Journal of Chemical Ecology, Vol. 30, No. 8, August 2004 (© 2004)

BEHAVIORAL RESPONSE OF *Lygus hesperus* TO CONSPECIFICS AND HEADSPACE VOLATILES OF ALFALFA IN A Y-TUBE OLFACTOMETER

J. L. BLACKMER,[1,*] C. RODRIGUEZ-SAONA,[1,2] J. A. BYERS,[1] K. L. SHOPE,[1] and J. P. SMITH[1]

[1]*Western Cotton Research Laboratory, USDA-ARS, 4135 E. Broadway Rd., Phoenix, Arizona 85040, USA*

(Received April 8, 2003; accepted April 7, 2004)

Abstract—The western tarnished plant bug, *Lygus hesperus* Knight, feeds and develops on a variety of weeds in the spring, with later generations moving to alfalfa and cotton where severe damage to reproductive structures can occur. A synthetic attractant for monitoring or mass-trapping *L. hesperus*, or the identification of potential attractants for natural enemies, would be useful tools for integrated pest management programs. Studies investigated the response of naive and experienced fifth-instar and adult *L. hesperus* to odors associated with conspecifics and alfalfa, *Medicago sativa* L. Fifth-instar *L. hesperus* responded to all plant/insect combinations, whereas female *L. hesperus* only responded preferentially to vegetative and flowering alfalfa where conspecifics had fed for 24–72 hr, and to vegetative alfalfa where conspecifics were added approximately 30 min before the test began. Males were not attracted to headspace volatiles from any of the alfalfa treatments. Analysis of headspace volatiles showed that (*E*)-2-hexanal, (*Z*)-3-hexen-1-ol, α-pinene, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, limonene, (*Z*)-ocimene, (*E*)-β-ocimene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E*, *E*)-α-farnesene are emitted from both vegetative and flowering alfalfa. Indole and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were only detected in flowering alfalfa. Damage to alfalfa by *L. hesperus* increased emissions of (*Z*)-ocimene, (*E*)-β-ocimene, (*E*)-β-caryophyllene, and (*E*, *E*)-α-farnesene, while β-pinene, myrcene, methyl salicylate, and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were only detected from damaged plants. Thus, individual or mixtures of these alfalfa volatiles may be useful

[2]Current address: Department of Botany, University of Toronto, Toronto, ON M5S 3B2, Canada
[*]To whom correspondence should be addressed. E-mail: jblackmer@wcrl.ars.usda.gov

0098-0331/04/0800-1547/0

as attractants for capturing nymphs and adult females of *L. hesperus* in the field.

Key Words—Miridae, western tarnished plant bug, Y-tube olfactometer, alfalfa, host location, herbivore-induced volatiles

INTRODUCTION

Lygus hesperus Knight (Heteroptera: Miridae), the western tarnished plant bug, is the most common species of *Lygus* bug found in the western United States. It is an extremely polyphagous species found on over 100 species of plants in 24 families (Scott, 1977). Many important crops such as beans, strawberries, peaches, cotton, and various seed crops are damaged by this insect. *Lygus* bugs prefer to feed on the meristematic and developing reproductive tissues of their hosts, which causes abortion of buds, blooms, and fruits, destruction of seeds or ovules, deformation of fruits, initiation of abnormal secondary vegetative growth, and necrotic spotting of fruits (Strong, 1970; Leigh, 1976; Mauney and Henneberry, 1984; Leigh et al., 1988). Although *L. hesperus* is also a facultative predator of insects (Wheeler, 1976; Agusti and Cohen, 2000), potential benefits are outweighed when large numbers move into crops where the preferred feeding habit might be herbivory.

A synthetic attractant would be a useful tool for monitoring or mass trapping *Lygus* spp. Most efforts to develop an attractant have focused on identifying sex pheromones of plant bugs (Gueldner and Parrott, 1978; Aldrich et al., 1988; Chinta et al., 1994; Groot et al., 1999; Ho and Millar, 2002), and although likely components have been determined, responses to these compounds in the field have been disappointing (Hedin et al., 1985; McLaughlin, 1998; Ho and Millar, 2002). A complimentary approach would be to identify the host-plant cues that attract *Lygus* spp., which in turn could be used in combination with visual sticky traps. Additionally, as herbivore-induced volatiles enhance the foraging success of many natural enemies (Dicke and Sabelis, 1988; Turlings et al., 1990; Agelopoulus and Keller, 1994; Powell et al., 1998), a better understanding of *Lygus*-plant interactions could lead to novel ways of using attractants to control this pest. Alfalfa, *Medicago sativa*, was chosen for our study because it is a preferred host for *L. hesperus*, and most likely to provide compounds that would be highly attractive to this species (Sevacherian and Stern, 1974, 1975; Whitbey, 1999).

The present study was undertaken to determine whether headspace volatiles released by alfalfa are used by fifth-instar and adult *L. hesperus* during host location. We also were interested in establishing whether volatiles associated with conspecifics feeding on alfalfa enhanced or inhibited the host-location response, and whether experienced *L. hesperus* were better at locating the host plant than naive individuals. Furthermore, volatile constituents relative to plant phenology

(vegetative or flowering), time of day, and insect damage were identified and their potential roles discussed.

METHODS AND MATERIALS

Insect Rearing and Maintenance. Lygus hesperus nymphs and adults were collected from alfalfa fields located at The University of Arizona—Maricopa Agricultural Center, Maricopa, AZ. To maintain genetic diversity, feral individuals were added to the colony 3–4 times per year. Green beans, carrots, pink bollworm eggs, and 10% sucrose solution were provided as food, and were changed every other day. The green beans and carrots also served as oviposition substrates, which were placed in 2 × 14-cm diam Petri dishes lined with filter paper, and then maintained in an incubator until first-instar *L. hesperus* emerged. Newly emerged nymphs were placed in 8.5 × 12.5-cm diam paper cartons where the center of each lid had been replaced with nylon organdy to allow air circulation. Nymphs were maintained with food and sucrose solution until they were needed for experiments. Insects were maintained in an incubator at 23 ± 2°C, 55 ± 15% RH, under a light–dark regime of 14L:10D.

Plant Maintenance. Medicago sativa (cv.'Cuf 101') was planted in 1-l pots containing a standard soil mixture and maintained in a greenhouse at 25 ± 5°C and 50–85% RH. Natural lighting provided a light-dark regime of 12L:12D during the trials. Plants were watered and fertilized regularly by means of a drip irrigation system. A 1:1 mixture of all-purpose Scotts Miracle-Gro Excel (21-5-20) and cal-mag Miracle-Gro Professional (15-5-15) was applied at a rate of 1/100 l of water.

Y-tube Olfactometer Setup. Bioassays were conducted in a 40-mm diam × 36-cm long glass, Y-tube olfactometer that had 50° inside angle. Incoming air was filtered through activated charcoal and humidified with doubly distilled, deionized water. The filtered air was split between two, 2-l holding chambers; one chamber served as a control (clean air) and the other chamber held the test material (i.e., plant or plant + insect). From each holding chamber, the air passed into the respective arms of the Y tube, and then through a series of screens before entering the main tube of the olfactometer. Airflow through the system was maintained at 4.8 l/min (=3.8 m/min inside the tube) by an inline flowmeter (Gilmont Instr., Barnant Co., Barrington, IL.). A smoke test demonstrated laminar airflow in both arms and throughout the olfactometer.

A 60-cm long, wide-spectrum fluorescent lamp (GE, F20T12-PL/AQ) was positioned 22 cm above the arms of the Y tube. Before each trial, light intensity over each arm was measured with a light meter (ExTech Instr. Model 401025, Zefon International, St. Petersburg, FL), and the tube was adjusted until intensity was the same in both arms. Light intensity averaged 810.6 ± 7.2 (mean ± SE) lux. The Y-tube setup was surrounded by a 50 × 70 × 60-cm black fabric enclosure, and

the holding chambers containing the treatments were placed outside the enclosure to eliminate visual cues. Holding chambers were illuminated by 40-W incandescent bulbs, which provided approximately 3000 lux to the plant material during the trials.

Bioassays. Approximately 30 min before trials were initiated, fifth-instar or 7- to 10-d-old adult *L. hesperus* were placed into individual holding/release tubes. Each tube was constructed from a 15-cm long, 5-ml plastic pipette from which 0.5 cm of the bulb and 8 cm of the pipette tip were removed. The cut end of the pipette tip was covered with organdy. A nymph or adult was placed inside the tube, and the end where the bulb tip had been removed was sealed with a cork. Tubes containing bugs were then placed into a separate holding container, so they would not be exposed to test odors before their release. Experienced nymphs and adults were obtained by allowing insects to feed on plants that were the same age as the treatment for 24–72 hr before being placed into the holding/release tubes.

Plants, with or without *L. hesperus*, were then placed into one of the holding chambers. For vegetative alfalfa treatments, we used 30-cm tall, intact plants in which their root system was wrapped with moist paper towels and then placed inside a plastic sleeve. Because plants are much larger (0.5 m^3) by the time they begin to flower, we used five stems, which were cut 30 cm below the flowers and treated as indicated above for vegetative plants. Headspace volatile profiles of these vegetative and flowering alfalfa treatments were comparable (in terms of compounds detected and relative amounts) to the headspace volatiles from greenhouse collections (unpublished data). For the treatments that consisted of plants plus conspecifics, we used a 1:1 sex ratio of adults, when adults were tested, and similar aged nymphs, when nymphs were tested. For longer term (24–72 hr) feeding treatments, we placed adults or nymphs inside fine-mesh bags, on the plants that were to be tested. Halfway through each olfactometer trial, fresh plant material was placed in the holding chamber, and the chamber was moved to the opposite side of the Y-tube setup. This eliminated any potential bias due to odor-source location.

At the beginning of each trial, the cork was removed from the holding/release tube, and the open end was placed at the downwind end of the Y tube. Each insect was given 5 min to respond to the treatment, and a choice for the left or right arm of the olfactometer was noted when the insect went 1 cm past the Y junction. The following measurements were recorded for all individuals: time when insect exited the release tube, percentage leaving the release tube, percentage that walked upwind and selected an arm of the Y tube, and response time to first choice. Temperature and RH in the olfactometer were maintained at 24.9 $\pm$ 0.1°C and 80.5 $\pm$ 8.5%, respectively.

Trials usually consisted of 20 insects that were tested only once, and for each individual a clean Y tube was used. Trials were replicated until plant or plant/insect treatments had a minimum of 60 individuals that had responded. The

null hypothesis that *L. hesperus* showed no preference for either olfactometer arm (a response equal to 50:50) was analyzed with a Chi-square goodness of fit test after correcting for continuity with Yates' correction factor (Zar, 1984). Time required for nymphs and adults to exit the release tube, percentage leaving the release tube, percentage that walked upwind to an arm, and response time to first choice for controls versus treatments were compared by two-way ANOVAs. Count data were transformed using square-root or log transformations and percentage data were arcsine transformed to meet the requirements of normality and homogeneity of variance before analysis.

Headspace Volatile Collections. To determine the types of compounds that *L. hesperus* might use to locate a host plant, we investigated the effects of plant phenology (vegetative or flowering), time of day, and insect damage by *L. hesperus* nymphs and adults on volatile emissions in alfalfa. Volatiles from vegetative and flowering alfalfa were collected using a push/pull apparatus constructed after Heath and Manukian (1994), and described in detail in Rodriguez-Saona et al. (2001), which allowed for simultaneous collections from four different plants. Plants were maintained in an air-conditioned greenhouse under natural light (~55,000 lux) and Arizona summer–autumn conditions (12L:12D regime, and 28°C day, 24°C night). On each sampling date, five to 10 stems from each plant were inserted into each glass cylinder (42.5 cm high and 18 cm diam; Analytical Research Systems Inc., Gainesville, FL), cotton was packed around the stems, and the cylinders were sealed off with guillotine-like bases that contained circular openings for the stems. Moist and dry air passed over the plants within the cylinders at a rate of 3 l/min. Near the base of the glass cylinders, eight openings allowed for the attachment of Super Q adsorbent collection traps (Alltech Assoc. Inc., Deerfield, IL). The tip of each collection trap was placed a few millimeters from the plant, and air was pulled through the trap at a rate of 1 l/min.

To test for diurnal variation in volatile emissions from vegetative and flowering alfalfa, Super Q traps were collected and processed at 4-hr intervals during the day (06:00–10:00, 10:00–14:00, and 14:00–18:00 hr). A total of eight vegetative and six flowering plants were sampled. In a second experiment, we tested the effect of damage by *L. hesperus* on volatile emissions from vegetative and flowering alfalfa. Thirty 4–5th instar nymphs or adults (7–10 days old; 1:1 sex ratio) were allowed to feed on the plant material for 48–72 hr before collecting headspace volatiles. These time periods and densities of *L. hesperus* are sufficient to induce volatile emissions in plants (Rodriguez-Saona et al., 2002). Nymphs and adults remained on the plant material during aeration. Volatiles were collected for eight consecutive hours (09:00–17:00 hr). Volatiles from damaged and undamaged plants were collected concurrently on a particular date. Each treatment was replicated 4–7 times.

After volatile collections, traps were rinsed with 180 μl of methylene chloride for extraction of the volatile compounds. Samples (1 μl) were analyzed with a

Hewlett-Packard gas chromatograph (GC model 6890) equipped with an HP1 methyl siloxane column (30 m × 0.32 mm ID, 0.25 μm film), a capillary injector system, and a flame ionization detector. Helium was used as the carrier gas at a linear flow velocity of 40 cm/sec. After injection, column temperature was maintained at 50°C for 3 min, increased by 5°C/min to 190°C, and then maintained at this temperature for 5 min. Individual compounds in the blend were identified using synthetic standards from commercial sources and by GC-mass spectroscopy (as described by Rodriguez-Saona et al., 2001). Spectral data were compared with spectra from the National Institute of Standards and Technology (NIST, 1995) database.

Amounts of each compound (ng/hr) were estimated by comparison of their peak areas with that of the internal standard (600 ng of *n*-octane in 5 μl of methylene chloride). Although this method to analyze plant volatiles does not take into account the fact that different compounds with the same amount might have different responses in terms of GC areas, it has commonly been used to compare volatile emissions between insect-damaged and undamaged plants (e.g., Turlings et al., 1998; Rodriguez-Saona et al., 2002). The effects of plant phenology (vegetative and flowering) and time of day (06:00–10:00, 10:00–14:00, and 14:00–18:00 hr) on volatile emissions in alfalfa were analyzed by using two-way MANOVA (SYSTAT, 1998). Similarly, the effect of damage by *L. hesperus* nymphs and adults on volatile emissions from vegetative and flowering alfalfa was analyzed with MANOVA. Amounts were log transformed before analyses to meet the assumptions of normality and homogeneity of variance. Compounds were classified based on their biosynthetic origin, so that lipoxygenase products (which include the so-called green-leaf volatiles = GLVs), monoterpenes, homoterpenes, and sesquiterpenes were analyzed as groups (*sensu* Paré and Tumlinson, 1998). In the same manner, indole and methyl salicylate (shikimic acid pathway) were analyzed as a group. Only groups containing a minimum of two compounds sharing the same biosynthetic pathway were considered for MANOVA. The insect-produced hexyl butyrate (Ho and Millar, 2002), was not included in the analysis.

RESULTS

Bioassays. The response of naive and experienced fifth-instar *L. hesperus* was similar ($t = -0.002$, df = 14, $P = 0.99$), so data were pooled before Chi-square analyses were performed. For fifth-instar *L. hesperus*, volatile compounds emitted from plant and plant-insect combinations were always preferred over clean air ($P < 0.05$ in all cases; Figure 1). The strongest upwind responses were to vegetative alfalfa on which nymphs had fed for 24–72 hr, and to flowering alfalfa where nymphs were added approximately 30 min before the test began (86.6 and 81.1 % responded to treatments, respectively).

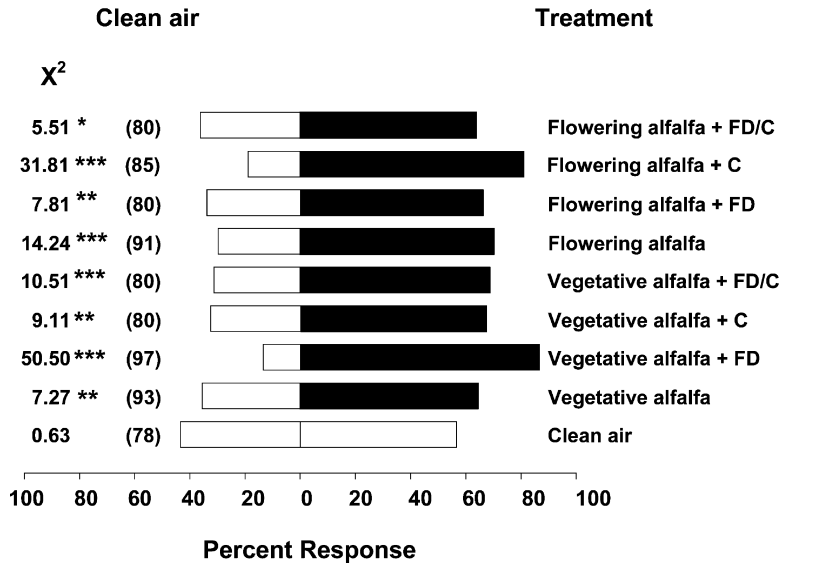

FIG. 1. Response of fifth-instar *Lygus hesperus* to headspace volatiles associated with vegetative and flowering alfalfa with or without feeding damage (FD) and conspecifics (C). Number in parentheses represents sample size; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Similar to nymphs, previous exposure to the treatments did not enhance or inhibit adult response ($t = -0.27$, df $= 14$, $P = 0.79$ for males; $t = -0.16$, df $= 14$, $P = 0.87$ for females) so data for naive and experienced adults were pooled before analyses. Females exhibited a significant response to vegetative and flowering alfalfa on which conspecifics had fed for 24–72 hr, and to vegetative alfalfa where conspecifics were added before the test began (63.5, 71.0, and 65.3 % responded to treatments, respectively; Figure 2). Males, however, never exhibited a significant preference to any of the plant or plant-insect combinations when compared to clean air (Figure 3). Male response was significantly inhibited by the vegetative and flowering alfalfa treatments, and to a lesser extent, by vegetative alfalfa on which conspecifics had fed for 24–72 hr.

In terms of their behavioral response to the various treatments, exit time from the release tube was significantly influenced by the insect stage and sex. Males exited the release tube after approximately 13 sec, females after 23 sec, and nymphs after 45 sec (Table 1A). The percentage of bugs that left the release tube was similar (94%) regardless of the treatment; however, the stage of the insect was important (Table 1B). Only 85 % of the nymphs left the release tubes, while 97% of the adults exited. The propensity to walk upwind and make a choice was influenced by the stage of the insect. Only 44% of the nymphs made a choice,

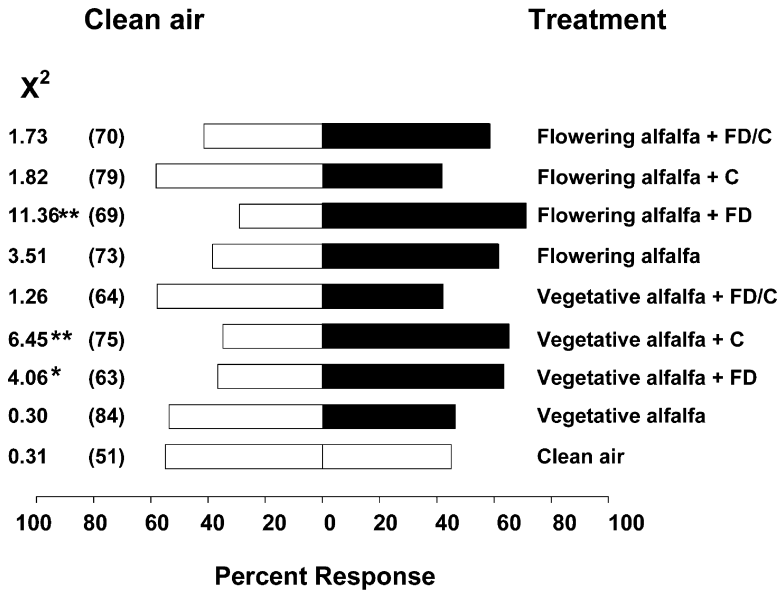

FIG. 2. Response of female *Lygus hesperus* to headspace volatiles associated with vegetative and flowering alfalfa with or without feeding damage (FD) and conspecifics (C). Number in parentheses represents sample size; $^{*}P < 0.05$; $^{**}P < 0.01$.

while 80 % of the females and 82% of the males made a choice between arms of the olfactometer (Table 1C). The time required to make a choice was not influenced significantly by the treatment, but stage was critical (Table 1D). Nymphs made their first choice in 169 sec, females required approximately 108 sec, and males chose after 75 sec.

Headspace Volatile Collections. All three factors that we evaluated: plant phenology (vegetative or flowering), time of day, and insect damage by *L. hesperus*, affected volatile emissions in alfalfa (Tables 2 and 3; Figures 4 and 5). Flowering alfalfa emitted higher amounts of volatiles as compared to vegetative alfalfa (Tables 2 and 3, MANOVA, significant Plant effect, Figures 4 and 5). Compounds such as (*Z*)-3-hexenyl butyrate from the lipoxygenase pathway, the monoterpenes α-pinene, limonene, (*Z*)-ocimene, (*E*)-β-ocimene, and linalool were emitted in higher quantities from flowering alfalfa. Indole and the homoterpene (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene were only detected from flowering alfalfa (Figures 4 and 5).

Volatile emissions in alfalfa were also affected by time of day (Table 2; significant Time effect, Figure 4). Most compounds were emitted in larger quantities from 06:00–14:00 hr, and tended to decline from 14:00–18:00 hr (Figure 4).

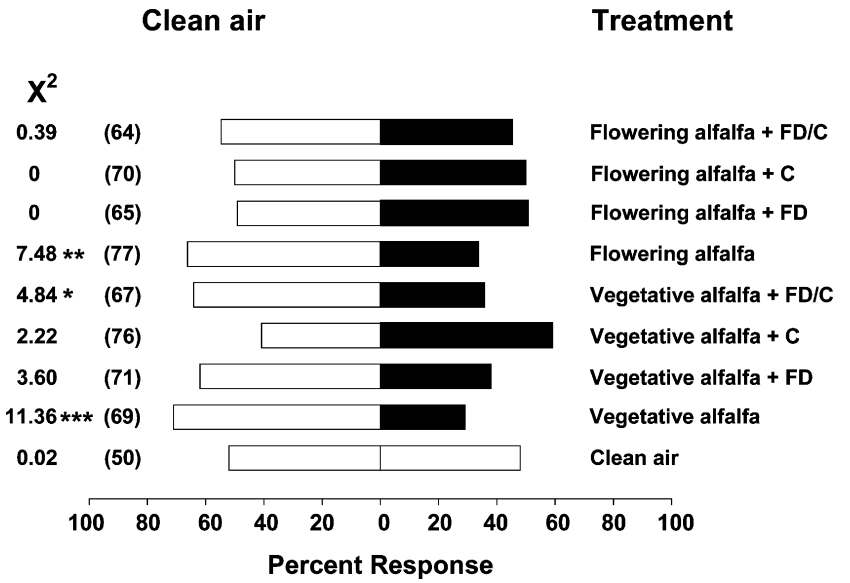

FIG. 3. Response of male *Lygus hesperus* to headspace volatiles associated with vegetative and flowering alfalfa with or without feeding damage (FD) and conspecifics (C). Number in parentheses represents sample size; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

However, there was a clear pattern of emissions for each volatile group (Table 2). Lipoxygenase products such as (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate were emitted in larger amounts soon after the beginning of photophase (06:00–10:00 hr), while several terpenes were emitted in larger amounts at midphotophase, from 10:00–14:00 hr. Compounds specific to flowering alfalfa were also emitted in larger quantities from 10:00–14:00 hr (Figure 4). This latter time interval was used to conduct our behavioral bioassays.

Damage to alfalfa by *L. hesperus* feeding increased volatile emissions (Table 3, significant Damage effect, Figure 5). Damage by adults caused greater induced responses in alfalfa as compared to nymphs. *Lygus* feeding caused increased emissions of the monoterpenes (*Z*)-ocimene, (*E*)-β-ocimene, and linalool, and the sesquiterpenes (*E*)-β-caryophyllene and (*E*,*E*)-α-farnesene. However, the effects of damage on sesquiterpene emissions were dependent on plant phenology (significant Plant $\times$ Damage interaction; Table 3). Damage by *L. hesperus* increased sesquiterpene emissions in vegetative but not flowering alfalfa. Compounds detected only from damaged plants included β-pinene, myrcene, methyl salicylate, and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. The *L. hesperus*-produced volatile, hexyl butyrate, also was detected in large quantities when adults were present (data not shown).

TABLE 1. TWO-WAY ANOVAS FOR EXIT TIME(S), PERCENTAGE EXITING RELEASE TUBE, PERCENTAGES THAT PROGRESSED UPWIND, AND TIME TO FIRST CHOICE(S) FOR FIFTH-INSTAR, AND MALE AND FEMALE *L. hesperus*

Source of variation	df	Mean square	*F* ratio	*P*
A. Treatment[a]	1	1.0	0.9	0.4
Stage[b]	2	56.8	51.64	<**0.001**
Interaction	2	0.3	0.3	0.8
Residual	66	2.8		
Total	71			
B. Treatment[a]	1	0.00005	0.003	1.0
Stage[b]	2	0.5	33.1	<**0.001**
Interaction	2	0.02	1.2	0.3
Residual	66	0.03		
Total	71			
C. Treatment[a]	1	0.002	0.1	0.8
Stage[b]	2	1.2	40.2	<**0.001**
Interaction	2	0.004	0.2	0.9
Residual	66	0.1		
Total	71			
D. Treatment[a]	1	22.8	0.05	0.8
Stage[b]	2	51294.7	107.8	<**0.001**
Interaction	2	396.5	0.8	0.4
Residual	66	475.5		
Total	71			

Note. Bold type indicates significant treatment effects ($P < 0.05$).
[a] Treatment refers to clean air versus plant and plant–insect combinations.
[b] Stage refers to fifth-instar or adult (male and female) *L. hesperus*.

DISCUSSION

Our data demonstrate that developmental stage and sex of *L. hesperus* influence their responsiveness and attractiveness to alfalfa odors. In our trials, nymphs that progressed upwind were more responsive to odors emanating from plant and plant-insect combinations than adults (Figures 1–3). The fact that nymphs are capable of making such choices would enable them to locate their host if they became dislodged, which would be critical as their dispersal ability is limited. For the relatively large percentage of nymphs that did not walk upwind, we suspect that the physiological state of the nymph greatly influenced its response. In several instances, nymphs molted shortly after the bioassay ended, and these individuals never moved upwind. Additionally, the type of food consumed (i.e., pink bollworm eggs versus plant material), time since last meal, and size of the meal could influence their tendency to respond to plant odors. The nymph's slower upwind progress, which would make them more susceptible to predation under

TABLE 2. TWO-WAY MANOVA FOR EFFECTS OF PLANT PHENOLOGY (VEGETATIVE AND FLOWERING) AND TIME OF DAY (MORNING: 06:00–10:00 HR; MIDDAY: 10:00–14:00 HR; AND AFTERNOON: 14:00–16:00 HR) ON ALFALFA VOLATILE EMISSIONS

	Plant			Time			Plant × Time		
	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*
Lipoxygenase products[a]	0.674	3.381	**0.014**	0.383	4.309	**<0.001**	0.936	0.235	0.992
Monoterpenes[b]	0.416	9.825	**<0.001**	0.596	2.068	**0.039**	0.627	1.842	0.069
Sesquiterpenes[c]	0.965	0.690	0.508	0.797	2.285	0.068	0.922	0.782	0.540

Note. Bold type indicates significant treatment effects ($P < 0.05$).

[a] Lipoxygenase products include (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, and (*Z*)-3-hexenyl butyrate.

[b] Monoterpenes include α-pinene, limonene, (*Z*)-ocimene, (*E*)-β-ocimene, and linalool.

[c] Sesquiterpenes include (*E*)-β-caryophyllene and (*E*, *E*)-α-farnesene.

TABLE 3. TWO-WAY MANOVA FOR EFFECTS OF PLANT PHENOLOGY (VEGETATIVE AND FLOWERING) AND FEEDING DAMAGE BY *Lygus hesperus* (NYMPHS AND ADULTS) ON ALFALFA VOLATILE EMISSIONS

	Plant			Damage			Plant x Damage		
	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*	Wilks' λ	*F*	*P*
Lipoxygenase products[a]	0.532	4.049	**0.009**	0.673	1.007	0.452	0.528	1.728	0.103
Monoterpenes[b]	0.184	13.277	**<0.001**	0.282	2.653	**0.007**	0.466	1.397	0.197
Homoterpenes[c]	0.848	2.323	0.118	0.783	1.690	0.166	0.690	2.652	**0.043**
Sesquiterpenes[d]	0.788	3.499	**0.045**	0.664	2.954	**0.028**	0.684	2.716	**0.040**
Shikimic acid pathway[e]	0.212	48.286	**<0.001**	0.637	3.290	**0.018**	0.729	2.223	0.079

Note. Bold type indicates significant treatment effects ($P < 0.05$).

[a] Lipoxygenase products include (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, and (*Z*)-3-hexenyl butyrate.

[b] Monoterpenes include α-pinene, β-pinene, myrcene, limonene, (*Z*)-ocimene, (*E*)-β-ocimene, and linalool.

[c] Homoterpenes include (3*E*)-4,8-dimethyl-1,3,7-nonatriene and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

[d] Sesquiterpenes include (*E*)-β-caryophyllene and (*E*, *E*)-α-farnesene.

[e] Shikimic acid pathway include indole and methyl salicylate.

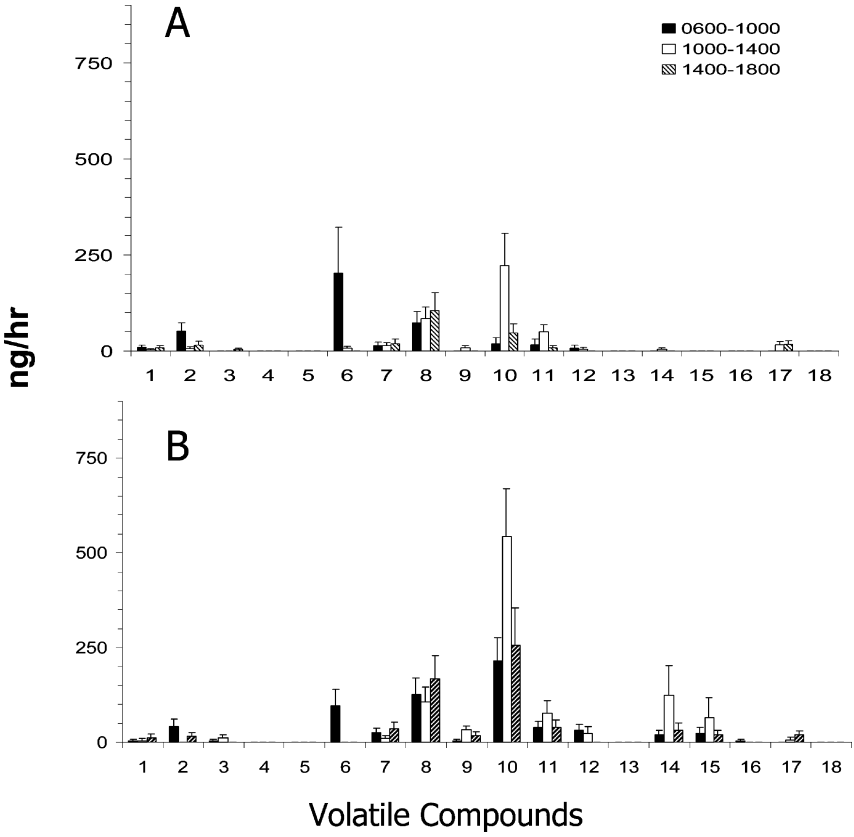

FIG. 4. Volatiles collected from the aerial portions of vegetative (A) or flowering (B) alfalfa. Volatile collections were taken at 4-hr intervals starting at 06:00 hr and ending at 18:00 hr. Each bar represents the mean ± SE for 6–8 replicates. 1 = (*E*)-2-hexenal, 2 = (*Z*)-3-hexen-1-ol, 3 = α-pinene, 4 = β-pinene, 5 = myrcene, 6 = (*Z*)-3-hexenyl acetate, 7 = (*E*)-2-hexenyl acetate, 8 = limonene, 9 = (*Z*)-ocimene, 10 = (*E*)-β-ocimene, 11 = linalool, 12 = (3*E*)-4,8-dimethyl-1,3,7-nonatriene, 13 = methyl salicylate; 14 = (*Z*)-3-hexenyl butyrate, 15 = indole, 16 = (*E*)-β-caryophyllene, 17 = (*E*, *E*)-α-farnesene, and 18 = (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

field conditions, might necessitate a higher behavioral threshold for host finding relative to adults.

The fact that nymphs responded to all alfalfa treatments implies a lack of specificity in their response to changes in alfalfa volatiles due to phenology (i.e., flowering) or insect damage. For instance, considerable amounts of lipoxygenase products (so-called GLVs) and several monoterpenes were emitted from alfalfa

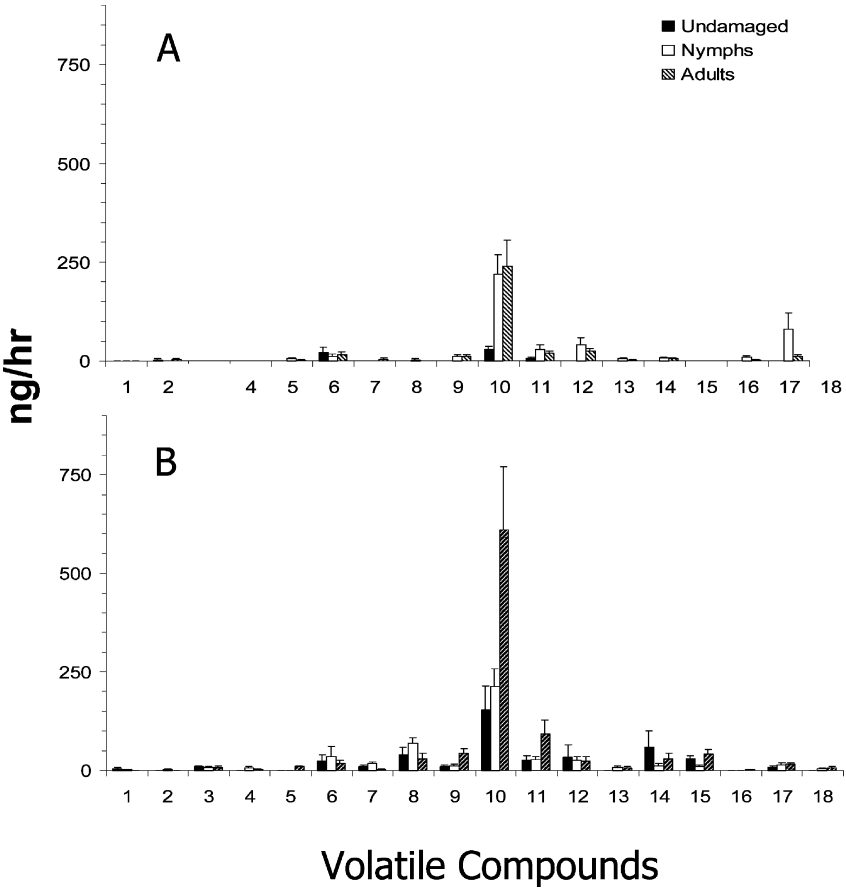

FIG. 5. Volatiles collected from the aerial portions of vegetative (A) or flowering (B) alfalfa damaged by nymphs and adults of *Lygus hesperus*. Volatiles were collected continuously for 8 hr during photophase. Each bar represents the mean ± SE for 4–7 replicates. Volatile designations are the same as described in Figure 4.

regardless of its phenology or amount of insect damage (Figures 4 and 5). GLVs and monoterpenes may serve in host location by *L. hesperus* nymphs. These compounds are produced by several other species of plants and their emissions are elevated after herbivore damage at the site of feeding and also systemically from undamaged tissues of damaged plants (Mattiacci et al., 2001). Therefore, it is likely that a polyphagous herbivore such as *L. hesperus,* that feeds on over 100 plant species, may utilize volatile cues common to a variety of plants during host location.

Female *L. hesperus* were more responsive to alfalfa headspace volatiles than males, of which the latter failed to show a preference for any alfalfa treatments (Figures 2 and 3). *Lygus hesperus* females deposit their eggs into plant tissue, so this sex-related difference in response is probably related to the female's need to find suitable substrates for oviposition. In contrast, males showed a negative response to plant odors when offered alone (without conspecifics). The strong negative response to both vegetative and flowering alfalfa is probably adaptive as it would decrease or eliminate the time males spend in areas that lack potential mates. In fact, for two other species of Miridae, *Lygus lineolaris* (Palisot de Beauvois) and *Lygocoris pabulinus* (L.), electroantennograms (EAGs) indicated that the antennal responses of female bugs were far more pronounced to plant compounds than the antennal responses of male bugs (Chinta et al., 1994; Groot et al., 1999). These studies also indicated that two ubiquitous groups of plant volatiles, GLVs (produced from the lipoxygenase pathway) and monoterpenes, caused greater responses in females as compared to male antennae. These two groups of compounds were collected from both vegetative and flowering alfalfa (Figures 4 and 5).

In addition to the differences in emissions due to plant phenology, GLVs and monoterpenes varied with time of day (Table 2; Figure 4). Similarly, Loper and Lapioli (1971) demonstrated a daily cycle for alfalfa volatile emissions that resembled a bell-shaped curve with emanations reaching a maximum 5–7 hr after the beginning of photophase. Under field conditions, Pecetti and Tava (2000) found similar patterns of emissions for the second and third flowerings of alfalfa. Our results indicated that GLVs were emitted from alfalfa soon after photophase (06:00–10:00 hr) and prior to emissions of monoterpenes that occurred primarily at midday (10:00–14:00 hr; Figure 4). Changes in the volatile composition of blends during photophase have also been reported for caterpillar-damaged maize and cotton (Turlings et al., 1998; Rodriguez-Saona et al., 2001). We also found that headspace volatile emissions for flowering alfalfa were maximal from midday to late afternoon (Figure 4). Marletto et al. (1985) demonstrated that these peaks in emissions of alfalfa volatiles at midday corresponded with increased activity in insect pollinators.

Another environmental factor that can potentially influence volatile emissions in alfalfa is insect damage (Figure 5). Damage by adults of both sexes and nymphs of *L. hesperus* induced local and systemic emissions of several GLVs and monoterpenes in cotton and maize (Rodriguez-Saona et al., 2002). Monoterpenes, such as (*E*)-β-ocimene, were also induced by *L. hesperus* feeding in alfalfa (Table 3), which may explain the greater responsiveness of females to plants that had been fed upon by conspecifics (Figure 2).

The lower-level response to alfalfa and conspecific cues exhibited by adult *L. hesperus,* in comparison to the nymphal response, suggests that for adults additional cues (i.e., visual or the combination of visual and volatile cues) could

be more important than volatile cues during the host-location process. Landis and Fox (1972) demonstrated that *L. hesperus* is strongly attracted to visual cues. Alternatively, because these insects are facultative predators, we may find that volatile cues associated with prey items are relatively important in mediating their behavior. Defensive compounds produced in the metathoracic glands (Gupta, 1961), sex pheromones, and possibly aggregation pheromones could also influence foraging decisions (Groot et al., 2001).

Finally, for adult insects that engage in flight, Y-tube olfactometers may not be the best way to examine their response to plant odors. Orientation during flight may be an integral part of the behavioral sequence that leads the adult to the host plant. Several of these possibilities are currently being investigated in our laboratory. With a better understanding of these various interactions, we hope to develop a trapping system that will be effective in monitoring both male and female *L. hesperus*. Furthermore, we know that herbivore-induced volatiles play an important role in the host-searching behavior of natural enemies (Dicke and Sabelis, 1988; Turlings et al., 1990; Agelopoulus and Keller, 1994; Powell et al., 1998); however, almost nothing is known about the cues used by natural enemies of *Lygus* spp. The fact that feeding by nymphs and adults caused increased releases of monoterpenes and sesquiterpenes, which have been found to be attractive to beneficial insects in other cropping systems (Turlings et al., 1993; Birkett et al., 2000), may enable us to monitor abundance of, or attract natural enemies of *L. hesperus*.

Acknowledgments—We gratefully acknowledge the assistance of Terry Steele in the construction of the Y-tube olfactometer setup, Linda Lee for collecting and rearing *Lygus*, Thomas Henneberry and Steven Crafts-Brandner for providing support and facilities to conduct these studies, Mohamed Farag for assistance in the identification of volatiles, and Glen Jackson for sharing knowledge of *Lygus* biology with us. For critical reviews of previous versions of this manuscript, we thank Glen Jackson, Athayde Tonhasca Jr., and two anonymous reviewers.

REFERENCES

AGELOPOULOS, N. G. and KELLER, M. A. 1994. Plant-natural enemy association in the tritrophic system, *Cotesia rubecula-Pieris rapae-Brassicae* (Cruciferae) I: Sources of infochemicals. *J. Chem. Ecol*. 20:1725–1748.

AGUSTI, N. and COHEN, A. C. 2000. *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: Evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. *J. Entomol. Sci*. 35:176–186.

ALDRICH, J. R., LUSBY, W. R., KOCHANSKY, J. P., HOFFMAN, M. P., WILSON, L. T., and ZALOM, F. G. 1988. *Lygus* bug pheromone vis-à-vis stink bugs. *Proc. Beltwide Cotton Conf.* 213–216.

BIRKETT, M. A., CAMPBELL, C. A. M., CHAMBERLAIN, K., GUERRIERI, E., HICK, A. J., MARTIN, J. L., MATTHES, M., NAPIER, J. A., PETTERSSON, J., PICKETT, J. A., POPPY, G. M., POW, E. M., PYE, B. J., SMART, L. E., WADHAMS, G. H., WADHAMS, L. J., and WOODCOCK, C. M. 2000. New

roles for *cis*-jasmone as an insect semiochemical and in plant defense. *Proc. Natl. Acad. Sci. USA* 97:9329–9334.

CHINTA, S., DICKENS, J. C., and ALDRICH, J. R. 1994. Olfactory reception of potential pheromones and plant odors by tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae). *J. Chem. Ecol.* 20:3251–3267.

DICKE, M. and SABELIS, M. W. 1988. How plants obtain predatory mites as bodyguards. *Neth. J. Zool.* 38:148–165.

GROOT, A. T., DRIJFHOUT, F. P., HEIJBOER, A., VAN BEEK, T. A., and VISSER, J. H. 2001. Disruption of sexual communication in the mirid bug *Lygocoris pabulinus* by hexyl butanoate. *Agric. Forest Entomol.* 3:49–55.

GROOT, A. T., TIMMER, R., GORT, G., LELYVELD, G. P., DRIJFHOUT, F. P., VAN BEEK, T. A., and VISSER, J. H. 1999. Sex-related perception of insect and plant volatiles in *Lygocoris pabulinus*. *J. Chem. Ecol.* 25:2357–2371.

GUELDNER, R. C. and PARROTT, W. L. 1978. Volatile constituents of the tarnished plant bug. *Insect Biochem.* 8:389–391.

GUPTA, A. P. 1961. A critical review of the studies of the so-called stink or repugnatorial glands of Heteroptera with further comments. *Can. Entomol.* 93:482–486.

HEATH, R. R. and MANUKIAN, A. 1994. An automated system for use in collecting volatile chemicals released from plants. *J. Chem. Ecol.* 20:593–607.

HEDIN, P. A., PARROTT, W. L., TEDDERS, W. L., and REED, D. K. 1985. Field responses of the tarnished plant bug to its own volatile constituents. *J. Miss. Acad. Sci.* 30:63–66.

HO, H. and MILLAR, J. G. 2002. Identification, electroantennogram screening, and field bioassays of volatile chemicals from *Lygus hesperus* Knight (Heteroptera: Miridae). *Zool. Stud.* 41:311–320.

LANDIS, B. J. and FOX, L. 1972. Lygus bugs in eastern Washington: Color preference and winter activity. *Environ. Entomol.* 1:464–465.

LEIGH, T. F. 1976. Detrimental effect of *Lygus* feeding on plants, p. 38, *in* D. R. Scott and L. E. O'Keeffe (eds.). *Lygus* Bug: Host Plant Interactions. University Press of Idaho, Moscow, Idaho.

LEIGH, T. F., KERBY, T. A., and WYNHOLDS, P. F. 1988. Cotton square damage by the plant bug, *Lygus hesperus* (Hemiptera: Heteroptera: Miridae), and abscission rates. *J. Econ. Entomol.* 81:1328–1337.

LOPER, G. M. and LAPIOLI, A. M. 1971. Photoperiodic effects on the emanation of volatiles from alfalfa (*Medicago sativa* L.) florets. *Plant Physiol.* 49:729–732.

MARLETTO, F., MANINO, A., and BARBERO, R. 1985. Indagini sui pronubi in coltivazioni di erba medica per la produzione di seme. *Apicolt. Mod.* 76:95–102.

MATTIACCI, L., AMBÜHL ROCCA, B., SCASCIGHINI, H., D'ALESSANDRO, M., HERN, A., and DORN, S. 2001. Systemically induced plant volatiles emitted at the time of "danger." *J. Chem. Ecol.* 27:2233–2252.

MAUNEY, J. R. and HENNEBERRY, T. J. 1984. Causes of square abscission in cotton in Arizona. *Crop Sci.* 24:1027–1030.

MCLAUGHLIN, J. R. 1998. The status of *Lygus* pheromone research. *Proc. Beltwide Cotton Conf.* Vol. 2:938–940.

NIST. 1995. Mass Spectral Library on CD-rom, Version 1.0. NIST, Gaithersburg, MD.

PARÉ, P. W. and TUMLINSON, J. H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47:521–526.

PECETTI, L. and TAVA, A. 2000. Effect of flower color and sampling time on volatile emanation in alfalfa flowers. *Crop Sci.* 40:126–130.

POWELL, W., PENNACHIO F., POPPY, G. M., and TREMBLAY, E. 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidinae). *Biol. Control* 11:104–112.

RODRIGUEZ-SAONA, C., CRAFTS-BRANDNER, S. J., PARÉ, P. W., and HENNEBERRY, T. J. 2001. Exogenous methyl jasmonate induces volatile emissions in cotton plants. *J. Chem. Ecol*. 27:679–695.

RODRIGUEZ-SAONA, C., CRAFTS-BRANDNER, S. J., WILLIAMS, L., III, and PARÉ, P. W. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *J. Chem. Ecol*. 28:1733–1747.

SCOTT, D. R. 1977. An annotated listing of host plants of *Lygus hesperus* Knight. *ESA Bull*. 23:19–22.

SEVACHERIAN, V. and STERN, V. M. 1974. Host plant preferences of Lygus bugs in alfalfa-interplanted cotton fields. *Environ. Entomol*. 3:761–766.

SEVACHERIAN, V. and STERN, V. M. 1975. Movements of Lygus bugs between alfalfa and cotton. *Environ. Entomol*. 4:163–165.

STRONG, F. E. 1970. Physiology of injury caused by *Lygus hesperus*. *J. Econ. Entomol*. 63:808–814.

SYSTAT, 1998. SPSS Science, Version 9. Chicago, IL.

TURLINGS, T. C. J., LENGWILER, U. B., BERNASCONI, M. L., and WECHSLER, D. 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207:146–152.

TURLINGS, T. C. J., TUMLINSON, J. H., and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–1253.

TURLINGS, T. C. J., WÄCKERS, F. L., VET, L. E. M., TUMLINSON, J. H., and LEWIS, W. J. 1993. Learning of host-finding cues by Hymenopterous parasitoids, pp. 51–78, *in* D.R. Papaj and A.C. Lewis (eds.). Insect Learning. Chapman & Hall, New York.

WHEELER, A. G., JR. 1976. *Lygus* bugs as facultative predators, pp. 28–35, *in* D. R. Scott and O'Keeffe, L. E. (eds.). *Lygus* Bug: Host Plant Interactions. University Press of Idaho, Moscow, Idaho.

WHITBEY, R. M. 1999. Green bean extract-induced oviposition site preference in laboratory reared western tarnished plant bug (Heteroptera: Miridae). *Environ. Entomol*. 28:201–204.

ZAR, J. H. 1984. Biostatistical Analysis. Prentice Hall, New Jersey.