

# Volatiles from Intact and *Lygus*-Damaged *Erigeron annuus* (L.) Pers. are Highly Attractive to Ovipositing *Lygus* and its Parasitoid *Peristenus relictus* Ruthe

Sean T. Halloran · Kerry E. Mauck · Shelby F. Fleisher · James H. Tumlinson

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**Abstract** Trap cropping and biological control can provide a sustainable means of controlling insect pests. Insects in the genus *Lygus* (Hemiptera: Miridae) are major pests on cotton and horticultural crops throughout the United States, and pesticide resistance within *Lygus* populations necessitates more sustainable long-term management techniques. Here, we explore behavioral responses of *Lygus* bugs (*L. rubrosignatus* Knight) and an introduced parasitoid, *Peristenus relictus* (Hymenoptera: Braconidae), to a common field edge plant, *Erigeron annuus*, which has the potential to serve as a trap host. *Erigeron annuus* is attractive to *Lygus* in the field, with *Lygus* preferentially moving to *Erigeron* patches compared to more abundant cotton plants. To determine the role of odor cues in mediating this attraction, we collected volatiles from *E. annuus* with and without *Lygus* damage, and then tested the attractiveness of these volatiles vs. those of cotton to *Lygus* females and female *P. relictus* wasps using Y-tube and wind tunnel bioassays. We found that undamaged *E. annuus* emits high concentrations of a complex volatile blend (60+ compounds), with novel compounds induced and constitutive compounds up-regulated in response to damage. Additionally, both female *Lygus* bugs and female *P. relictus* wasps are highly attracted to *E. annuus* volatiles over those of cotton in almost every combination of damage treatments. Our results suggest that *Erigeron annuus* would be an effective trap plant to control *Lygus* in cotton, since it is highly attractive to both the pest and its natural enemy.

**Keywords** *Lygus* bugs · Trap crop · *Gossypium hirsutum* · Volatile release · Hemiptera · Miridae · Hymenoptera · Braconidae · Push-pull · Sustainable pest management

## Introduction

The use of trap crops can be an effective means of managing insect pests within an agricultural system, especially in situations involving subsistence agriculture, where growers cannot afford to purchase pesticides or spraying equipment. Herbivorous insects often use plant-produced volatile organic compounds (VOCs) as species-specific cues for locating plant hosts (Bernays and Chapman 1994; Bruce et al. 2005) and for discriminating among hosts of varying age, quality, or damage status (Blackmer et al. 2004; De Moraes et al. 2001; Mauck et al. 2010). Effective trap crops or trap plants should exploit volatile-mediated host-finding behavior to draw a target pest away from the economically valuable crop (Cook et al. 2007; De Camelo et al. 2007). Additionally, a trap crop/plant will be even more viable as a control measure if it not only pulls a pest out of the target crop, but also serves to attract and/or provision food to natural enemies of the pest. This scenario could lead to an increase in natural enemy populations or increased predation/parasitization of the pest species (Hokkanen 1991). One of the most well known of these systems is the so-called “push-pull” system developed for subsistence cereal farmers in sub-Saharan Africa (Pickett et al. 1997; Khan et al. 2000). In this system, maize or sorghum is intercropped with another plant (such as *Desmodium uncinatum* Jacq. DC., or *Melinis minutiflora* P. Beauv.) that produces semiochemicals repellent to lepidopterous pests. Adjacent to this intercrop field, a trap crop of Napier grass (*Pennisetum purpureum* Schumach.) or Sudan grass (*Sorghum sudanensis* Piper) is planted. These two plants are highly attractive to the pests and serve to pull them

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S. T. Halloran · K. E. Mauck · S. F. Fleisher · J. H. Tumlinson (✉)  
Department of Entomology, The Pennsylvania State University,  
University Park, PA 16802, USA  
e-mail: jht2@psu.edu

out of the cereal field. Additionally, the *M. minutiflora* also leads to an increase in parasitism of the Lepidopteran pests, since it is highly attractive to parasitoids (Khan et al. 2000). Trap crops that are attractive to natural enemies of the target pest also have been successful in reducing stink bug populations in soybean (Corrêa-Ferreira and Moscardi 1996), attracting *Peristenus relictus* wasps in strawberry fields (Pickett et al. 2009), and have been shown in other systems to be an important refuge for natural enemies during mowing or harvesting of the target crop (Hickman and Wratten 1996; Lys et al. 1994). These studies indicate that trap crops, particularly in combination with natural enemies, are a viable alternative to chemical controls in some agricultural systems.

A trap crop/natural enemy approach may be a particularly useful management strategy to explore for a crop in which few species achieve pest status. Additionally, systems in which major crop pests show insecticide resistance also would benefit from a more sustainable management strategy like trap cropping and natural enemy conservation. In the United States, cotton, *Gossypium hirsutum* L., is one such crop. Due to advances in the development of non-pesticide control strategies for management of key pests, such as the boll-weevil, over the past 40 years, cotton now can be described as a low-spray crop (Hardee et al. 2001; Layton 2000; Naranjo 2011). However, polyphagous *Lygus* bugs (Hemiptera: Miridae), commonly referred to as ‘tarnished plant bugs’, remain a key pest of cotton and are still controlled by chemical spraying. Due to this reliance on chemical controls, some *Lygus* species have shown a consistent ability to develop resistance to organophosphate, pyrethroid, and cyclodiene insecticides (Snodgrass 1996; Snodgrass and Scott 2000; Snodgrass et al. 2009). Additionally, the use of insecticides can disrupt the activity of *Lygus* natural enemies already present within the crop (Williams et al. 2003). A renewed focus on alternative strategies for management of *Lygus* bugs in cotton is necessary to prolong the usefulness of existing chemical controls and to move towards an overall more sustainable management regime for this pest. A trap crop that functions to attract *Lygus* bugs via VOCs is one promising strategy that could reduce the reliance on chemical controls. *Lygus* bugs have been shown to respond to a number of plant-produced compounds by orienting towards the source of those compounds, indicating that VOC blends are likely important in their host-finding in nature (Blackmer et al. 2004; Blackmer and Cañas 2005; Williams et al. 2010). Furthermore, *L. hesperus* Knight adults have been shown to be attracted to alfalfa plants either recently damaged or currently being damaged by conspecifics (Blackmer et al. 2004; Blackmer and Cañas 2005), which suggests that *Lygus* feeding and/or arrestment on a potential trap crop may serve to enhance rather than suppress its attractive quality.

In the present study, we examined interactions among *Lygus*, a parasitoid natural enemy, and a promising potential trap crop (annual fleabane: *Erigeron annuus*), with the goal of evaluating

the feasibility of this plant as a sustainable means of controlling *Lygus* in cotton. *Erigeron annuus* is a fast-growing annual wild plant that is consistently preferred by *Lygus* in nature over other plants and many target crops. Previous research has shown that *Erigeron annuus* growing wild in highway right-of-ways harbors significantly more adult *Lygus lineolaris* Knight bugs than other co-occurring wild plants, which indicates that adults are preferentially choosing pre-flowering and flowering *Erigeron* as a feeding and oviposition site (Fleischer and Gaylor 1987). One study also used rubidium-marked individuals to examine the movement of *Lygus* in relation to patches of *E. annuus* planted within and adjacent to a cotton field (Fleischer et al. 1988). When pre-infested plots of *E. annuus* within the cotton field were destroyed, more marked individuals were recovered from the trap plots of *E. annuus* on the edge of the cotton field than within the cotton crop itself (Fleischer et al. 1988). Cage studies also showed that when *E. annuus* was available, *Lygus* will leave cotton and move onto *E. annuus* (Fleischer et al. 1988). Limited research also suggests that flowering *E. annuus* is a good-quality food source for parasitoids, including those that attack *Lygus* bugs (Streams et al. 1968), and that higher densities of *E. annuus* are associated with higher parasitism rates on *Lygus* (Shahjahan and Streams 1973). Thus, *E. annuus* may fulfill a dual role in attracting and retaining *Lygus* bugs and attracting and provisioning *Lygus* natural enemies. However, native natural enemies of *Lygus* are often inadequate for reducing pest populations enough to avoid economic losses (Carignan et al. 2007; Clancy and Pierce 1966; Day et al. 1990), and native egg parasitoids have varying success in accessing and parasitizing *Lygus* eggs on different hosts (Williams and Zhu 2012). As a result, non-native parasitoids that attack nymphs of several problematic *Lygus* species have been introduced to the United States (specifically for use in alfalfa, another crop heavily impacted by *Lygus*) (Day et al. 1990; Day 1996). One parasitoid native to Mediterranean countries, *Peristenus relictus*, is a promising candidate for biological control of *Lygus* in cotton, since it is adapted to regions that experience similar weather conditions and temperatures (Hoelmer et al. 2008; Pickett et al. 2007). Presently, researchers are attempting to establish permanent populations of *P. relictus* across the western portion of New Jersey, and permanent populations appear to have established in the Monterey Bay region of coastal Central California (Pickett et al. 2009). Additionally, rearing programs are established for *P. relictus*, so production and release is already streamlined. However, all current research on preferences, performance, and effectiveness of *P. relictus* is limited to alfalfa, even though this wasp has the potential to control *Lygus* in many agricultural systems in the southern U.S.

Here, we report on the role of VOCs in mediating interactions among *Lygus rubrosignatus*, *P. relictus*, *Erigeron annuus*, and cotton using a combination of volatile-based behavioral assays and chemical analytical techniques to identify, quantify, and compare volatile emissions from these major hosts.

Through a series of choice test experiments, we determined the relative attractiveness of *E. annuus* (with and without damage from conspecifics) to female *Lygus* bugs, and relate our results to the differences in volatile emissions among damage treatments within each host plant species, and between the two host plant species. Additionally, we assessed the host finding ability and odor-based preferences of *P. relictus* when given a choice between cotton and *E. annuus* plants subjected to various damage treatments by *Lygus*. By undertaking this study, we hope to provide a mechanistic basis for *Lygus* movement in the field that will aid in the implementation of *Erigeron* species as *Lygus* trap plants, and inform efforts to combine this strategy with parasitoid biological controls.

## Methods and Materials

### Insect Rearing and Maintenance

*Lygus rubrosignatus* adults and nymphs were obtained from The Phillip Alampi Beneficial Insect Laboratory, West Trenton, NJ, USA. This species of *Lygus* bug was used due to the ease of rearing in a lab setting, as well as a shared host range with *Lygus lineolaris* along the Northeast US and Southern Canada (Goeden and Ricker 1974; Kelton 1980; Scudder 1997; Young 1986). The colony was kept in a rearing room maintained at  $22 \pm 2$  °C,  $60 \pm 10$  % RH, and a L:D regimen of 14:10 hr. Individuals were reared on Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA) packets of *Lygus* artificial diet purchased from Bio-serv, while adult females were allowed to oviposit on similar packets full of Carrageenan Gelcarin GP812 (PhytoTechnology Laboratories, Shawnee Mission, KS, USA). Diet packs were changed every other day, while oviposition packs were removed when they became full of eggs. Oviposition packs then were placed into new containers where the nymphs were allowed to hatch and grow.

*Peristenus relictus* wasps were obtained as adults from the Phillip Alampi Beneficial Insect Research Lab (New Jersey Department of Agriculture) in West Trenton, New Jersey. This colony of wasps was made up of individuals originally obtained from southern Spain (Andalucia) and central Italy (Umbria). Upon arrival, wasps were maintained in a large screen cage with a dilute wasp diet consisting of honey and other essential nutrients (yeast extract) in the same temperature and humidity conditions as for the *L. rubrosignatus* bugs.

### Plant Maintenance

All plant species were grown in a pest-free greenhouse with a L:D regimen of 16:8 hr and a relative humidity of  $50 \pm 10$  %. Plants were grown in Metro-Mix potting soil with 5 g of Osmocote Plus slow release fertilizer (Scotts) mixed in at the time of potting. All plants were bottom-watered daily with a hose.

*Erigeron annuus* was grown from seed in 6-inch diam pots, while *Gossypium hirsutum* (cultivar DPL90) was grown from seed in 4-inch diam pots. Both species were maintained at  $27 \pm 2$  °C. *Erigeron annuus* was allowed to grow through its rosette stage and into its bolting stage (approximately 5–6 wk). Bolting *E. annuus* (buds present but not yet flowering) were used for the volatile collections and behavioral assays. *Gossypium hirsutum* plants were grown until early squares began to appear (approximately 4 wk), at which point the plants were used in experiments.

### Volatile Collection and Analysis

To quantify and identify VOCs released by the study plants, the headspace around the plants was collected. An automatic volatile collection system built by Analytical Research Systems (Gainesville, FL, USA) was used to control sampling periods. The system was capable of controlling simultaneous collections from 12 individual treatment plants at any chosen set of intervals 24 hr a day.

A portion of each non-excised plant was enclosed in a 7 L glass bell jar with a base consisting of two metal plates with a hole in the center for the plant stem to fit through. Clean cotton-balls were packed around the junction of the stem and base to prevent air from lower portions of the plants or the soil from entering the bell jars. The portion of the plant being collected from was a single stem with buds for *E. annuus* and the apical portion of the main stem with three developing squares for *G. hirsutum*. Charcoal-purified air was pumped through the top of the bell jars at an average rate of  $4 \text{ L min}^{-1}$  and allowed to pass over the plant. Volatiles from headspace were sampled by pulling air at a rate of  $1 \text{ L min}^{-1}$  through an adsorbent volatile trap (45 mg of Super-Q 80/100 mesh, Alltech). Since more air entered the bell jar than was sampled through the volatile trap, the excess air exited through imperfections along the bottom of the bell jar (helping to maintain positive pressure and prevent accidental sampling of impurities from the outside air).

The plants were divided into three treatments during the collections: (1) control (undamaged); (2) damage from *L. rubrosignatus* adult feeding; and (3) damage from *L. rubrosignatus* nymph feeding. For treatments involving insect damage, either 12 adult (1:1 sex ratio) or 16 nymph *L. rubrosignatus*, were placed in the bell jars an hour before collections began (4 extra nymphs were added to account for the fragility of this life stage during collection and movement). All insects remained in the bell jars and were allowed to feed constantly over the duration of the collections. The collections were checked several times a day and any dead insects were aspirated out and replaced with new individuals. Sample sizes were between 4 and 8 plants per species  $\times$  damage treatment.

The collections were run during the day over a 12-h period starting at 9:30 and ending at 21:30. These daytime collections

were divided between 3 collection periods of 4 hr each. This prevented breakthrough or loss of small molecular weight compounds. All collections occurred within a pest-free greenhouse maintained at 25 °C, 50%RH and a L:D cycle of 16:8 hr (supplementary lighting was provided by high-pressure sodium vapor bulbs to achieve the desired photoperiod).

Samples were analyzed by first eluting the compounds off of each filter using 125 µl of 1:1 dichloromethane:hexanes (Burdick and Jackson) and then adding 200 ng *n*-octane and 400 ng nonyl acetate to each eluted sample to act as internal standards. Eluted samples then were analyzed on an Agilent 6890 analytical gas chromatograph (GC) equipped with an FID detector, with a splitless injector, and an HP-1 column (15 m × 0.25 mm × 0.25 µm, Agilent). The column was held at 35 °C for half a minute and then increased by 4 °C per min to 160 °C, and then further increased by 20 °C per min to reach a maximum temperature of 220 °C. The column flow rate was 1.7 ml/min with a helium carrier gas. In order to identify compounds, selected samples were run on an Agilent 6890 N GC equipped with an Agilent 5973 N mass selective detector configured for electron impact mode and a HP-1MS column (30 m × 0.25 mm × 0.25 µm, Agilent). The column was held at 40 °C for one min and then increased by 10 °C per min to reach a maximum temperature of 300 °C. The column flow rate was 0.7 ml/min. Mass spectra were compared to spectra for standards available in the National Institute of Standards and Technology (NIST) library as well as known standards from the lab. The Kovats Index (KI) of each compound was also determined (Kováts 1965) and used to tentatively identify some compounds by comparing the unknown sample KIs to those of known standards run on the same type of GC column (HP-1). These indices also were used to ensure consistency of identification between experiments over time.

#### Lygus Bug Behavioral Assays

We used a vertical Y-tube olfactometer (2.5 cm diam, base 18 cm, arms 11 cm) to determine up-wind, volatile based orientation preferences of *Lygus* bugs for *E. annuus* vs. crop plants under different damage conditions. The choice tests performed are

**Table 1** Choice tests performed to examine movement of *Lygus* bugs in response to volatile cues

Choice 1	Choice 2	Treatment applied to choice plants	Number of plant pairs	Number of bugs tested
Cotton	<i>Erigeron</i>	Both undamaged	6	89
Cotton	<i>Erigeron</i>	Both damaged by 12 female adults	6	119
Cotton	<i>Erigeron</i>	Both damaged by 12 male adults	4	65
Cotton	<i>Erigeron</i>	Both damaged by 16 nymphs	4	53
Cotton	<i>Erigeron</i>	Cotton damaged by nymphs, <i>Erigeron</i> undamaged	2	41
Cotton	<i>Erigeron</i>	Cotton damaged by female adults, <i>Erigeron</i> undamaged	3	68
Cotton	Clean air	Cotton undamaged	2 plants (1 per test)	62

outlined in Table 1, and all tests assessed the behavior of adult females, as these are the most relevant life stage due to their mobility and the fact that they are searching for feeding and oviposition sites. Assays were carried out over multiple days, with one pair of plants used per day. Plant odors were derived from intact portions of whole plants enclosed in 7 L glass bell jars (1–2 stalks of pre-flowering *E. annuus* and the apical squaring portion of cotton plants). Clean, humidified air was delivered to each bell jar at a rate of 4 L min<sup>-1</sup> and a Teflon tube connected the headspace within each dome to one of the Y-tube arms using a ground-glass connector. To perform each test, a single adult female bug was placed in a ground-glass connecting chamber at the base of the Y-tube. The connector was fitted immediately into the Y-tube base and a vacuum (1 L min<sup>-1</sup>) pulled air through the Y-tube from each of the odor sources via the connecting Teflon tubes. The bug was given 5 min to walk up the Y-tube and enter one of the arms (with the aid of traction provided by a Y-shaped stick that had been positioned inside the tube). If the bug entered an arm and walked the entire length to the endpoint, then that was considered a choice. Bugs that did not choose within 5 min were excluded from the analysis. The Y-tube set up was tested with clean air vs. a squaring cotton plant to ensure that bugs could locate and orient towards cotton volatiles even in the absence of another choice. The bioassay materials were cleaned with acetone, and the treatment input arms were switched every 4 bugs. Volatile samples were collected from several of the odor sources simultaneous to performing choice tests to verify that the cues being delivered were similar to those we detected during the more detailed collections performed independent of the choice tests.

After the first round of data collection, an additional choice combination of undamaged *E. annuus* vs. cotton damaged by 40 *Lygus* nymphs was added to the treatment list to attempt to correct for the large difference in total volatiles released from *E. annuus* and cotton plants when the same damage treatment was applied to both plants.

#### Parasitoid Host Choice Assay

Bioassays to determine up-wind, volatile-based orientation preferences of *P. relictus* females for *E. annuus* vs. *G. hirsutum*



plants under different *Lygus* damage conditions were performed in a wind tunnel. The wind tunnel was a  $0.61 \times 0.61 \times 1.83$  m acrylic glass wind tunnel with a charcoal-filtered air input and a wind velocity of 0.5 m/s. The wind tunnel was maintained at 25–29 °C and 50 % relative humidity during the bioassays. Bioassays were carried out during the months of September through October between noon and 5:00 p.m.

Twenty-four hours before the start of the behavioral assays with the wasps, 3<sup>rd</sup>-instar *L. rubrosignatus* nymphs were placed in “clip cages” on the squares of *G. hirsutum* or the buds of *E. annuus* plants that would be used during the next day as choice plants. To ensure plants were of similar sizes, and to enable them to fit in the wind tunnel, plants used in wasp choice tests were cut and placed in vials of water the day before being used in the assays. Volatile collections using cut plants were carried out using the previous methods in order to insure that the volatile profiles were similar to those seen in intact plants (see Online Resource 1). Additionally, to give the wasps the experience of stinging nymphs on *G. hirsutum* plants, a second *G. hirsutum* plant also was infested with 3<sup>rd</sup>-instar nymphs. The next morning the damaged portions of this *G. hirsutum* plant were cut off and placed in a large glass Petri dish with the nymphs still feeding. Female *P. relictus* wasps then were placed inside the dish and allowed to parasitize 2–5 nymphs in the presence of nymph damaged *G. hirsutum* plant volatiles. After 10 min of resting time, each female wasp then was flown in the wind-tunnel assay.

The day of the bioassay, a single *E. annuus* and *G. hirsutum* plant were placed in the wind tunnel equidistant from the side-walls and each other as choice plants in the upwind position, and a starting *G. hirsutum* plant infested with a single *Lygus* nymph was placed 1 m downwind. At the beginning of the assay, a single female *P. relictus* was placed on the starting *G. hirsutum* plant and was allowed to patrol the area and orient upwind. We recorded the first choice of the female after initiating up-wind flight. A wasp was considered to have made a choice if it landed on either of the two plant choices. Some individuals landed on the walls or ceiling before re-orienting and then landing on one of the choice plants, and these also were recorded as choices. If a wasp flew to the wall or ceiling and then did not reorient and fly upwind within 30 sec then it was recorded as a ‘no choice’. Wasps were allowed 10 min to take flight before they were removed. Wasps removed from the starting location in this manner were not recorded as ‘no choice’, as it usually meant that the conditions of the wind tunnel were not conducive to flight on that day potentially due to differences in humidity or temperature due to rainy weather in the outside environment.

In the first set of trials, the *E. annuus* plants were undamaged while the *G. hirsutum* plants were damaged by 16 3<sup>rd</sup>-instar *L. rubrosignatus* nymphs (5 pairs of plants, 31 wasps). In a second trial, the damage treatments was reversed, with the *G. hirsutum* plant left undamaged, and the *E. annuus*

plant damaged by *Lygus* nymphs (3 pairs of plants, 22 wasps), followed by both plant species damaged by *Lygus* (4 pairs of plants, 34 wasps), and finally neither plant species damaged by *Lygus* (3 pairs of plants, 17 wasps). Only *Lygus* nymph damage treatments were used in these series of experiments because this is the life stage that is attacked by *P. relictus* females. Assays were carried out over multiple days, with one pair of plants used per day.

## Statistical Analysis

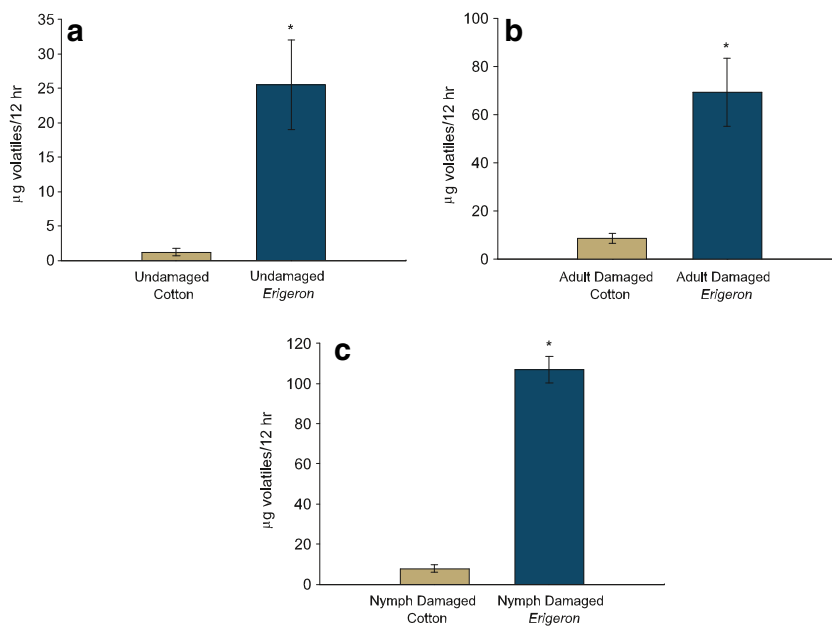
Total volatile emissions over the daytime period were calculated for each replicate plant by summing the amounts of each recorded compound across the three samples collected over 12 h (amounts calculated relative to the internal standard nonyl acetate based on peak area). Total volatiles emitted by cotton and *Erigeron* were compared within each bug damage treatment using GLM (Minitab) with plant species as the main factor. Compound means and standard errors were also calculated, and each damage treatment was compared to the control to determine if a compound was novel, or if it was a constitutive compound that was up-regulated (increased levels of release relative to controls), or down-regulated (lower levels of release relative to controls) based on non-overlap of two times each standard error surrounding the means (as in Eigenbrode et al. 2002; Mauck et al. 2010). Behavioral tests were analyzed using *chi-square* tests, and volatiles collected during choice tests were analyzed as for the larger collections.

## Results

### Volatile Collection and Analysis

*Erigeron annuus* released significantly more total volatiles than cotton both in the presence and absence of different types of *Lygus* damage (Fig. 1). This was despite the larger size and total leaf mass of the cotton plants relative to *E. annuus*. Induction of VOCs also was observed for both plant species in the presence of *Lygus* bug feeding (Fig. 1, note differences in scale of the y-axis between a-c, Tables 2 and 3) (data for cut plants in Online Resource 1). Table 2 displays the compounds emitted by potted *E. annuus* across the three damage treatments, as well as the mean and standard error for each compound within each treatment. In addition to an increase in the number of compounds (from 93–114), many compounds that are released constitutively (released from the plant even when undamaged) in low amounts are up-regulated when *E. annuus* is damaged by adult *Lygus* bugs. Nymphs also induce novel compounds and cause up-regulation of constitutive compounds, but several constitutive compounds also are absent from the nymph-induced blend (Table 2). Differences in the blends among *E. annuus* damaged by adults and nymphs also

**Fig. 1** Mean total volatiles  $\pm$  standard error for *Erigeron* and cotton under different damage treatments: **a** both plant species undamaged, **b** both plant species damaged by a mixture of male and female adults, **c** both plant species damaged by 3–4<sup>th</sup> instar nymphs. In graphs \* indicates significant difference between the two treatments at  $P < 0.05$



are apparent, with some constitutively released compounds up-regulated differently between the two treatments (Table 2). Overall, both constitutive and induced blends released from *Erigeron* are rich in terpenes, with induced blends showing considerable up-regulation of constitutive terpenes as well as induction of novel terpenes. Cotton plants also showed induction of new compounds in response to both adult and nymph feeding, with more novel compounds being induced by nymph feeding than adult feeding (Table 3). However, cotton plants, even under damage, released less than half the number of compounds relative to *Erigeron* (Table 3). There is some overlap with *Erigeron* treatments in the identities of terpenes induced by damage (Tables 2 and 3), indicating that in both instances feeding may be inducing similar pathways. Additionally, both plant species emit more alcohols, aldehydes, and acetates (“green leaf volatiles” or GLVs) in response to feeding. Collectively, these results indicate that *E. annuus* releases a highly complex blend of compounds in large amounts, both constitutively and when attacked by *Lygus* bugs, and that volatile emissions from *E. annuus* are significantly higher (sometimes up to 10-fold) than emissions from the co-occurring cotton crop plant.

#### Lygus Bug Behavioral Assays

In all choice combinations where both plants had the same treatment applied (undamaged, nymph damaged, female adult damaged, male adult damaged), a significant percent of the female *Lygus* adults chose to move towards the *E. annuus* odor source as compared to the cotton odor source (Fig. 2). However, when cotton damaged by female *Lygus* bugs was tested against undamaged *E. annuus*, there was no significant

difference between the two choices (Fig. 3), while the combination of cotton plants damaged with 40 nymphs vs. undamaged *E. annuus* plants showed a significant percent of the females choosing the *Erigeron* odor (Fig. 3). In combinations involving a cotton plant vs. clean air, a significant percentage of the females moved towards the cotton (Fig. 3).

Total volatiles released from both the nymph damaged cotton and the undamaged *E. annuus* used in the choice tests were not significantly different (Fig. 4). Total volatiles released from the undamaged *E. annuus* vs. female damaged cotton used in the choice tests do show a significant difference in total emissions (Fig. 4). These data demonstrate that a quantitative difference in volatile production alone does not fully explain the attractiveness of *E. annuus* has towards *Lygus*.

#### Parasitoid Host Choice Assay

Despite only receiving oviposition experience on nymph damaged *G. hirsutum* plants, female *P. relictus* wasps still flew upwind towards *E. annuus* plants in several of the damage treatments (Fig. 5). When both choice plants were left undamaged, a little over 70 % of the parasitoids flew upwind towards *Erigeron*. Although this percentage was trending towards significance, it was not different from those that went to the undamaged cotton plants ( $Chi\ squared = 2.882$ ,  $df = 1$ ,  $P = 0.089$ ). However, when both plants were damaged by *Lygus* nymphs, parasitoids were more attracted to the *E. annuus* plants ( $Chi\ squared = 8.048$ ,  $df = 1$ ,  $P = 0.005$ ), and when nymph damaged *E. annuus* was compared to undamaged cotton plants 100 % of the females wasps flew to the *E. annuus* plants ( $Chi\ squared = 14.000$ ,  $df = 1$ ,  $P < 0.001$ ). In contrast, when only the cotton plants were

**Table 2** Mean amounts of each compound +/- standard error from undamaged, adult damaged, and nymph damaged *Erigeron annuus* plants

Compound	Undamaged <sup>a</sup>	Adult-damaged <sup>a</sup>	Nymph-damaged <sup>a</sup>
KI 702	16 +/- 5	30 +/- 12	<b>64 +/- 11</b>
4-methylpent-3-en-2-one	0	5 +/- 3	12 +/- 5
KI 745	9 +/- 3	<b>57 +/- 22</b>	<b>153 +/- 11</b>
Toluene	5 +/- 3	14 +/- 5	<b>42 +/- 5</b>
KI 757	14 +/- 8	22 +/- 2	24 +/- 4
KI 762	0	4 +/- 1	33 +/- 9
KI 768	4 +/- 2	<b>97 +/- 37</b>	<b>371 +/- 61</b>
Hexanal	0	214 +/- 36	32 +/- 10
(E)-2-hexenal *	7 +/- 2	<b>62 +/- 10</b>	<b>80 +/- 5</b>
(Z)-3-hexen-1-ol *	36 +/- 15	<b>671 +/- 101</b>	<b>398 +/- 44</b>
(E)-2-hexen-1-ol	116 +/- 32	<b>485 +/- 86</b>	<b>900 +/- 85</b>
1-hexanol	38 +/- 11	<b>195 +/- 26</b>	<b>292 +/- 28</b>
Dimethylbenzene <sup>b</sup>	0	1 +/- 1	18 +/- 1
KI 864	0	16 +/- 7	32 +/- 13
KI 866	9 +/- 3	29 +/- 13	<b>108 +/- 15</b>
KI 870	3 +/- 2	25 +/- 12	0
α-pinene *	50 +/- 6	82 +/- 10	77 +/- 5
Camphene	17 +/- 6	63 +/- 15	41 +/- 3
Propylbenzene	11 +/- 7	24 +/- 11	0
6-methyl-5-hepten-2-one	1 +/- 1	13 +/- 7	<b>32 +/- 5</b>
β-pinene *	68 +/- 8	<b>112 +/- 11</b>	<b>95 +/- 5</b>
Myrcene *	383 +/- 114	676 +/- 203	<b>1615 +/- 129</b>
(Z)-3-hexenyl acetate *	614 +/- 175	<b>4234 +/- 432</b>	<b>4284 +/- 541</b>
α-phellandrene	67 +/- 14	<b>807 +/- 155</b>	<b>1768 +/- 187</b>
Benzyl alcohol	2 +/- 2	1 +/- 1	0
Eucalyptol	25 +/- 9	<b>266 +/- 84</b>	<b>246 +/- 24</b>
Limonene	31 +/- 8	<b>107 +/- 12</b>	<b>128 +/- 3</b>
(Z)-β-ocimene *	961 +/- 106	1395 +/- 163	<b>1601 +/- 77</b>
(E)-β-ocimene *	12659 +/- 4580	16609 +/- 6602	<b>34760 +/- 1381</b>
Linalool oxide (furan)	0	65 +/- 11	59 +/- 3
4-carene	33 +/- 11	<b>1030 +/- 139</b>	<b>1259 +/- 78</b>
2-isopropyl-3-methoxypyrazine	0	46 +/- 12	111 +/- 11
Linalool *	204 +/- 48	<b>3207 +/- 611</b>	<b>4803 +/- 704</b>
KI 1093	1 +/- 1	<b>56 +/- 9</b>	<b>75 +/- 9</b>
Chrysanthenone	0	6 +/- 2	15 +/- 2
KI 1103	1429 +/- 543	0	0
(E)-4,8-dimethyl-1,3,7-nonatriene *	1850 +/- 316	<b>14372 +/- 3157</b>	<b>26073 +/- 3201</b>
Cosmene <sup>b</sup>	1 +/- 1	<b>87 +/- 12</b>	<b>132 +/- 23</b>
Menthatriene	8 +/- 0.3	4 +/- 3	18 +/- 7
Alloocimene	0	3 +/- 1	8 +/- 2
KI 1146	19 +/- 6	40 +/- 16	<b>70 +/- 4</b>
Borneol	4 +/- 2	<b>42 +/- 9</b>	<b>49 +/- 6</b>
Linalool oxide (pyran)	0	5 +/- 2	4 +/- 2
(Z)-3-hexenyl butyrate *	50 +/- 39	28 +/- 10	60 +/- 14
Methyl salicylate *	407 +/- 221	<b>1569 +/- 165</b>	<b>1537 +/- 337</b>
Unknown monoterpene alcohol (KI 1170)	19 +/- 1	19 +/- 6	10 +/- 6
Unknown monoterpene alcohol (KI 1186)	10 +/- 3	21 +/- 8	<b>35 +/- 4</b>

Values in bold indicates compounds that are up-regulated (non-overlap of standard errors) relative to undamaged plant emissions

Compounds marked with a (\*) were verified using standards. All other compounds were IDed based on matches in the NIST library

<sup>a</sup> Amounts are nanograms of volatiles/12 h collection period

<sup>b</sup> Due to not having a reference standard, the configuration of this compound cannot be confirmed

**Table 2** (continued)

Compound	Undamaged <sup>a</sup>	Adult-damaged <sup>a</sup>	Nymph-damaged <sup>a</sup>
KI 1188	4 +/- 3	<b>56 +/- 8</b>	<b>29 +/- 3</b>
3-hexenyl isovalerate	0	10 +/- 5	6 +/- 3
KI 1219	11 +/- 7	<b>139 +/- 35</b>	<b>85 +/- 17</b>
Piperitone	7 +/- 3	<b>84 +/- 32</b>	<b>163 +/- 26</b>
Cinnamaldehyde	5 +/- 3	13 +/- 5	0
Geranial	0	110 +/- 42	212 +/- 40
Indole *	23 +/- 6	<b>371 +/- 41</b>	<b>425 +/- 77</b>
Citronellyl formate	37 +/- 14	78 +/- 32	<b>139 +/- 24</b>
Cinnamyl alcohol	0	45 +/- 17	103 +/- 14
Bornyl acetate	0	11 +/- 4	25 +/- 5
KI 1284	0	35 +/- 13	101 +/- 18
2,4-dodecadienal	0	16 +/- 7	15 +/- 4
KI 1306	24 +/- 9	52 +/- 22	<b>88 +/- 18</b>
KI 1316	0	27 +/- 11	18 +/- 5
4-hydroxybenzaldehyde	0	24 +/- 11	6 +/- 2
KI 1330	0	5 +/- 3	9 +/- 3
( <i>Z</i> )-jasmone *	5 +/- 2	7 +/- 3	3 +/- 3
KI 1360	26 +/- 10	37 +/- 18	33 +/- 18
<i>cis</i> -jasmone	8 +/- 3	<b>247 +/- 29</b>	<b>239 +/- 42</b>
KI 1378	7 +/- 3	<b>95 +/- 14</b>	<b>99 +/- 7</b>
KI 1385	10 +/- 7	<b>53 +/- 7</b>	<b>58 +/- 4</b>
$\beta$ -elemene	13 +/- 8	<b>204 +/- 160</b>	<b>56 +/- 4</b>
$\beta$ -bourbonene	35 +/- 12	0	8 +/- 8
$\beta$ -caryophyllene *	893 +/- 307	3025 +/- 1155	<b>5292 +/- 333</b>
$\alpha$ -cedrene	129 +/- 68	<b>866 +/- 366</b>	20 +/- 1
$\beta$ -cubebene	7 +/- 7	<b>173 +/- 25</b>	<b>231 +/- 30</b>
Bergamotene	220 +/- 159	644 +/- 244	0
2-phenylethyl isothiocyanate	0	48 +/- 19	0
$\alpha$ -humulene *	80 +/- 13	<b>311 +/- 53</b>	<b>403 +/- 29</b>
( <i>E</i> )- $\beta$ -farnesene *	60 +/- 13	<b>286 +/- 27</b>	<b>280 +/- 26</b>
KI 1452	0	19 +/- 7	43 +/- 1
KI 1456	0	9 +/- 6	43 +/- 17
KI 1460	11 +/- 9	<b>121 +/- 47</b>	0
KI 1469	83 +/- 33	<b>1487 +/- 587</b>	<b>3351 +/- 535</b>
KI 1473	30 +/- 6	<b>331 +/- 41</b>	<b>242 +/- 24</b>
Germacrene D	229 +/- 80	<b>2178 +/- 747</b>	386 +/- 20
$\alpha$ -bergamotene	86 +/- 47	<b>5838 +/- 2137</b>	<b>10898 +/- 34</b>
$\beta$ -selinene	11 +/- 8	<b>88 +/- 33</b>	0
Germacrene B	3456 +/- 1093	925 +/- 414	0
( <i>E,E</i> )- $\alpha$ -farnesene <sup>b</sup>	13 +/- 8	50 +/- 19	0
$\alpha$ -amorphene	46 +/- 30	157 +/- 53	24 +/- 10
KI 1512	4 +/- 4	<b>66 +/- 26</b>	0
Delta-cadinene	37 +/- 24	173 +/- 66	0
KI 1527	1 +/- 1	<b>32 +/- 12</b>	0
KI 1533	15 +/- 10	67 +/- 25	0
KI 1547	53 +/- 35	105 +/- 42	0
Nerolidol *	45 +/- 13	447 +/- 159	660 +/- 93
KI 1555	77 +/- 19	82 +/- 22	87 +/- 43
( <i>E,E</i> )-4,8,12-trimethyl-1,3,7,11-tridecatetraene *	194 +/- 59	1142 +/- 563	672 +/- 334



**Table 2** (continued)

Compound	Undamaged <sup>a</sup>	Adult-damaged <sup>a</sup>	Nymph-damaged <sup>a</sup>
KI 1575	6 +/- 4	65 +/- 28	0
Caryophyllene oxide	5 +/- 5	45 +/- 18	0
Hexadecane	7 +/- 7	<b>84 +/- 19</b>	7 +/- 7
KI 1613	11 +/- 7	94 +/- 37	0
KI 1620	14 +/- 4	<b>50 +/- 10</b>	<b>35 +/- 5</b>
KI 1630	6 +/- 6	<b>105 +/- 38</b>	23 +/- 10
KI 1641	13 +/- 9	123 +/- 51	0
KI 1658	4 +/- 4	48 +/- 19	0
KI 1666	2 +/- 2	<b>30 +/- 12</b>	0
KI 1670	14 +/- 11	135 +/- 55	0
KI 1693	3 +/- 2	41 +/- 21	<b>30 +/- 8</b>
KI 1716	6 +/- 2	<b>53 +/- 20</b>	<b>81 +/- 13</b>
KI 1727	78 +/- 54	116 +/- 47	0
Octadecane	1 +/- 1	<b>20 +/- 8</b>	0
KI 1833	6 +/- 6	<b>62 +/- 22</b>	14 +/- 8
KI 1838	30 +/- 16	61 +/- 25	0
KI 1844	74 +/- 29	217 +/- 89	<b>427 +/- 10</b>
Anthrone	0	10 +/- 4	0.8 +/- 0.8
KI 2012	12 +/- 6	187 +/- 132	0
1,4-anthraquinone	6 +/- 4	185 +/- 130	87 +/- 84

damaged by *L. rubrosignatus* nymphs there was no difference between the number of wasps that flew to either choice plant ( $Chi\ squared=0.429$ ,  $df=1$ ,  $P=0.5127$ ).

## Discussion

Our data demonstrate that *Erigeron annuus* produces large quantities of a complex blend of volatile organic compounds both constitutively and in response to *Lygus* feeding. Between damaged and undamaged *Erigeron* plants, the blends differ in both the quantity of total volatiles as well as in the identity of the compounds that make up the blend (Table 2) (Online resource 1). Relative to the crop of interest in this study (cotton), *E. annuus* plants release both more compounds and greater amounts of compounds shared between the two blends. Our behavioral data demonstrate that this volatile blend plays a strong role in mediating the attraction of *Lygus* bugs to *Erigeron*, which has previously been observed in the field (Fleischer et al. 1988).

Despite the overwhelming volume of volatile compounds that it emits, the attractiveness of *E. annuus* to *Lygus* bugs cannot be explained by quantity of volatiles alone. When the same damage treatment was applied to both cotton and *Erigeron* plants, the *Erigeron* plants emitted a much larger total volume of volatiles relative to the cotton (Fig. 1), and were more attractive in all cases (Fig. 2). However, when different

damage treatments were applied to the two choice plants (nymph damaged cotton vs. undamaged *Erigeron*), total volatiles were similar (Fig. 4), but female adult *Lygus* bugs still were attracted to *Erigeron* over the cotton treatment (Fig. 3). Similarly, when bugs were presented with a choice between undamaged *Erigeron* and female adult damaged cotton, they did not display a preference (Fig. 3) even though *Erigeron* released more total volatiles than the damaged cotton (Fig. 4). Additionally, nearly half of the compounds present in nymph or adult damage cotton treatments also are present in all of the blends released by the various *Erigeron* damage treatments (Tables 2 and 3), so it is unlikely that our results are simply due to cotton being repellent when damaged by adults. Therefore, these data indicate that specific qualitative aspects of the odor blend from *Erigeron* mediate the increased attraction (possibly the large diversity and volume of terpenes emitted from both undamaged and damaged *Erigeron* plants (Table 2)).

Previous studies on *Lygus* attraction towards specific plant volatiles have shown that several green leaf volatiles (GLVs), terpenes, and aromatic compounds are detected by *Lygus* bugs and elicit a variety of responses. Blackmer et al. (2004) demonstrated that female adult *L. hesperus* were attracted to *Medicago sativa* L. plants that had either been damaged by conspecifics for several days or recently had conspecifics added onto them. Furthermore, the compounds (*Z*)- $\beta$ -ocimene, (*E*)- $\beta$ -ocimene,  $\beta$ -caryophyllene, and  $\alpha$ -farnesene were upregulated by *L. hesperus* damage, while  $\beta$ -pinene, myrcene, methyl salicylate, and

**Table 3** Mean amounts of each compound +/- standard error from undamaged, adult damaged, and nymph damaged cotton plants

Compound	Undamaged <sup>a</sup>	Adult damaged <sup>a</sup>	Nymph damaged <sup>a</sup>
KI 778	1657 +/- 7	8125 +/- 42	895 +/- 43
( <i>E</i> )-2-hexenal *	36 +/- 2	<b>4725 +/- 76</b>	<b>468 +/- 18</b>
( <i>Z</i> )-3-hexen-1-ol *	51 +/- 1	<b>406.3 +/- 129</b>	<b>7308 +/- 351</b>
KI 850	0	0	1033 +/- 8
KI 854	0	4675 +/- 11	3383 +/- 16
KI 857	0.9 +/- 0.9	<b>297 +/- 37</b>	217 +/- 13
KI 864	4571 +/- 2	0	8167 +/- 5
KI 887	6857 +/- 3	0	4.7 +/- 3
KI 894	0	1075 +/- 3	2033 +/- 9
$\alpha$ -pinene *	536 +/- 7	<b>9303 +/- 424</b>	<b>4807 +/- 87</b>
KI 939	0.7 +/- 0.7	<b>62 +/- 17</b>	367 +/- 1
$\beta$ -pinene *	0.7 +/- 0.7	<b>146 +/- 69</b>	<b>777 +/- 15</b>
Myrcene *	0	256.1 +/- 79	2093 +/- 42
( <i>Z</i> )-3-hexenyl acetate *	61 +/- 20	<b>1990 +/- 744</b>	<b>2491 +/- 1076</b>
KI 996	0	161 +/- 63	265 +/- 9
KI 1007	257 +/- 1	1275 +/- 7	1267 +/- 9
KI 1013	393 +/- 10	<b>231 +/- 21</b>	<b>1273 +/- 31</b>
Limonene *	0	5275 +/- 26	2917 +/- 10
( <i>E</i> )- $\beta$ -ocimene *	67 +/- 4	<b>6105 +/- 115</b>	<b>753 +/- 146</b>
KI 1060	286 +/- 1	<b>3875 +/- 11</b>	<b>3617 +/- 8</b>
KI 1074	571 +/- 2	<b>5275 +/- 14</b>	<b>43 +/- 15</b>
Linalool *	116 +/- 5	<b>713 +/- 6</b>	<b>3293 +/- 71</b>
( <i>E</i> )-4,8-dimethyl-1,3,7-nonatriene *	3729 +/- 233	0	4055 +/- 301-
KI 1088	614 +/- 4	0	3833 +/- 17
KI 1134	2571 +/- 1	<b>875 +/- 0</b>	85 +/- 3
( <i>Z</i> )-3-hexenyl butyrate *	649 +/- 18	<b>4088 +/- 138</b>	3737 +/- 179
KI 1186	886 +/- 2	<b>3275 +/- 5</b>	85 +/- 3
KI 1201	0.4 +/- 0.4	<b>10 +/- 2</b>	1083 +/- 5
KI 1216	0	625 +/- 2	14 +/- 8
KI 1220	0	625 +/- 3	8667 +/- 3
Indole *	0	9025 +/- 29	385 +/- 13
KI 1396	2071 +/- 9	0	1667 +/- 10
KI 1415	0	4033 +/- 138	1752 +/- 58
$\alpha$ -humulene *	0	0	1317 +/- 8
( <i>E</i> )- $\beta$ -farnesene *	27 +/- 1	<b>1155 +/- 36</b>	<b>668 +/- 14</b>
KI 1496	0.7 +/- 0.7	<b>484 +/- 107</b>	<b>3873 +/- 134</b>
KI 1507	0	1125 +/- 41	573 +/- 11
KI 1514	274 +/- 10	<b>1295 +/- 26</b>	675 +/- 21
KI 1540	0.8 +/- 0.8	675 +/- 3	<b>1083 +/- 4</b>
KI 1553	453 +/- 19	1558 +/- 57	1925 +/- 63
KI 1560	0	25 +/- 2	5 +/- 2
Nerolidol *	1343 +/- 13	0	6 +/- 4
( <i>E,E</i> )-4,8,12-trimethyl-1,3,7,11-tridecatetraene *	4021 +/- 259	0	2755 +/- 191

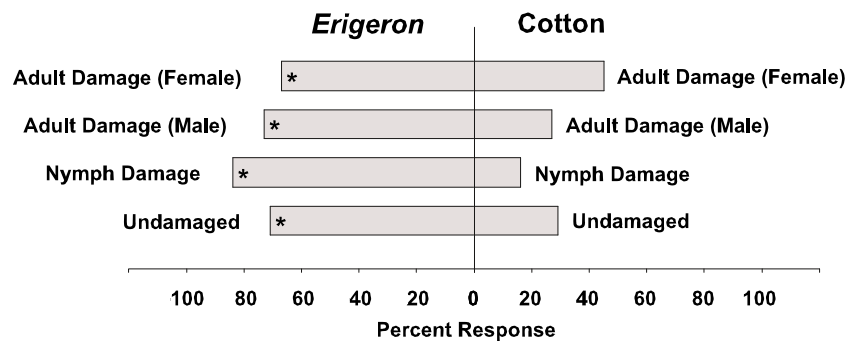
Values in bold indicates compounds that are up-regulated (non-overlap of standard errors) relative to undamaged plant emissions

Compounds marked with a (\*) were verified using standards. All other compounds were IDed based on matches in the NIST library

<sup>a</sup> Amounts are nanograms of vol-atiles/12 h collection period

tridecatetraene were all novel compounds induced by bug feeding. While Blackmer et al. (2004) did not test these compounds individually, Williams III et al. (2010) carried out both electroantennogram (EAG) tests and Y-tube olfactometer bioassays that tested the response of *L. hesperus* to individual

compounds. They found a strong antennal response to the GLVs (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, 1-hexenol, (*E*)-2-hexenyl acetate, and (*E*)-2-hexenal, and a moderate response to the terpenes (*E*)- $\beta$ -ocimene,  $\alpha$ -farnesene, and linalool and the aromatic methyl salicylate. All of these compounds, as well as many



**Fig. 2** Female adult *Lygus* behavioral responses to odor cues from *Erigeron* or cotton plants with the same damage treatments. Analysis by *chi-square* tests determined if the distribution of bug choices deviated from 50:50 (\* indicates significant difference at  $P < 0.05$ ). The number of responding females used for each set of choice tests (and the number of

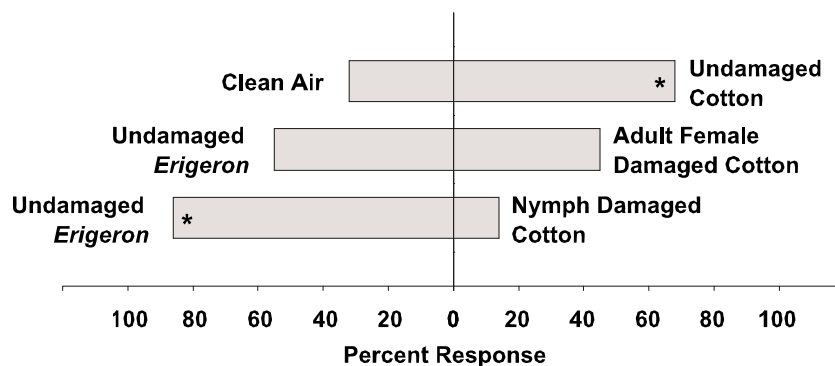
plant pairs) was: adult damaged (female)  $N=112$  (7 non-responders) (6 pairs), adult damaged (male)  $N=59$  (6 non-responders) (4 pairs), nymph damaged  $N=46$  (7 non-responders) (4 pairs), and undamaged  $N=75$  (14 non-responders) (6 pairs)

other GLVs and monoterpenes, appear in the volatile blend from *E. annuus* in both undamaged and *Lygus* damaged treatments (Table 2). However, the Y-tube tests carried out by Williams III et al. (2010) were less consistent, with male and female *L. hesperus* showing no positive response to several of these individual compounds and in some cases even repellent responses. This may be due to the fact that herbivorous hemipteran insects often are attracted to specific blends of volatiles rather than to any one volatile within a blend (Ngumbi et al. 2007; Webster et al. 2010). Our data support this previous finding.

While the large total amount of volatiles produced by *E. annuus* may not play a direct role in making the plant attractive to *Lygus*, this characteristic may make the plant ideal for use as a trap crop. A single *Erigeron* plant emits a far greater amount of volatiles than a much larger *G. hirsutum* plant, which means that a travelling *Lygus* bug may be able to locate and preferentially move to a small patch of *Erigeron* even if it is located in a large cotton field. In fact, this seems to be the case as seen in field studies carried out by Fleischer et al. (1988) where *L. lineolaris* adults were shown to disperse through a 4.7 ha field of cotton into 4 6 m by 6 m plots of *E. annuus* located at the

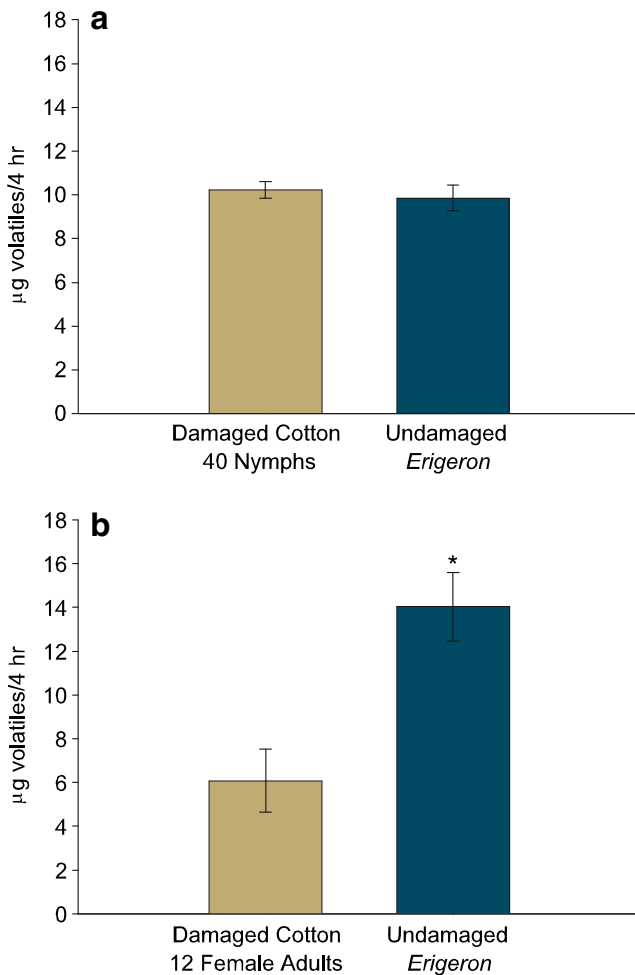
edges of the field. More importantly, our data show that *Erigeron* remains an attractive host plant for *Lygus* females relative to cotton under a wide variety of damage treatments (consistent with previous research on alfalfa [Blackmer et al. 2004]), suggesting that *Erigeron* will remain attractive even as it is colonized by ovipositing females and developing nymphs.

Simply ‘pulling’ *Lygus* bugs off of cotton may not be sufficient to make *Erigeron* a successful trap crop. A higher density of *Lygus* on the trap plant species relative to the economically important crop does not always lead to a decrease in the pest population on the crop or a decrease in the damage caused to the crop. This can be seen in attempts to manage *L. rugulipennis* Poppius in strawberry using *Matricaria recutita* L. and *Medicago sativa* as trap crops (Easterbrook and Tooley 1999). Additional management steps may be necessary, such as mowing of the trap crop, or spraying of pesticides into the trap crop patches. Some successful attempts at controlling *Lygus* species by using trap crops have used these methods. Accinelli et al. (2005) found that plots of *Medicago sativa* grown alongside lettuce plots did not reduce the damage on lettuce by *L. rugulipennis* feeding alone, but when combined

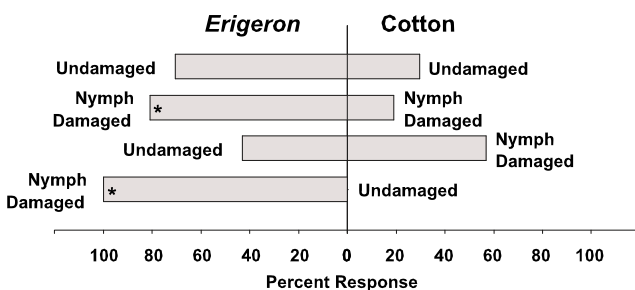


**Fig. 3** Female adult *Lygus* behavioral responses to odor cues from cotton vs. clean air and undamaged *Erigeron* vs. cotton with different damage treatments. Analysis by *chi-square* tests determined if the distribution of bug choices deviated from 50:50 (\* indicates significant difference at  $P < 0.05$ ). The number of responding females used for each set of choice tests

(and the number of plant pairs) was: undamaged cotton vs. clean air  $N=59$  (3 non-responders) (2 plants), undamaged *Erigeron* vs. adult (F) damaged cotton  $N=65$  (3 non-responders) (3 pairs), undamaged *Erigeron* vs. nymph damaged cotton  $N=37$  (4 non-responders) (2 pairs)



**Fig. 4** Mean total volatiles  $\pm$  standard error for *Erigeron* and cotton plants under different damage treatments during the Y-tube choice tests. **a** choice test using 40 actively feeding nymphs on a cotton plant vs. an undamaged *Erigeron* plant, **b** choice test using 12 actively feeding and ovipositing female *Lygus* bugs vs. an undamaged *Erigeron* plant (\* indicates significant difference at  $P=0.05$ )



**Fig. 5** Percent response of female *Peristenus relictus* wasps to volatiles from undamaged and *Lygus* nymph damaged *Erigeron annuus* (left) and *Gossypium hirsutum* (right) plants. All wasps were given experience only on nymph damaged cotton and were started on a cotton leaf downwind of the choice plants. The \* symbol indicates a significant preference at  $P < 0.05$  (*Chi-squared* test). The number of responding female wasps used for each set of choice tests was: undamaged *E. annuus* vs. undamaged cotton  $N=17$  (0 non-responders), nymph-damaged *E. annuus* vs. nymph-damaged cotton  $N=21$  (13 non-responders), undamaged *E. annuus* vs. nymph-damaged cotton  $N=29$  (2 non-responders), nymph-damaged *E. annuus* vs. undamaged cotton  $N=22$  (0 non-responders)

with spraying of pesticides in the alfalfa plots, damage was significantly reduced on the lettuce. Godfrey and Leigh (1994) also found some success in controlling *L. hesperus* numbers in cotton that was interplanted with strips of alfalfa that were then cut over the course of the growing season (see also Stern et al. 1969), while Swezey et al. (2007) demonstrated that vacuuming the alfalfa trap crop greatly reduced the damage caused by *L. hesperus* to organic strawberries. However, Godfrey and Leigh (1994) also noted that increased frequency of mowing in the alfalfa patches led to decreases in populations of natural enemies of *Lygus*. If a trap crop is sufficiently attractive to effective natural enemies, control may be achieved within the trap crop – and by extension the crop – simply by relying on non-chemical measures (e.g., natural enemy release or conservation).

The results of the wind tunnel bioassays demonstrate that this strategy may be possible for the *Erigeron*-cotton system since *Erigeron* is highly attractive to female *Peristenus relictus* wasps, which are an effective control for *Lygus* in other crops (Day et al. 2003; Day 2005). In every treatment, the *E. annuus* plants attracted a large proportion of the responding female wasps, even when the *Erigeron* plant was undamaged and the cotton plant was damaged by nymphs (Fig. 5). This was the case despite the fact that these wasps had been allowed previous oviposition experience only on *Lygus* nymph damaged cotton. This finding is surprising given that parasitoid wasps will learn the odor cues associated with hosts or with host-damaged plant tissues and will then use these long-term associative memories to guide foraging for additional hosts (Lewis and Tumlinson 1988; Lewis and Takasu 1990; Papaj et al. 1994; reviewed in Vet et al. 1995). Parasitoid wasps are capable of developing these memories after only a single positive experience in the presence of an odor (Smid et al. 2007), and can maintain different odor memories for locating food sources or suitable hosts (Lewis and Takasu 1990). In our study, wasps were given between 2 and 5 positive oviposition experiences in the presence of volatile odors from nymph damaged cotton, but consistently preferred the odors of nymph damaged *E. annuus* over both undamaged and nymph damaged cotton plants (Fig. 5). This may be due to the fact that damaged *E. annuus* plants release many of the same compounds as nymph damaged cotton, and release a large number of additional compounds in high amounts (particularly terpenes, which are attractive to natural enemies [e.g., Kessler and Baldwin 2001; Turlings et al. 1990]) (see Online Resource 1). The addition of these other compounds to the blend already released by cotton (those compounds shared between the two treatments) does not seem to interfere with the ability of the wasps to respond to the blend it has learned in the presence of hosts, and appear to enhance the attractiveness of *E. annuus* relative to cotton.

In the choice tests, undamaged *E. annuus* plants also were attractive to the *P. relictus* females, as roughly half of the wasps flew to undamaged *E. annuus* even in the presence of a nymph



infested cotton plant. Comparing undamaged *E. annuus* to damaged cotton, many compounds found in the damaged cotton (except unknown compound KI 823, unknown compound KI 888, 1-methyl-2-(1-methylethyl)-benzene KI 1011, and unknown compound KI 1500) are shared by the undamaged *Erigeron* (Online Resource 1). However, the undamaged blend of *E. annuus* has fewer compounds overall than the damaged *E. annuus* blend, which may indicate that these extra compounds are part of what makes the damaged *Erigeron annuus* plants so attractive.

The situation we have observed here is similar to what was reported by Khan et al. (2000) regarding the plant, *Melinis minutiflora*. In that case, the constitutive volatiles produced by the *M. minutiflora* were similar to the induced volatiles produced by pest-damaged maize. Khan et al. (2000) theorized that this compound overlap (which had already been shown to be attractive to parasitoids) would act to repel lepidopterous pests, and this theory was proven correct as *Melinis minutiflora* intercropped amongst cereal fields does reduce the population of stem borers in those fields. Volatiles from plants damaged by herbivores being repellent to ovipositing female Lepidoptera has been shown in other cases as well, such as the night-time volatiles from *Heliothis virescens* Fabricius damaged tobacco plants being repellent to females searching for oviposition sites (De Moraes et al. 2001). However, herbivore-repellent effects of conspecific-damaged host plant blends do not appear to be universal, as our results demonstrate that *Lygus* are highly attracted to damaged *Erigeron*.

Our results indicate that *Lygus* attraction to *E. annuus* is influenced by volatile emissions, and is due to both qualitative and quantitative aspects of the *E. annuus* blend, which has significant overlap with the target crop host blend in addition to including a large number of additional compounds primarily volatile terpenes. The dual attraction of both *Lygus* females and females of an introduced natural enemy, *P. relictus*, to *E. annuus* under a variety of damage treatments suggests that this plant would function well as a trap to both pull *Lygus* out of the target crop and focus biological control efforts into areas of maximum pest density. These results, considered alongside previous work showing that *E. annuus* serves as a high quality nectar source for other, native, *Peristenus* species (Streams et al. 1968), indicates that *E. annuus* could fulfill a third role in the system by provisioning food resources to *P. relictus* wasps released into the area. Future work should focus on performing trials in the field to verify that *Erigeron* can fulfill the roles of trap plant, natural enemy attractor, and natural enemy provisioner under real-world conditions that include other aspects of cotton management.

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