua* larvae (> 70 s) compared to controls (< 70 s) (mechanical, $F = 2.55$, $df = 3, 124$, $P =$

0.06; *S. exigua*, $F = 1.58$, $df = 3$, 120, $P = 0.20$) (Figure 2). Significant differences were not detected among mean times spent in treatment odors in each of the experiments ($\chi^2 = 5.95$, $df = 4$, 146, $P = 0.203$).

A. iole females entered the selection regions of treatment odors more frequently than that of controls when plants were damaged either by *L. hesperus* females or males (females, $F = 3.47$, $df = 3$, 124, $P = 0.02$; males, $F = 5.04$, $df = 3$, 112, $P = 0.003$) (Figure 3). In contrast, 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, $P = 0.12$; *S. exigua*, $F = 0.63$, $df = 3$, 120, $P = 0.59$) (Figure 3). 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, $P = 0.35$) (Figure 3).

Attraction of A. iole Females Toward Plant-Host Complexes

Significant differences were not detected among the total times spent in each arm of the olfactometer independent of the assigned odor in all the experiments conducted (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 indicated that responses to individual olfactometer arms were unbiased.

Individual wasps spent significantly more time in the treatment odor fields (> 120 s) compared to control odor fields (< 50 s) of the olfactometer in all plants tested, with the exception of annual ragweed (cotton, $F = 5.75$, $df = 3$, 124, $P = 0.002$; alfalfa, $F = 4.08$,

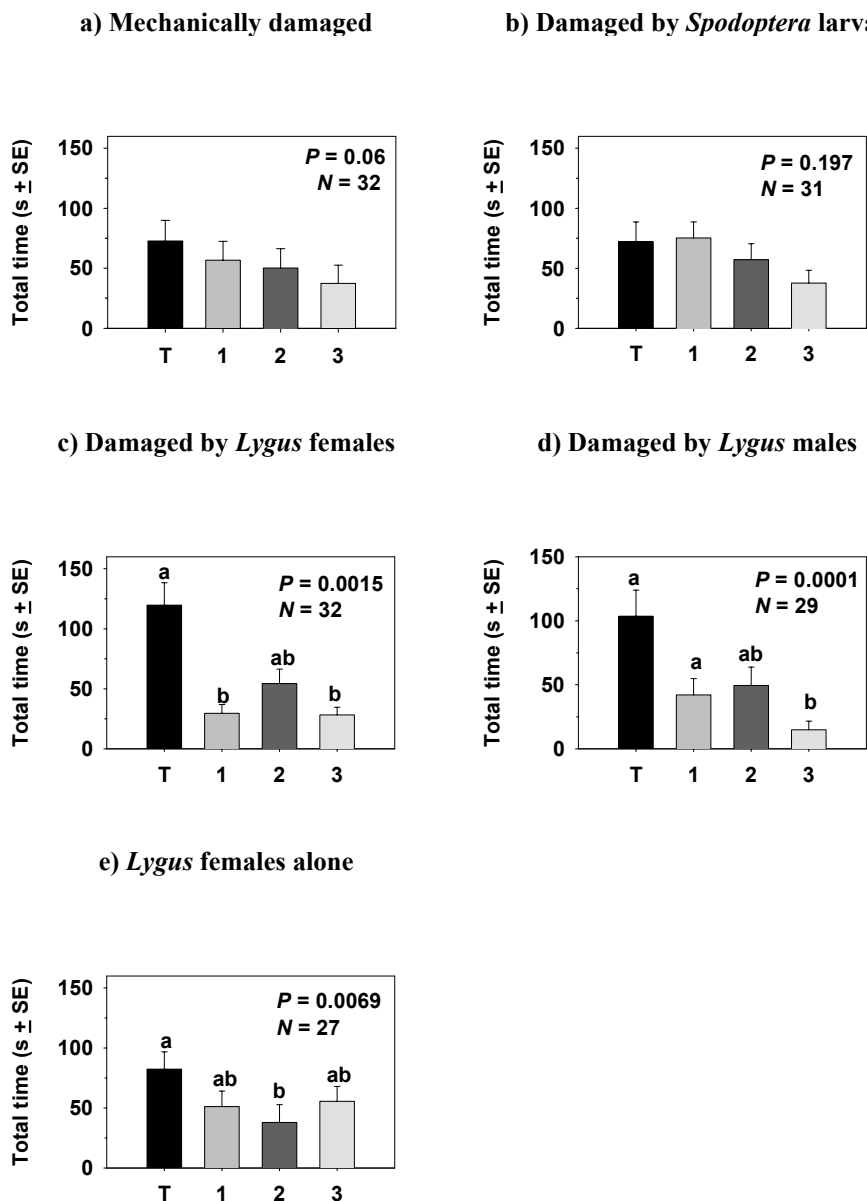


Figure 2. Mean total times ($s \pm SE$) *Anaphes iole* females spent in each odor field of a four-arm olfactometer. T = field with treatment odor; 1, 2, 3 = control (odorless) fields. Treatment odors (a) cotton plant damaged mechanically, (b) cotton plant damaged by *Spodoptera exigua* larvae, (c) cotton plant damaged by *L. hesperus* females, (d) cotton plant damaged by *L. hesperus* males, (e) *L. hesperus* females alone. 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 HSD ($P < 0.05$).

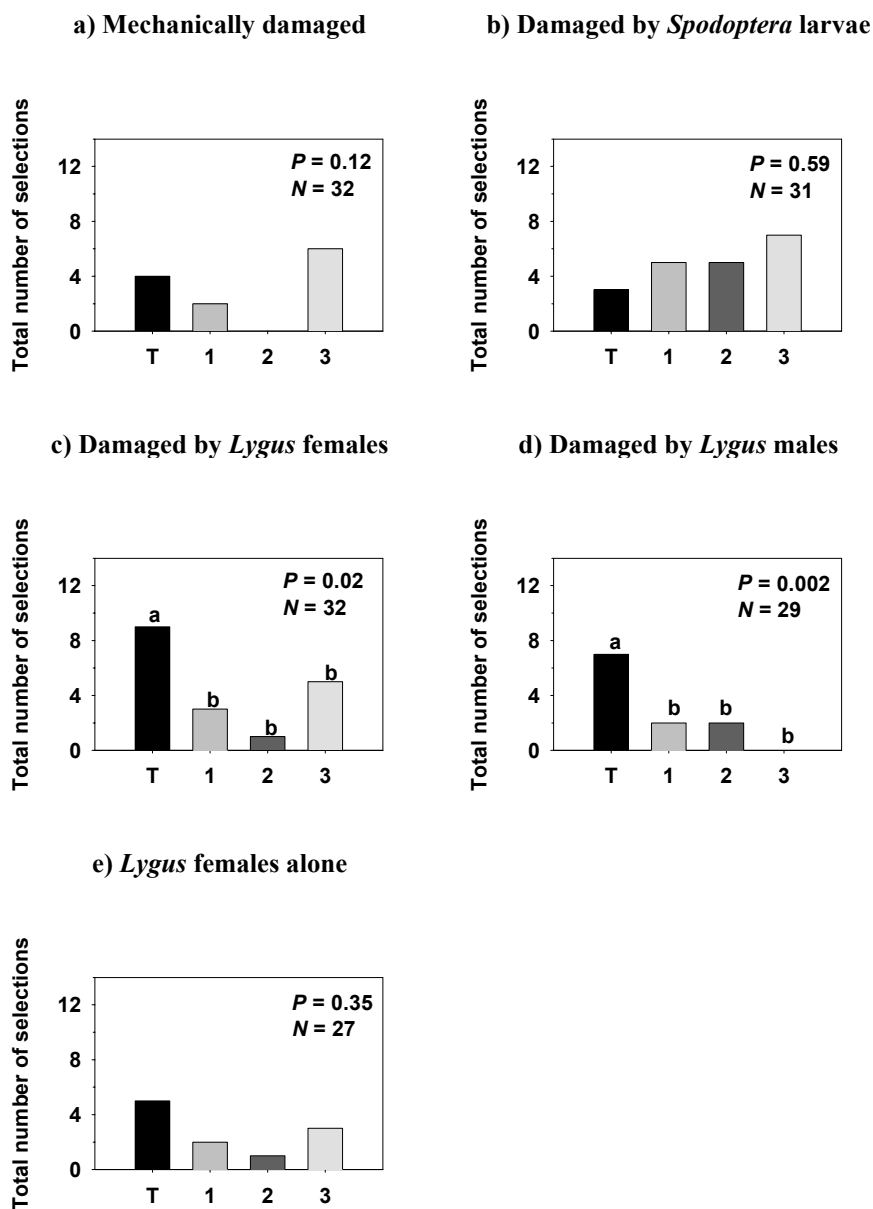


Figure 3. Total number of times *Anaphes iole* females entered the selection region of a four-arm olfactometer. T = field with treatment odor; 1, 2, 3 = three fields with control air. Treatment odors (a) cotton plant damaged mechanically, (b) cotton plant damaged by *Spodoptera exigua* larvae, (c) cotton plant damage by *L. hesperus* females, (d) cotton plant damaged by *L. hesperus* males, (e) *L. hesperus* females alone. *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 HSD ($P < 0.05$).

$df = 3, 148, P = 0.008$; common groundsel, $F = 7.08, df = 3, 140, P < 0.001$; redroot pigweed, $F = 2.86, df = 3, 120, P = 0.04$; annual ragweed, $F = 1.21, df = 3, 108, P = 0.30$) (Figure 4).

A. iole females selected more frequently treatment odor fields compared to control odors in all plant species evaluated (cotton, $F = 3.47, df = 3, 124, P = 0.018$; alfalfa, $F = 11.47, df = 3, 148, P < 0.001$; common groundsel, $F = 5.79, df = 3, 140, P < 0.001$; redroot pigweed, $F = 4.46, df = 3, 120, P = 0.005$; annual ragweed, $F = 4.75, df = 3, 108, P = 0.0038$) (Figure 5). However, significant differences were not detected among mean total times spent in treatment odor fields among plant species ($\chi^2 = 1.427, df = 4, 160, P = 0.84$) (Figure 4).

Discussion

Herbivore-induced plant odors play a major role in the foraging behavior of natural enemies. This study provides the first experimental data demonstrating that the egg parasitoid *A. iole* is attracted to volatiles released by different plants species following *L. hesperus* feeding and oviposition, while it is not attracted to odors derived from cotton plants damaged by *S. exigua* larvae or mechanically. 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 laid in a variety of plant species (Scott, 1977; Jackson and Graham, 1983; Graham et al., 1986). Results from olfactometry experiments suggest that this egg parasitoid relies on volatile cues derived from plant-*Lygus* complexes to locate its host's habitat.

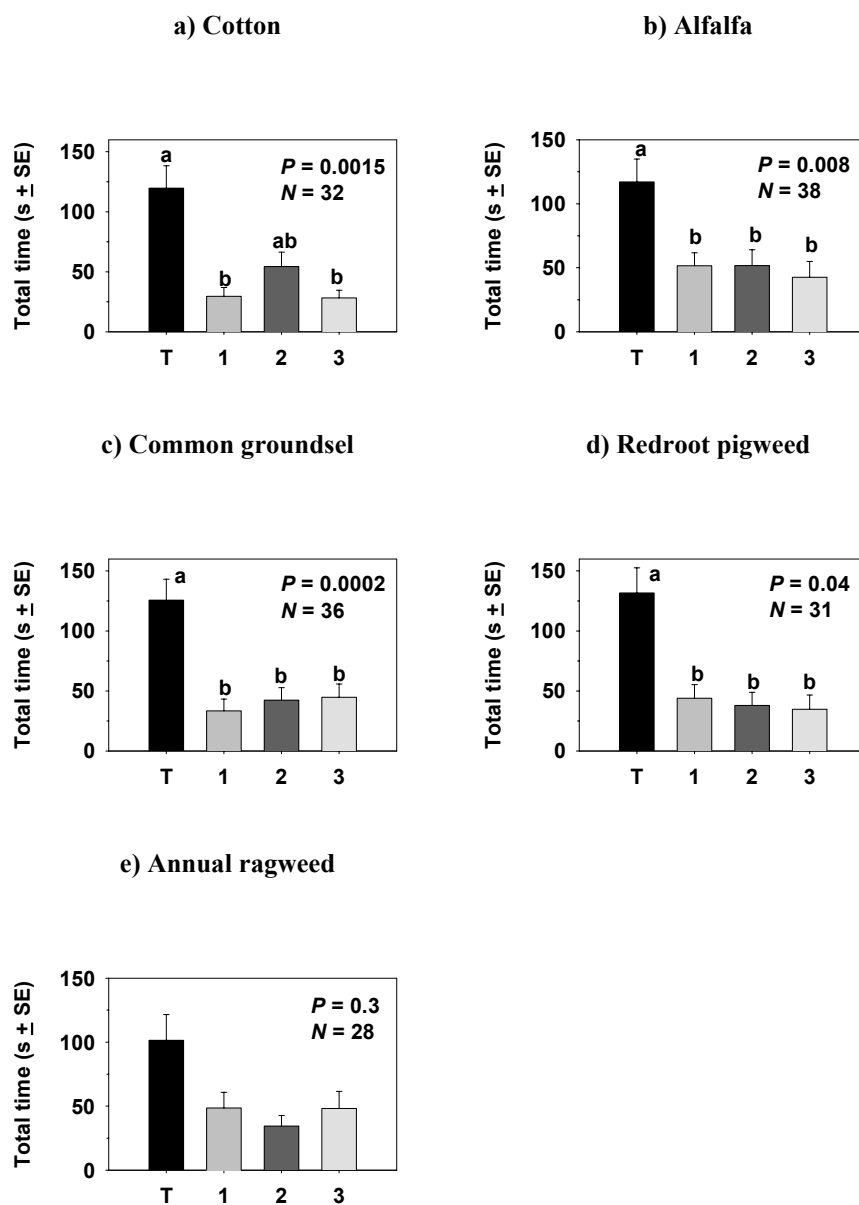


Figure 4. Mean total times ($s \pm SE$) *Anaphes iole* females spent in each odor field of a four-arm olfactometer using different host plants. T = field with treatment odor; 1, 2, 3 = control (odorless) fields. Treatment odors consisted of plant's species exposed to feeding and oviposition by *L. hesperus* 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 HSD ($P < 0.05$).

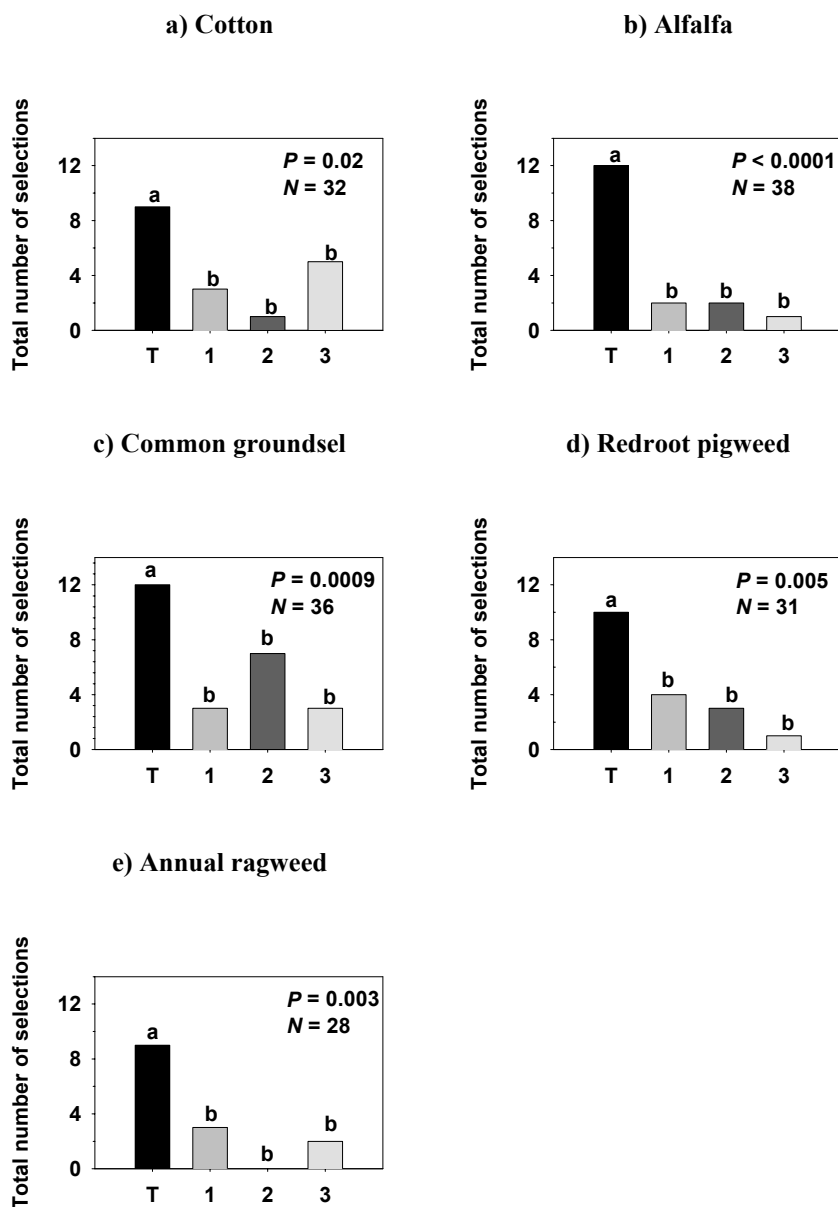


Figure 5. Total number of times *Anaphes iole* females entered the selection region of a four-arm olfactometer using different host plants. T = field with treatment odor; 1, 2, 3 = control (odorless) fields. Treatment odors consisted in different plant species exposed to feeding and oviposition by *L. 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 HSD ($P < 0.05$).

Similar results were obtained by Meiners et al. (2000), who showed that oviposition by the elm leaf beetle *Xanthogaleruca luteola* on field elm leaves (*Ulmus minor* Miller) induced elm to emit volatiles attractive to the specialist parasitoid *Oomyzus gallerucae*. Similarly, the egg parasitoid *Trissolcus basalus* Wollaston was attracted to odors derived from leaves of *Vicia faba* L. laden with eggs of *Nezara viridula* L. (Colazza et al., 2001). According to Hilker et al. (2002) odors from twigs of *Pinus sylvestris* L. bearing egg masses of the pine sawfly *Diprion pini* L. attracted *Chrysonotomyia ruforum*. The elicitor of the attractive volatiles was shown to be present in oviduct secretions coating the eggs of *D. pini*. In contrast, *L. hesperus* eggs are partially embedded in plant tissues, and the release of constitutive compounds seem to be associated with the number of eggs laid on the plant (Rodriguez-Saona et al., 2002). Thus, plants laden with host eggs are attracted to egg parasitoids in olfactometry studies.

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 volitacin isolated from oral secretions of *S. exigua* larvae. The present study showed that *A. iole* females were attracted to odors derived from plants damaged by both genders of *L. hesperus*, whereas they were not attracted to plants damaged by *S. exigua* larvae. These results suggest that *A. iole* discriminates among volatile blends released following feeding damage by different insect species. Several differences among volatile

blends could account for this discrimination, e.g. differences in volatile concentrations and/or changes in the ratios of certain chemicals among treatment odors. In addition, odors emanating from *L. hesperus* frass or other substances left on the plant may account for *A. iole* females attraction. However, the volatile blend emitted by plants following damage by *L. hesperus* females, did not contain the pheromone (*E*)-2-hexenyl butyrate after insects were removed from the plants (Rodriguez-Saona et al., 2002). Further research is needed to elucidate the specific compounds eliciting olfactory responses in *A. iole* females.

Different elicitors have been isolated from oral secretions of several species of lepidopteran larvae, and have been identified as the active compounds involved leading to systemic release of plant volatiles (Mattiacci et al., 1995; Alborn et al., 1997; Baldwin et al., 2001). In the case of *L. hesperus*, insect feeding *per se* was the predominant factor leading to emission of non-constitutive compounds from cotton plants (Rodriguez-Saona et al., 2002). The suitability of *Lygus* eggs as hosts for *A. iole* decreases with time (Stoner and Surber, 1969), thus there should be a strong selective pressure toward finding their host eggs soon after oviposition. The attraction of *A. iole* to plant volatiles induced by *L. hesperus* feeding alone may increase the chances of finding recently deposited eggs, resulting in successful parasitism. However, the elicitors and pathways involved in the release of *L. hesperus*-induced volatiles that are attractive to *A. iole* are unknown.

Since *L. hesperus* adults co-occur with egg stages during reproduction, *A. iole* females may use other indirect cues in addition to plant volatiles to finding its host's

habitat. According to this study, *A. iole* females spent more time in treatment odors derived from *L. hesperus* females alone, though no differences were detected in the frequency of selections compared to controls. This discrepancy between behavioral variables may suggest that the set of cues emanating from *L. hesperus* alone is incomplete. In contrast, odors derived from host-plant complexes elicited more selections by *A. iole* females, pointing to the importance of plant volatiles in host habitat location by this egg parasitoid. Several species of *Trichogramma* have been shown to respond to the sex pheromones of their hosts (Lewis et al., 1982; Kaiser et al., 1989; Noldus et al., 1988, 1991; McGregor and Henderson, 1998). Similarly, the sex pheromone of the spined soldier bug *Podisus maculiventris* Say, contains volatiles used by the egg parasitoid *Telenomus calvus* Johnson (Aldrich, 1996). *L. hesperus* females are known to produce sex pheromones (Strong et al., 1970; Graham, 1988), and the compound (*E*)-2-hexenyl butyrate accounts for the 70-80% of the total volatiles emitted from males and females adults (Aldrich et al., 1988). In addition, calm and agitated females of *Lygus lineolaris* Palisot de Beauvois 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). Thus, *A. iole* females may use cues from adult hosts to reach areas where host matings occur or where eggs were recently laid, increasing the chances of successful parasitism.

Even though volatile blends may vary among the plant species tested in this study, *A. iole* female wasps were similarly attracted to odors derived from different plant-host complexes in the olfactometer. In general, the results of this study suggest that

the various plant-host complexes emit a blend of volatiles that is attractive to *A. iole* females. Previous studies showed that the quality and quantity of the volatiles emitted from plants under herbivore attack varies with many factors, and several natural enemies were able to discriminate among them (Sabelis and van de Baan, 1983; Geervliet et al., 1997). For example, the specialist parasitoid *Toxoneuron (Cardiochiles) nigriceps* preferred odors emanating from cotton, tobacco, and maize infested with its host, *H. virescens*, compared to *H. zea* (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 attracted to *A. iole* females.

In summary, *A. iole* females were attracted to odors derived from plants damaged by *L. hesperus* feeding and oviposition, but they were not attracted to plants damaged mechanically or by *S. exigua* larvae. In the field, *A. iole* females face more complex environments, in which diverse plant-herbivore interactions likely result in emissions of different volatile blends coming from potential host sites. The results of this study provide only 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.

CHAPTER III

**FLIGHT RESPONSE OF *ANAPHES IOLE* TOWARD PLANTS
BEARING HOSTS OR TREATED WITH METHYL JASMONATE
IN LABORATORY AND GREENHOUSE**

Introduction

The role of plant volatiles in host habitat location by natural enemies is well documented (Sabelis and Van de Baan, 1983; Elzen et al., 1986; Geervliet et al., 1997; Tumlinson et al., 1993; Turlings et al., 1991a,b; Birkett et al., 2003). A wide variety of bioassay arenas have been used to study the responses of parasitoids and predators toward odors cues; including olfactometers (Kudon and Berisford, 1981; Sabelis and Van de Baan, 1983; Vet et al., 1983), wind tunnels (Drost et al., 1986; Noldus et al., 1988), and field tests (Nealis, 1986; Papaj and Vet, 1990). The behaviors observed in each arena vary: e.g. wind tunnel and field experiments are used to evaluate flight responses, whereas in olfactometers only walking responses can be measured. According to Steinberg et al. (1992), *Cotesia glomerata* (L.) females showed higher and more consistent responses toward plant-host complexes when tested in a glasshouse flight chamber, compared to wind-tunnel and Y-tube olfactometer assays. These findings suggest that using a variety of bioassay set-ups may provide a better understanding of the complex processes involved in host finding behavior of parasitoids.

In order to determine the nature of long distance cues used by natural enemies, flight responses to both infested and uninfested plants has been traditionally evaluated.

For example, Elzen et al. (1986) found that *Camponotus sonorensis* Cameron females flew toward undamaged plant leaves devoid of hosts and associated products when tested in a wind tunnel. Similarly, *Cotesia flavipes* Cameron and *Cotesia sesamiae* Cameron preferred odors from infested plants over odors from uninfested plants, showing that both specialist parasitoids were able to identify plants attacked by its hosts (Ngi-Song et al., 1996). In the case of *Aphidius colemani* Viereck, female parasitoids were significantly attracted to plant-aphid complexes in a wind tunnel after a pre-flight experience (Grasswitz, 1998). In addition, 40 *Acyrtosiphon pisum* Harris, feeding on broad bean plants for 60-72 hr, induced production of volatiles attractive to *Aphidius ervi* Haliday females, while plants infested by 10 or 20 aphids did not elicit such response (Guerrieri et al., 1999). Maize plants began releasing compounds attractive to *Cotesia marginiventris* Cresson females only several hours after initial injury by the herbivore *Spodoptera exigua* Hubner (Turlings et al., 1990b). Therefore, the processes leading to volatile emissions attracted to natural enemies seems to depend on both herbivore infestation levels and duration of feeding activity (Dicke et al., 1993; Tumlinson et al., 1993).

Jasmonic acid, an elicitor produced from linolenic acid via the octadecanoid pathway in plants, has potential in IPM both for decreasing herbivory (Staswick and Lehman, 1999; Thaler et al., 2001) and increasing natural enemy activity (Dicke et al., 1999; Thaler, 2002; Hilker et al., 2002). Application of jasmonic acid results in emission of volatiles that mimic the volatiles released when plants are fed upon by herbivores (Boland et al., 1995; Dicke and Vet, 1999). For example, tomatoes treated with

exogenous jasmonic acid induced the same array of chemicals as those produced by *Helicoverpa zea* Boddie feeding (Thaler et al., 1996). Field studies on tomatoes showed that *Hyposoter exiguae* Viereck was not affected by induced resistance, whereas syrphid flies were negatively affected (Thaler, 2002). However, arthropod pests including beet armyworms (*S. exigua*), flea beetles (*Epitrix hirtipennis* Melsheimer), aphids (*Myzus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas), and thrips (*Frankliniella occidentalis* Pergande) were reduced in tomato plots treated with jasmonic acid (Thaler et al., 2001). In addition, several egg parasitoids were attracted to volatiles released from the elm and pine twigs treated with jasmonic acid (Meiners and Hilker, 2000; Hilker et al., 2002). Methyl jasmonate, a derivative of jasmonic acid, is another commonly used elicitor. Studies on cotton plants indicated that methyl jasmonate application directly and systemically induced emission of volatiles that may serve as odor cues for natural enemies searching for hosts (Rodriguez-Saona et al., 2001).

Lygus hesperus Knight is an important pest of many crops in the U.S. including cotton and alfalfa (Clancy, 1968; Jackson and Graham, 1983). Management of *Lygus* populations is difficult, mainly because of their high mobility, broad host range, cryptic damage, and poorly understood population dynamics (Ruberson and Williams, 2000). *Anaphes iole* Girault attacks *Lygus* eggs on a variety of host plants, and has potential for use in inundative and conservation biological control programs (Ruberson and Williams, 2000). Rodriguez-Saona et al. (2002) showed that oviposition by *L. hesperus* females induced emission of significant amounts of constitutive volatiles from cotton, and the level of emission was associated with the number of eggs laid on a plant. In addition, the

blend of volatiles induced by *L. hesperus* damage was similar to those released by plants treated with methyl jasmonate (Rodriguez-Saona et al., 2002).

The goal of the present study was to evaluate the flight responses of *A. iole* females toward induced cotton plants in laboratory and greenhouse experiments. The attraction of *A. iole* females toward cotton plants with different levels of *L. hesperus* infestation or treated with methyl jasmonate was examined in a flight chamber. In addition, flight responses of *A. iole* females toward plants infested with *L. hesperus* eggs or treated with methyl jasmonate were assessed in greenhouse cage experiments.

Materials and Methods

Insects

The *L. hesperus* colony was started from cultures obtained from USDA-ARS, Weslaco, Texas. *L. hesperus* were maintained on fresh green beans in the laboratory at $27 \pm 1^\circ\text{C}$, 10:14 (L:D) photoperiod and 50-70% RH (Beards and Leigh, 1960). Mated *L. hesperus* adults (10-15 days old) were starved for 4 h before caging them with plants for experiments (see below).

A. iole were obtained from a laboratory colony maintained on *L. hesperus* eggs at the USDA-ARS, Mississippi State, Mississippi, and USDA-ARS, Weslaco, Texas laboratories. Parasitized *L. hesperus* eggs were held in Plexiglass cages ($28 \times 28 \times 28$ cm) under the environmental conditions noted above. Upon emergence, adult parasitoids were provided *ad lib* with distilled water via absorbent matting and food (honey:water 1:1). *A. iole* females used in the experiments were <4 days old, and were assumed mated

(*A. iole* is protandrous and copulate soon after emergence). All experiments were conducted between 8:00 and 17:00 h.

Plants

Cotton (*Gossypium hirsutum* L., var. Deltapine 491) was planted from seeds in individual pots (13 cm diameter) in the greenhouse under natural light and Texas summer conditions (16:8 (L:D) photoperiod, 50-70% RH, and 25-35°C). The seeds were obtained from commercial suppliers (Delta and Pine Land Company, Scott, Mississippi). Cotton plants (23-30 cm tall) with 4-6 fully expanded true leaves were used in all experiments.

Flight Chamber

A flight chamber was constructed to compare *A. iole* female attraction to plants with different levels of *L. hesperus* infestation. A rectangular Plexiglas box (65 cm long, 35 cm high, 40 cm wide) with two compartments at one end was used (Figure 6); each compartment received either a treatment or control plant (see below). The air was pulled from the outside, charcoal-filtered (Carbon Cap, WhatmanR, Clifton, New Jersey) and humidified before entering the chamber. Each compartment had 4 holes (1.5 cm diam) to allow flowing air to enter the cage, and a flowmeter (Riteflow, Bel-Art Products, Pequannok, New Jersey) was used to regulate the overall airflow at 15ml/min/hole. At the other end of the box, the door had a rectangular window covered with mesh to allow air to flow out of the box. In order to minimize visual contrasts between plants and the background, green paper was placed externally on all walls of the box.

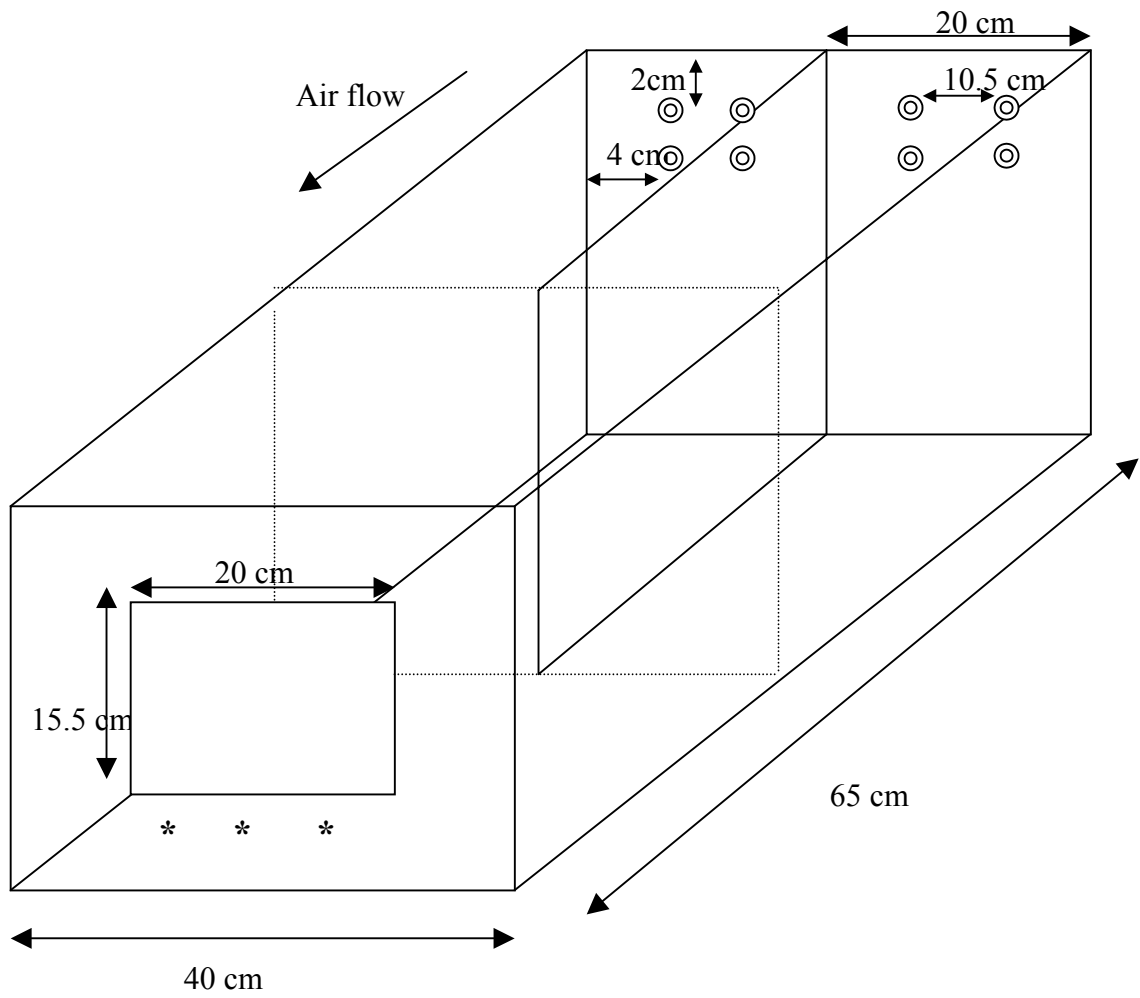


Figure 6. Flight chamber used in laboratory assays to measure flight responses of *Anaphes iole*.

* Release points

A. iole movement between compartments during trials was evaluated by placing 50 females on either treatment (infested plant) or control (uninfested plant) plants in the flight chamber, and counting the numbers moving to adjacent compartments after 2 h. Most females (>90%) were recovered in the compartment in which they were released compared to the adjacent compartment (<9%), and differences were not significant between the numbers of females moving from treatment to control compared to females moving from control to treatment compartments ($G = 0.046$, $df = 1$, $P = 0.83$) (Figure 7).

Flight Response of A. iole Toward Cotton Plants with Different Levels of Infestation

Cotton plants were treated with different infestation levels by exposing them either to 5 (low density), 10 (moderate density) or 20 (high density) *L. hesperus* females, for 24 h prior to experiments in laboratory at $27 \pm 1^\circ\text{C}$, 10:14 (L:D) photoperiod and 50-70% RH. *L. hesperus* females were removed from the cotton plants, and the plants infested with *L. hesperus* eggs were compared against control plants not exposed to *L. hesperus* using the flight chamber. After the airflow was stabilized within the chamber, 200 *A. iole* females were released at the end of the cage opposite from plants: 100 females were released in the center, and 50 wasps were released at 5 cm on either side of the center. After 2 hours the assay was stopped, and the numbers of wasps on each plant and on compartment walls were counted. Six replicates were conducted for each level of infestation, and in each case the infested and uninfested plants were alternated between flight chamber compartments. *L. hesperus* eggs on each plant were counted at the end of each assay using a microscope.

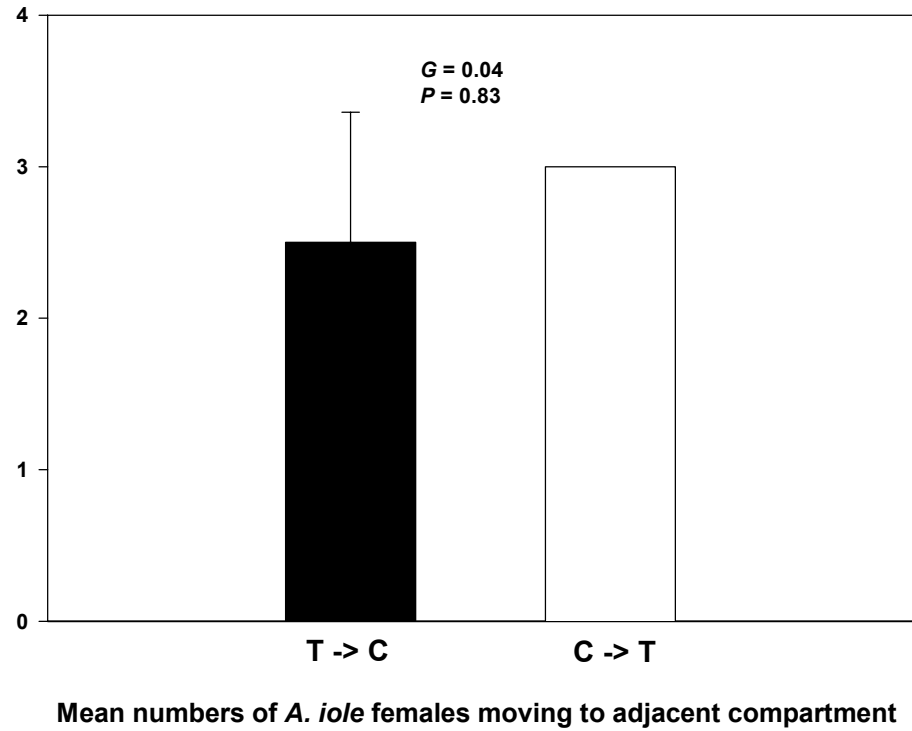


Figure 7. Mean numbers of *Anaphes iole* females moving between compartments of the flight chamber. T->C: wasps that moved from treatment side to control side. C->T: wasps that moved from control side to treatment side.

Flight Response of A. iole Toward Cotton Plants Treated with Methyl Jasmonate

The attraction of *A. iole* females toward cotton plants treated with methyl jasmonate was tested using the flight chamber as mentioned above. Cotton plants were treated with methyl jasmonate (3 ml methyl jasmonate dissolved in 3 ml of acetone and dispersed in 8 liters of water) (treatment plants), or were treated with a control solution (3 ml of acetone per 8 liters of water) (control plants). Applications were made by spraying the top of leaves until run-off using an atomizer, and plants were placed in cages in a greenhouse under natural light and Texas summer conditions (16:8 (L:D) photoperiod, 50-70% RH, and 25-35°C) for 72 h prior to assays.

Attraction of A. iole Females Toward Cotton Plants Bearing L. hesperus Eggs or Treated with Methyl Jasmonate in the Greenhouse

The possible attraction of *A. iole* females toward *Lygus*-infested plants and plants treated with methyl jasmonate was evaluated in greenhouse experiments during August of 2003. Two wood frame cages (1.2 × 1.2 × 1.2 m) made of white cotton fabric screen were placed in a greenhouse. Six potted cotton plants (3 treatment pots and 3 control pots) were arranged in an 80 cm-diam circle in each cage. One cage contained three *L. hesperus* infested plants and three uninfested plants; while the second cage contained three plants treated with methyl jasmonate and three plants treated with control solution. *L. hesperus* infested plants were obtained by caging 10 *L. hesperus* females for 24 h prior to the experiments. Plants treated with methyl jasmonate or controls (both prepared as above) were treated by spraying until run-off using an atomizer, 72 h prior to the experiments. Both cage and plant arrangement inside each cage, were randomized

between replicate trials. Two-hundred *A. iole* females were released in the center of each cage at the beginning of the experiment, and every 1.5 hour, the wasps arriving at each plant were counted and removed from the cage. In order to confirm that leaf size did not differ among plants and influence parasitoid response, all leaves were removed from the plants at the end of the experiments, photocopied and scanned into a computer, and total leaf surface area per plant was measured using Sigma Scan Program. The experiments were conducted between 8:00 and 13:00 h, and replicated six times (dates).

Statistical Analyses

The numbers of *A. iole* females arriving at each compartment of the flight chamber were compared using a heterogeneity chi-square test, and subsequently pooled and analyzed via log-likelihood ratio tests (Zar, 1999). The proportions of *A. iole* females recovered from treatment compartments were compared among the three infestation levels via Tukey-type tests for proportions (Zar, 1999). In addition, linear regression analysis was conducted to determine the relationship between the proportion (arcsine transformation) of female wasps recovered on infested plants and the number of *L. hesperus* eggs laid on the plant.

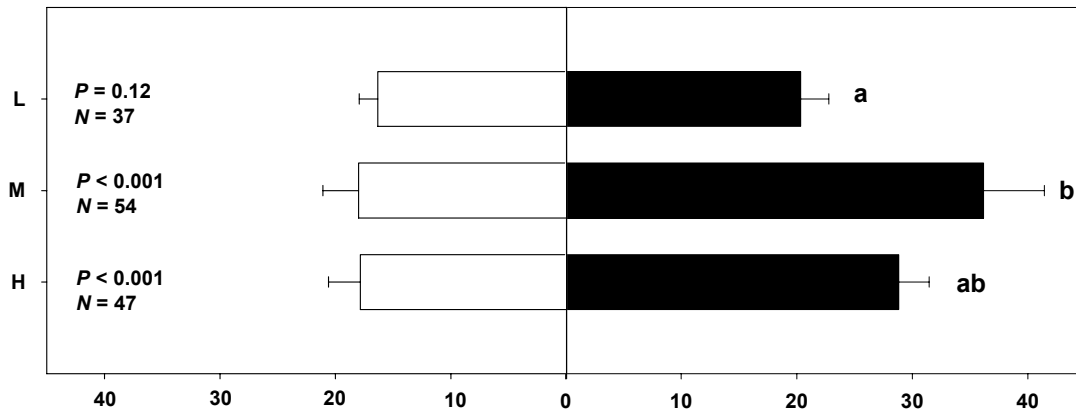
Data from the greenhouse experiments were subjected to two-way analysis of variance (ANOVA) using the proportion (arcsine transformation) of *A. iole* females recovered on cotton plants independent of treatments as the dependent variable, and date and plant position inside cage as factors (Zar, 1999). Attraction of *A. iole* females toward treatment versus control plants in each cage was analyzed via Friedman's two-way ANOVA by ranks, with treatments (*L. hesperus* infested versus controls, methyl

jasmonate-treated versus controls) and date as factors; leaf area was included as a covariable (Zar, 1999). The proportions of females (arcsine transformation) recovered on *L. hesperus* infested relative to control plants and methyl jasmonate-treated relative to control plants were compared via randomized block one-way ANOVA, with date as block and treatments (infested versus methyl jasmonate-treated) as factors (Zar, 1999). For all statistical analyses, a significance level of 0.05% was adopted.

Results

Flight Response of A. iole Toward Cotton Plants with Different Levels of Infestation

The mean numbers of wasps recovered on cotton plants with low *L. hesperus* infestations (7 ± 1 eggs) did not differ from the number recovered on control plants (no eggs) ($G = 2.41$, $df = 1$, $P = 0.12$) (Figure 8). In contrast, significantly more wasps arrived at plants with moderate (41 ± 8 eggs) and high (98 ± 10 eggs) *L. hesperus* infestation levels compared to uninfested plants ($G = 36.58$, $df = 1$, $P < 0.001$; $G = 15.64$, $df = 1$, $P < 0.001$; respectively) (Figure 8). Significantly higher proportions of *A. iole* females were recovered on moderately infested plants compared to low infestation levels ($q = 3.76$, $df = 1$, $P = 0.005$), while differences were not significant between low and high infestations ($q = 2.02$, $df = 1$, $P > 0.2$) (Figure 8). The proportions of females recovered from infested plants relative to uninfested plants were not significantly correlated with the numbers of *L. hesperus* eggs on plants ($F = 1.46$, $df = 1$, $P = 0.24$, $r^2 = 0.08$) (Figure 9).



Mean number of *A. iole* females arriving to each compartment of the olfactometer

Figure 8. Mean numbers (\pm SE) of *Anaphes iole* females recovered in each compartment of a flight chamber. Empty bars indicate unfested plants (controls), filled bars indicate plants infested with *Lygus hesperus* eggs. L = low infestation (7 ± 1 *L. hesperus* eggs); M = moderate infestation (41 ± 8 *L. hesperus* eggs); H = high infestation (98 ± 10 *L. hesperus* eggs). *P* values inset indicate significant differences within columns via heterogeneity *G*-test for pooled data. Different letters among filled bars indicate significant differences via Tukey-type tests for proportions ($P < 0.05$). *N* = mean total numbers of female wasps recovered in the flight chamber.

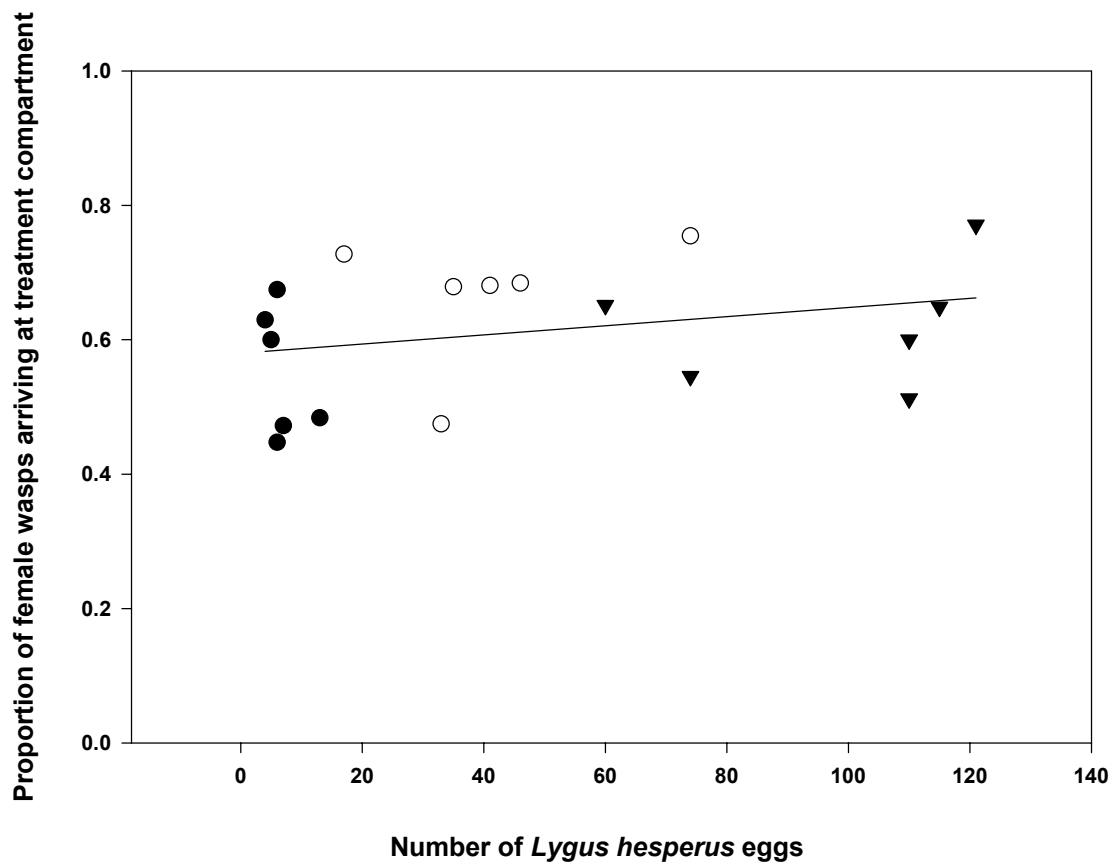


Figure 9. Proportion of *Anaphes iole* females recovered on treatment compartment as a function of *Lygus hesperus* eggs laid in cotton plants. Filled circles indicate plants with low *Lygus* infestations, empty circles indicate plants with moderate *Lygus* infestations, and filled triangles indicate plants with high *Lygus* infestations. ($F = 1.46$, $df = 1, 16$, $P = 0.24$, $r^2 = 0.08$; $y = a + bx$).

Flight Response of A. iole Toward Cotton Plants Treated with Methyl Jasmonate

Similar numbers of *A. iole* females were recovered on plants treated with methyl jasmonate (32 ± 3 wasps) compared to untreated plants (31 ± 4 wasps) in the flight chamber ($G = 0.09$, $df = 1$, $P = 0.76$).

Attraction of A. iole Females Toward Cotton Plants Bearing L. hesperus Eggs or Treated with Methyl Jasmonate in the Greenhouse

Differences were not detected in the proportions of *A. iole* females recovered in each position of the greenhouse cages independent of the assigned treatment during the six days of the experiment (date \times position, $F = 1.68$, $df = 25, 36$, $P = 0.08$; position, $F = 1.55$, $df = 5, 36$, $P = 0.19$; date, $F = 0.06$, $df = 5, 36$, $P = 0.997$). These results indicated that *A. iole* female response toward specific position inside cages were unbiased.

Date \times treatment interactions (*Lygus* infested plants, $F = 0.01$, $df = 5, 23$, $P = 0.94$; methyl jasmonate-treated plants, $F = 1.41$, $df = 5, 23$, $P = 0.26$) and leaf surface areas (*Lygus* infested plants, $t = 0.39$, $P = 0.69$, methyl jasmonate-treated plants, $t = 1.15$, $P = 0.26$) did not have significant effects on the numbers of *A. iole* females recovered from plants. Similarly, the numbers of *A. iole* females recovered from *Lygus* infested plants was not significantly affected by date ($F = 0.24$, $df = 5, 23$, $P = 0.94$), though these numbers varied among dates in the case of methyl jasmonate-treated plants ($F = 4.05$, $df = 5, 23$, $P = 0.008$). The numbers of *A. iole* females recovered from plants bearing *L. hesperus* eggs were > 7 -fold greater than those recovered from uninfested plants ($F = 84.45$, $df = 1, 23$, $P < 0.0001$) (Figure 10). In contrast, differences were not significant between wasps recovered on methyl jasmonate-treated plants and control

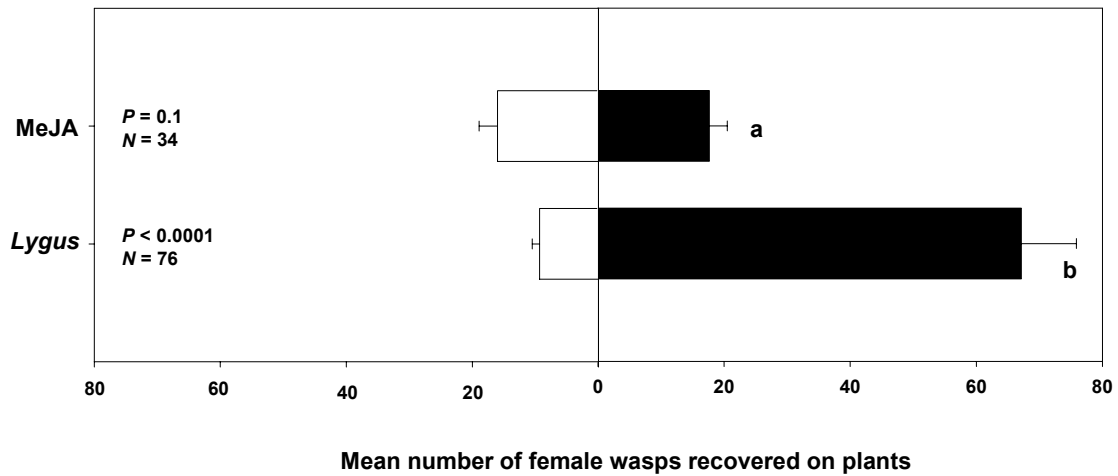


Figure 10. Mean numbers (\pm SE) of *Anaphes iole* females recovered on treatment and control plants in a greenhouse experiment. Empty bars indicate control plants, filled bars indicate treatment plants. MeJA = plants treated with methyl jasmonate versus plants treated with control solution. Lygus = plants infested with *Lygus hesperus* eggs versus uninfested control plants. P values inset indicate significant differences within columns via Friedman's one-way ANOVA by ranks ($P < 0.05$). Different letters above filled bars indicate significant differences via one-way ANOVA (arcsine transformation) ($P < 0.05$). N = total mean number of females wasps recovered.

plants ($F = 2.78$, $df = 1, 23$, $P = 0.1$) (Figure 10). The proportion of female parasitoids recovered from *Lygus* infested plants (88 %) (relative to control plants) was significantly higher than the proportion recovered from methyl jasmonate-treated plants (53 %) (females recovered, $F = 35.35$, $df = 1, 9$, $P = 0.0003$; leaf surface area, $t = 0.35$, $P = 0.73$).

Discussion

Host habitat location is an integral part of the general process of successful parasitism, and considerable difficulties can be encountered in laboratory studies because of the complexity associated with testing long-range attraction. A combination of laboratory and greenhouse experiments, such as that employed in this study, may provide additional insight into understanding the role of volatile signals in the searching behavior of parasitic wasps. Results from flight chamber bioassays showed that *A. iole* females discriminated between plants with moderate (41 eggs) and high (98 eggs) levels of *L. hesperus* infestations, while no differences were detected between methyl jasmonate treated-plants and control plants. The greenhouse experiment showed that *A. iole* females responded to *L. hesperus* infested plants but not to methyl jasmonate-treated plants. These findings suggest that *A. iole* females are attracted to plants laden with host eggs following *L. hesperus* damage, and that more female wasps will arrive at plants bearing high versus low numbers of eggs. Thus, field areas with high infestations of *L. hesperus* will likely attract greater numbers of *A. iole* females, which could result in higher levels of parasitism.

Similar results have been obtained in other tritrophic systems. For example, bean plants, *Phaseolus vulgaris* L., infested with greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, emitted at least three compounds that influenced the flight behavior of *Encarsia formosa* Gahan (Birkett et al., 2003). According to Röse et al. (1998), both *Cotesia marginiventris* and *Microplitis croceipes* Cresson responded strongly to cotton plants damaged by caterpillars compared to undamaged plants in a flight tunnel. Moreover, the specialist predatory mite, *Phytoseiulus persimilis* Athias-Henriot, was attracted to plants infested with its prey *Tetranychus urticae* Koch in olfactometer and greenhouse experiments (Dicke et al., 2003). These studies suggest that specific chemical signals emitted as a result of herbivore damage play important roles in guiding natural enemies toward the microhabitat where hosts or prey are located.

Even though a prior experience with the plant-host complex usually enhances behavioral responses in female parasitoids (Vinson et al., 1977a,b; Sandlan, 1980; Vet, 1983, 1985; Turlings et al., 1990b; Fritzsche-Hoballah et al., 2002), naïve *A. iole* females in this study discriminated between infested and uninfested plants in both flight chamber and greenhouse experiments. Similarly, *Cotesia plutellae* Kurdjumov and *Cotesia glomerata* showed an innate preference for host-infested plants over uninfested ones in flight chamber bioassays (Shiojiri et al., 2000). These results suggest that an innate response is involved in the host searching behavior of the egg parasitoid, *A. iole*.

Results from flight chamber experiments showed that plants with low infestation levels (< 7 eggs) did not elicit a significant response in *A. iole* females, while moderate

and high infestations (> 41 eggs) attracted higher numbers of parasitic wasps than uninfested plants. However, the proportions of *A. iole* females recovered from infested plants did not increase with the numbers of *L. hesperus* eggs on the plants. Assuming that *L. hesperus* feeding increases with the number of adult bugs confined on a plant, the number of *Lygus* eggs may represent an index of damage. Thus, the linear regression results may indicate that olfactory chemoreceptors of *A. iole* females can perceive volatiles induced by *L. hesperus* feeding and oviposition damage only above a certain threshold. However, the increased emissions of constitutive compounds associated with the number of *Lygus* eggs laid on a plant (Rodriguez-Saona et al., 2002) will not trigger higher responses in *A. iole* females. Further research should evaluate the role of constitutive and non-constitutive compounds in the olfactory behavior of this egg parasitoid.

Jasmonic acid is a key plant hormone responsible for a variety of plant responses to herbivory (Staswick and Lehman, 1999), including release of volatile compounds. The present study showed that *A. iole* females did not discriminate between methyl jasmonate-treated plants and control plants in both laboratory and greenhouse experiments. However, higher numbers of *A. iole* females (>87%) were recovered on *L. hesperus* infested plants relative to uninfested in the greenhouse. These findings suggest that plants treated with methyl jasmonate, in the manner and concentration employed in this study, do not elicit significant flight responses in *A. iole* females. Several explanations may account for this lack of response, including quantitative and qualitative differences among volatile blends induced by methyl jasmonate compare to *L. hesperus*

feeding. The same volatile organic compounds that are released after both *L. hesperus* (Rodriguez-Saona et al., 2002) and *S. exigua* feeding (Paré and Tumlinson, 1997a,b) in cotton are also released by methyl jasmonate-treated cotton plants (Rodriguez-Saona et al., 2001). However, plants treated with methyl jasmonate did not induce the emissions of constitutive compounds such as α -pinene, β -pinene, myrcene, limonene, (*E*)- β -caryophyllene, α -humulene, hexanal, (*Z*)-3-hexenol, and hexyl acetate (Rodriguez-Saona et al., 2001). These compounds result from the breakdown of stored lipids that are released after herbivore damage (McCall et al., 1994), and may be important components of volatile blends that are attractive to *A. iole* females. In addition, differences may exist in the ratios of certain volatiles released by cotton plants following *L. hesperus* feeding and methyl jasmonate-treated plants. Further studies are needed to examine the responses of *A. iole* females to induced plants treated with different methyl jasmonate concentrations or other elicitors involved in emission of plant volatiles.

In summary, this study provides valuable information concerning the role of plant volatiles in host habitat location by *A. iole*. Flight chamber and greenhouse experiments showed that *A. iole* females were attracted to cotton plants infested with *L. hesperus* eggs compared to uninfested plants, while plants treated with methyl jasmonate did not elicit significant responses. Greenhouse experiments support the conclusions obtained in the laboratory. Further research is needed to examine the individual volatile compounds involved in host habitat location by *A. iole*, and determine the chemical elicitors that attract *A. iole* females into crops. Laboratory and greenhouse bioassays enable the manipulation and simplification of tritrophic interactions by considering only

specific components of plant-herbivore-parasitoid systems. However, the effects of plant volatiles should be investigated under field conditions, because plants in the field are part of communities where host-infested plants are surrounded by other plants, and herbivore-induced volatile blends occur in mixtures from different herbivore-plant complexes.

CHAPTER IV

GENERAL CONCLUSIONS

Overall, the results of laboratory and greenhouse studies suggested that *Anaphes iole* Girault females use volatile signals when searching for host habitats. *A. iole* females were attracted to odors derived from plants damaged by *Lygus hesperus* Knight feeding and oviposition. Moreover, they discriminated between *L. hesperus* infested and uninfested plants in flight chamber and greenhouse bioassays. The results of both laboratory and greenhouse experiments point to similar conclusions.

The results presented in Chapter II showed that after a pre-assay experience with a plant-host complex, *A. iole* females were attracted to odors emanating from *L. hesperus* females or plants damaged by *L. hesperus* feeding and oviposition in olfactometry experiments. However, plants damaged by *Spodoptera exigua* Hubner or mechanically did not elicit significant responses in *A. iole* females. These results suggested that *A. iole* females use specific volatiles emanating from plant-host complexes when searching for *L. hesperus* eggs. Associative learning is frequently suggested as the underlying mechanism whereby responses to stimuli are acquired or enhanced by linking them to a reinforcing stimulus (Vet and Dicke, 1992). According to Grasswitz (1998), *Aphidius colemani* Viereck females were more attracted to odors derived from plant-host complexes after a brief pre-assay experience. In the case of *A. iole*, female wasps will likely encounter variable densities of hosts on different plant species, and learning and associations made during oviposition may strongly influence

subsequent responses to host-related cues. However, further studies are needed to examine the importance of associative learning in the searching behavior of *A. iole* females.

The results presented in Chapter III showed that *A. iole* females lacking pre-assay experience with plant-host complexes, were strongly attracted to cotton plants harboring *L. hesperus* eggs in flight chamber and greenhouse experiments. *A. iole* females discriminated between *L. hesperus* infested plants and uninfested plants when moderate (41 ± 8 eggs) and high (98 ± 10 eggs) infestations of *L. hesperus* were evaluated, but not when lower (7 ± 1 eggs) infestations were employed in a flight chamber. Thus, volatiles induced by plants damaged by >10 *L. hesperus* females attracted more female wasps than undamaged plants. However, cotton plants treated with methyl jasmonate and untreated controls attracted similar numbers of *A. iole* females in flight chamber and greenhouse experiments, suggesting that volatiles emanating from treated cotton plants were not attractive to *A. iole* females. Further research should examine the individual compounds involved in habitat location by *A. iole*, and determine whether specific chemical elicitors could be used to attract female wasps into crops. In addition, minute parasitoids are unable to fly against strong winds (Keller et al., 1985), thus the effect of air currents on dispersal of *A. iole* females should be considered in field studies.

Parasitoid foraging efficiency is influenced by the interactions of many factors including genetic variation between parasitoid individuals (Vet, 1983; Drost et al., 1988; Prevost and Lewis, 1990), phenotypic plasticity (Lewis and Tumlinson, 1988, Vet et al., 1990; Lewis et al., 1991), and parasitoid physiological state with regard to non-host

resources such as food, egg load, or mating opportunities (Takasu and Lewis, 1993; Jervis and Kidd, 1996). Thus, several factors may affect the behavioral responses of female parasitoids toward volatile signals when searching for hosts. Even though all females assayed in Chapter II were observed under similar environmental conditions, and all had pre-assay experience, high variability was evident among female parasitoids; e.g., 20-40% of *A. iole* females did not move from the center of the olfactometer. Differences in individual physiological states may account for this variability, e.g. food satiation, and oviposition experience. Similarly, *A. iole* females differed substantially in their flight responses to infested plants in flight chamber and greenhouse bioassays: only 20-40% of total females were recovered on plants following the releases. In contrast to olfactory bioassays, *A. iole* females lacked pre-assay experience with the plant-host complex in flight chamber and greenhouse experiments. While individual female parasitoids were allowed to walk in the olfactometer, numerous *A. iole* females were released and allowed to fly toward infested and uninfested plants in the flight chamber and greenhouse assays. Releasing large numbers of female parasitoids in these experiments had several advantages and disadvantages. For example, the release of many parasitoids allowed us to simultaneously test the response of numerous females in one replicate, however, the presence of conspecifics may affect the behavioral response of *A. iole* females. Future studies should examine these and others factors likely affecting the behavioral responses of *A. iole* females, and potential sources of variability must be included in the analyses.

In hymenopteran parasitoids, reproductive success depends on the ability of female wasps to find their hosts. Thus, the types of behavior associated with host searching and selection in parasitoids will be strongly influenced by natural selection. 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 host foraging. *L. hesperus* is a polyphagous herbivore, feeding on > 140 cultivated and non-cultivated plants (Scott, 1977). Thus, *A. iole* females in the field will face the challenge of finding hosts in a variety of plant species. In this scenario, some genetically fixed responses to volatiles from the most important host plants may be expected (Vet and Dicke, 1992), or responses may be elicited by a component that all host plants have in common (Martin et al., 1990). Results from this thesis suggest that *A. iole* females are attracted to odors emanating from different plant-*Lygus* complexes. Thus, the blends of volatiles released by plants following *L. hesperus* feeding may share chemical compounds that are attractive to *A. iole* females.

The role of volatile plant signals in recruiting natural enemies appears to be complex and dynamic. The chemical composition of herbivore-induced plant volatiles has been studied for numerous plant-herbivore systems (Dicke et al., 1990b; Turlings et al., 1993a, 1995; Takabayashi et al., 1995). In general, chemical analyses indicate that differences between blends of herbivore-induced plant volatiles are largest between plant species and smallest within plants species infested by different herbivores (Dicke et al., 1990a; Turlings et al., 1990a, 1993a; De Moraes et al., 1998; Du et al., 1998; Takabayashi et al., 1995). However, whether blend differences are biologically relevant

depends on the sensitivity of the animals perceiving them. Arthropods are well known for a well-developed sense of smell (Mustaparta, 1984). Neurophysiological studies coupled with behavioral analyses are necessary for a better understanding of the role of herbivore-induced plant volatiles, and their reliability as indicators of herbivore identity. According to Rodriguez-Saona et al. (2002), *L. hesperus* feeding and oviposition induces volatile emissions in cotton and maize. Results from this study revealed that *A. iole* females employ volatile signals to locate their host's habitat, and that they are attracted to plants damaged by *L. hesperus* feeding and oviposition. These results are the first experimental data showing that the egg parasitoid *A. iole* is attracted to odors derived from plant-host complexes. Further research should seek to identify the chemical elicitors involved in the release of plant volatiles attractive to *A. iole* females. Such knowledge may allow us to develop crop cultivars that produce *Lygus*-induced signals more quickly and at lower herbivore infestation levels. In addition, chemical elicitors could be used in the field to recruit and/or arrest more parasitoids in crops. However, both large-scale and long-term field studies are necessary for developing efficient and sustainable methods to exploit plant signaling in biological control.

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