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Induction of Plant Volatiles by Herbivores with Different Feeding Habits and the Effects of Induced Defenses on Host-Plant Selection by Thrips

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Abstract Induced plant responses to attack by chewing insects have been intensively studied, but little is known about plant responses to nonchewing insects or to attack by multiple herbivores with different feeding habits. We examined volatile emissions by tobacco, *Nicotiana tabacum*, in response to feeding by the piercing–sucking insect western flower thrips (WFT), *Frankliniella occidentalis*, the chewing herbivore *Heliothis virescens*, and both herbivores simultaneously. In addition, we examined the effects of herbivore-induced plant defenses on host-plant selection by WFT. Plants responded to thrips feeding by consistently releasing five compounds. Simultaneous feeding by WFT and *H. virescens* elicited the same 11 compounds emitted in response to caterpillar feeding alone; however, two compounds, α-humulene and caryophyllene oxide, were produced in greater amounts in response to simultaneous herbivory. In choice tests, thrips consistently preferred uninduced plants over all other treatments and preferred plants damaged by caterpillars and those treated with caterpillar saliva over those treated with caterpillar regurgitant. The results are consistent with a previous finding that caterpillar regurgitant induces the release of significantly more volatile nicotine than plants damaged by caterpillars or plants treated with caterpillar saliva. A repellent effect of nicotine on WFT was confirmed by encircling unwounded plants with septa releasing volatile nicotine. Our results provide the first direct evidence that thrips feeding induces volatile responses and indicates that simultaneous herbivory by insects with different feeding habits can alter volatile emissions. In addition, the findings demonstrate that induced plant responses influence host-plant selection by WFT and suggest that the induction of volatile nicotine may play a role in this process.

Keywords Plant–insect interactions · Induced defenses · Plant volatiles · Simultaneous herbivory · Ovipositional preference · Insect behavior · Host-plant selection · *Frankliniella occidentalis* · *Heliothis virescens* · *Nicotiana tabacum* · Nicotine

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Introduction

Herbivory induces plant defense responses including the production of toxic secondary metabolites and the synthesis and release of volatile chemical signals that are attractive to herbivore natural enemies (Turlings et al. 1990, 1995; Karban and Baldwin 1997; De Moraes et al. 1998; Dicke 1999; Pare and Tumlinson 1999). These induced responses can alter host-plant quality and impact the behavior and performance of subsequent herbivores (Karban and Baldwin 1997; Agrawal 1999; De Moraes et al. 2001; Wise and Weinberg 2002; De Moraes and Mescher 2004; Rodriguez-Saona et al. 2005). Interactions between plants and chewing insects (e.g., lepidopteran larvae) are well documented (Karban and Baldwin 1997; Walling 2000). Much less is known about plant responses to insects that employ other feeding modes, such as aphids, whiteflies, and bugs (Du et al. 1998; Turlings et al. 1998; Rodriguez-Saona et al. 2002, 2003; Williams III et al. 2005), although extensive research has been conducted on the spider mite *Tetranychus urticae*, a non-insect arthropod that also feeds in a piercing–sucking manner (Dicke and Sabelis 1988; Dicke et al. 1990a, b). Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is a piercing–sucking insect and a major worldwide pest of agriculture and horticulture (Lewis 1997; Kirk and Terry 2003). We are unaware of any previous research that has explicitly examined volatile induction in response to WFT feeding, but WFT infestation is known to influence subsequent insect behavior. For example, the predatory bug *Orius laevigatus* prefers cucumber plants infested with WFT (Venzon et al. 1999), whereas the spider mite *T. urticae* avoids them (Pallini et al. 1997). Thrips themselves may exploit cues from infested plants to avoid intraspecific competition (Agrawal and Colfer 2000).

Plant responses to feeding by multiple herbivore species that employ different feeding modes are also poorly understood, although attack by herbivores with diverse feeding strategies is common in nature (Strauss 1991; Vos et al. 2001). Simultaneous feeding by multiple herbivores may influence plant volatile responses by inducing competing plant defense pathways, with possible implications for interactions with the third trophic level. For example, feeding by two species of Lepidoptera, *Plutella xylostella* and *Pieris rapae*, causes cabbage plants to emit different volatile blends from those emitted in response to feeding by either species alone and alters the response of parasitoids (Shiojiri et al. 2001). Additionally, the mixing of different volatile blends emitted from neighboring plants simultaneously infested with different herbivore species has the potential to influence the foraging success of herbivore natural enemies (Dicke et al. 2003).

This paper explores plant volatile responses to feeding by WFT and to simultaneous feeding by WFT and the chewing insect *Heliothis virescens* (Fabricius) on tobacco, *Nicotiana tabacum* L. In addition, we document the impacts of induced plant responses on host-plant selection by WFT. Prior herbivory can influence the behavior of subsequent herbivores by impacting host-plant quality (Karban and Baldwin 1997), by eliciting volatile responses that serve as cues for foraging insects (e.g., De Moraes et al. 2001), and by inducing other biochemical changes in host plants (e.g., Duffey and Stout 1996). We previously found that tobacco plants treated with *H. virescens* regurgitant release significantly more volatile nicotine than plants treated with *H. virescens* saliva or plants damaged by *H. virescens* caterpillars (Delphia et al. 2006). In the current study, we examine the effects of *H. virescens* saliva- and regurgitant-induced plant responses, as well as other treatments, on the distribution and ovipositional preferences of WFT. Our results offer insight into the dynamics of plant–herbivore interactions when plants are simultaneously attacked by herbivores with different feeding habits, and into the role of induced defenses in influencing host-plant choice by an important insect pest.

Methods and Materials

Plants and Insects

Tobacco seeds (*N. tabacum* strain K326) were germinated in a peat-based, general-purpose potting soil (Pro-Mix, Premier Horticulture Inc., Quakertown, PA, USA) and transplanted as 2-wk-old seedlings into pots (16 cm tall × 16.5 cm diam) with fertilizer (Osmocote/ Hummert International, MO, USA) in a growth chamber [16:8 hr light:dark; 25:22°C day: night; 65% relative humidity (RH)]. Five-wk-old plants with three fully expanded leaves were used for all experiments. Eggs of *H. virescens* were obtained from the USDA/ARS Research Laboratory in Tifton, Georgia, and reared on an artificial casein diet in a growth chamber under the same conditions as above. Adult and larval *F. occidentalis* were obtained from infested greenhouse plants that were virus-free (i.e., plants free of symptoms typical for tomato spotted wilt virus: chlorotic rings, chlorotic mosaic patterns, mottled leaves, reduction of growth or deformation).

Induced Plant Volatiles

Simultaneous Herbivory

To examine induced plant responses to herbivory, we collected volatiles from tobacco plants receiving the following treatments: (1) feeding by adult and larval thrips, (2) mechanical wounding, (3) mechanical wounding plus feeding by adult and larval thrips, (4) feeding by three third-instar *H. virescens*, or (5) feeding by three third-instar *H. virescens* plus adult and larval thrips. Undamaged plants were used as controls. Treatments were applied daily, immediately before volatile collection, and volatiles were collected on d 1–4 to ensure induction in response to herbivory. Plants were mechanically wounded by using a razor blade to scrape an approximately 1 cm^2 area on each of the three leaves per plant. The same three leaves received new mechanical damage each day so that by the end of the 4-d experiment, plants had three leaves with four wounds each. Five adult and five first- or second-instar thrips were added to thrips treatments each day so that by the end of the 4-d experiment, 20 adult and 20 larval thrips had been added to each plant. Thrips were added daily to ensure that a minimum number of thrips were feeding each day, as they tended to escape from the volatile collecting chambers. The level of infestation was chosen based on the levels found in greenhouses that employ conservative action thresholds for monitoring thrips. *Heliothis virescens* were allow to feed continuously throughout the 4-d experiment. For treatments where both herbivores were allowed to feed on plants, the feeding location of each herbivore did not appear to be influenced by the presence of the other, and both thrips and caterpillars fed on the same leaves. This experiment was replicated four times.

Increased Levels of Thrips Feeding Damage

To determine if higher levels of thrips damage would alter emission profiles, we collected volatiles from tobacco plants exposed to two additional levels of thrips feeding. We employed (1) a medium-damage treatment where plants received 20 first- or second-instar thrips and 20 adult thrips on the first day of the experiment, followed by 10 additional adult thrips per day for the following 3 d for a total of 70 thrips added to each plant, and (2) a high-damage treatment where plants received 40 first- or second-instar thrips and 40 adult thrips on the first day of the experiment, followed by 20 additional adult thrips per day for the following 3 d for a total of 140 thrips added to each plant. Thrips were added immediately before volatile collection, and volatiles were collected on each of the 4 d to ensure induction in response to herbivory. This experiment was replicated three times.

Collection and Analysis of Volatiles

Volatiles were collected from tobacco plants by using a closed push/pull system. A two-piece Teflon® base with a hole for the plant stem rested on the pot. A glass dome chamber (15 cm tall×16 cm diam) enclosed plants and rested on the base. Filtered air was pushed through Teflon® tubing into the top of the chamber (3.0 l/min) and was pulled through side ports (0.8 l/min) across beds of adsorbent Super-Q® (25 mg, Alltech Associates, Deerfield, IL, USA). Plant volatiles were collected between 1000 and 2200 hours (light period: 0600 to 2200 hours). Super-Q® traps were rinsed with 150 μl of dichloromethane; 5 μl of *n*-octane (40 ng/μl) and *n*-nonyl-acetate (80 ng/μl) were added as internal standards. Samples were injected, using a splitless injector held at 220°C, in 1-μl aliquots into an Agilent model 6890 gas chromatograph fitted with a flame ionization detector. The column (15 m× 0.25 mm i.d., 0.1 μm film thickness, HP-1) was maintained at 35°C for 0.5 min and then increased by 12°C per min to 180°C. Quantifications of compounds were made relative to the internal standard using ChemStation software (Agilent Technologies, Wilmington, DE, USA). Samples were also analyzed by gas chromatography mass spectrometry by using electron ionization (6890 gas chromatograph interfaced to a Hewlett-Packard 5973N mass selective detector). The column (30 m×0.25 mm i.d., 0.25 μm film thickness, HP-1MS) was maintained at 35°C for 0.5 min and then increased by 12°C per min to 180°C. Identifications of all volatile compounds were confirmed by comparing retention times and mass spectra to commercial standards.

Leaf Measurements

To determine leaf area for plants used for volatile collections, we removed leaves from plants and photocopied them. Photocopies were digitized, and leaf areas and amount of damage were determined using an imaging program (SigmaScan® Pro, SPSS Inc., Chicago, IL, USA).

Collection of *H. virescens* Salivary Glands and Regurgitant

Labial salivary glands were dissected from artificial-diet-fed *H. virescens* larvae that had been fifth-instars for 48 hr and had been chilled on ice for approximately 5 min. Once removed, the salivary glands were placed into microcentrifuge tubes, kept on ice in groups of 10 pairs, and stored at −80°C until needed. Immediately before use, each 10-gland sample was extracted in 50 μl physiologically buffered saline (pH 7.2) by homogenizing the salivary glands with a hand-held pestle in a microcentrifuge tube kept on ice. This allowed salivary-gland extracts to be applied to mechanically wounded leaves with a pipette. Protein concentrations were determined by using the method of Bradford (1976). Total protein concentrations for our samples of homogenized salivary glands were around 700 μg per 40 μl (i.e., the amount applied to plants in experiments). This amount was based on a previously described method (Delphia et al. 2006).

Before collecting regurgitant, recently molted fifth-instar caterpillars were fed for 48 hr on tobacco leaves from approximately 7-wk-old plants. Regurgitant was collected from these larvae by gently squeezing the caterpillars, collecting the resulting oral secretions with a pipette, and dispensing them into a microcentrifuge tube on ice. Regurgitant was pooled from approximately 50 larvae and then separated into 50 μl aliquots and stored at −80°C until needed.

Thrips Distribution and Ovipositional Preferences

We conducted a series of choice tests to examine thrips behavior in response to induced and uninduced tobacco plants. Adult thrips were allowed to choose between undamaged control plants and plants that received various treatments including mechanical wounding, wounding plus the application of caterpillar saliva or regurgitant, and caterpillar feeding. We also explored the effects of volatile nicotine on thrips behavior by allowing thrips to choose between undamaged plants surrounded by rubber septa releasing nicotine and those surrounded by blank septa. In all choice tests, adult thrips (20–50 depending on the experiment) were released each day for 2 d then counted every day for 3, 4, or 6 d (depending on the experiment) to determine thrips distributional preferences. At the end of all experiments, the leaves of plants were removed, and the numbers of larvae were counted to determine ovipositional preferences. All choice experiments were conducted under greenhouse conditions except for the nicotine choice experiment that was done in a growth chamber.

Effects of Mechanical Wounding

To determine the effects of mechanical wounding on thrips distribution and oviposition preferences, we allowed thrips to choose between wounded plants and unwounded controls. Two mechanically wounded plants and two control plants were positioned 0.3 m from each other at one end of a greenhouse bench (approximately 1.5×3 m), creating a square with wounded plants on one side and control plants on the other. This design was replicated, with treatments reversed, at the other end of the bench. On the first day of the experiment, the plants were wounded by using a razor blade to damage three leaves (as above). This was repeated on d 2–5, wounding the same three leaves each day. On d 1 and 2, wounding was followed by the release of 20–30 adult WFT from a 150-ml beaker in the center of each set of four plants. On d 3–6, we recorded the number of adult thrips on each plant. On d 8, we removed leaves and counted the larvae. Observations and wounding occurred each morning between 0800 and 1000 hours. This experiment was replicated six times.

Effects of Caterpillar Feeding and Oral Secretions

To determine the effects of caterpillar feeding damage and the application of oral secretions associated with herbivore feeding on thrips distribution and ovipositional preferences, thrips were allowed to choose between tobacco plants receiving the following treatments: (1) prior feeding (~18 hr) by two third-instar *H. virescens* followed by continuous feeding by one third-instar *H. virescens* for 5 d, (2) mechanical wounding, (3) mechanical wounding plus the application of 40 μl of *H. virescens*-homogenized salivary glands distributed among three wounds per day, (4) mechanical wounding plus the application of 40 μl of *H. virescens* regurgitant distributed among three wounds per day, or (5) control (i.e., unwounded) plants. We were unable to include a treatment that examined thrips ovipositional preferences in response to plants induced by thrips infestation because of an inability to exclude or distinguish larvae resulting from prior infestation. The amounts of oral secretions applied to plants were based on a previously described method (Delphia et al. 2006).

Five plants were positioned in a circle equidistant from a central point (0.3 m) on each of the two greenhouse benches (approximately 1.5×3 m). The positions of the treatments on each bench were arranged so that no two treatments were adjacent to one another on both benches. On d 1, the plants were wounded by using a razor blade to damage three leaves (as above). Regurgitant and homogenized salivary glands were applied at this time using a pipette to evenly distribute the compounds among the damaged sites. This procedure was repeated on d 2–5. Also, on d 1, we removed one of the two third-instar *H. virescens*, which had been feeding for the previous 18 hr, from the caterpillar-damaged treatment to limit leaf tissue loss. The remaining caterpillar was allowed to feed continually on d 1–5. On d 1 and 2, wounding and the application of oral secretions were followed by the release of 30–40 adult WFT from a 150-ml beaker in the center of each set of five plants. On d 3–8, we recorded the number of adult thrips on each plant. On d 8, we removed leaves and counted the larvae. All observations and manipulations occurred between 0900 and 1100 hours. This experiment was replicated six times.

Effects of Volatile Nicotine

To determine the effects of volatile nicotine on thrips distribution and ovipositional preferences, thrips were allowed to choose between unwounded tobacco plants encircled by rubber septa that had been treated with either nicotine or left blank (i.e., treated with solvent alone). Two plants were positioned about 1 m from each other in a growth chamber (16:8 light:dark; 25:22°C day:night; 65% RH). Three rubber septa, which were previously conditioned with 150 μl dichloromethane, were positioned on metal stands around each of the two plants. With a pipette, 2 μl of nicotine was added to each of the septa in the nicotine treatment. Control septa were left blank. On the first 2 d of the experiment, 45–55 adult WFT were released from a 150-ml beaker centered between the two plants. On d 2, 3, and 4, we recorded the number of adult thrips per plant. On d 7, we removed leaves and counted the larvae. Observations and thrips release occurred each morning between 1030 and 1130 hours. This experiment was replicated three times.

Statistical Analyses

We analyzed the data collected on d 4 from volatile collection experiments to allow sufficient induction in response to herbivory. We quantified all volatile compounds released in measurable amounts. The data from the simultaneous herbivory experiment were $\log_e(x+1)$-transformed to satisfy the assumptions of normality and homogeneity of variance among treatments and were analyzed by analysis of variance (ANOVA), with trial treated as a random effect. The data from the levels of thrips feeding damage experiment were analyzed by ANOVA. All pairwise comparisons were conducted by using Tukey's honestly significant difference (HSD) test. The data from choice tests were square-root-transformed to homogenize variance among treatments. We analyzed both the mechanical wounding and the caterpillar feeding and oral secretions choice tests for adult thrips distribution over four and six sampling days, respectively, by repeated-measures ANOVA, with day treated as a fixed effect and with trial and bench position within trial treated as random effects. We analyzed the volatile nicotine choice test for adult thrips distribution over three sampling days by repeated-measures ANOVA, with day treated as a fixed effect. We used ANOVA to analyze the number of larvae per plant at the end of all three choice tests. All statistical analyses were conducted by using Minitab v. 14.1 (Minitab Inc., State College, PA, USA).

Results

Induced Plant Volatiles

Thrips Feeding Damage

When we compared volatile emissions in response to low, medium, and high levels of thrips feeding damage, we saw an apparent increase in the total amount of volatiles released, although this difference was not statistically significant (ANOVA: $F_{2,\ 7}=2.86$, $P=0.124$; Fig. 1a; Table 1). Plants responded to low levels of thrips feeding by releasing two compounds consistently ((*E*)-β-ocimene and β-caryophyllene; Table 1). Two additional

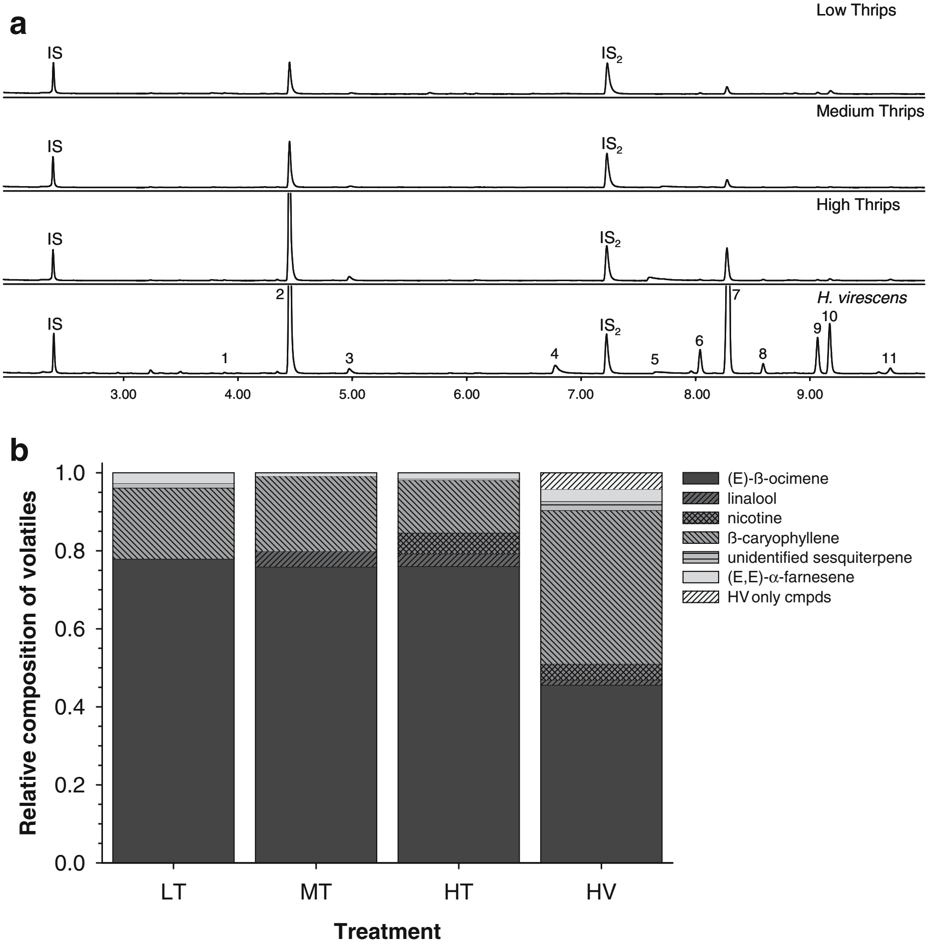

Fig. 1 Chromatograms showing typical daytime emission profiles (**a**) and composition (**b**) of volatiles released by tobacco plants receiving low (LT), medium (MT), and high levels of thrips feeding (HT) or *H. virescens* feeding (HV). Compounds are labeled as follows: *1*, myrcene; *2*, (*E*)-β-ocimene; *3*, linalool; *4*, indole; *5*, nicotine; *6*, β-elemene; *7*, β-caryophyllene; *8*, α-humulene; *9*, unidentified sesquiterpene; *10*, (*E,E*)-α-farnesene; *11*, caryophyllene oxide; *IS* and IS_2, internal standards (*n*-octane and *n*-nonyl-acetate)

Table 1 Amounts (nanogram per day; mean ± SE) of volatiles released on d 4 by tobacco plants receiving varying treatments

Compound	Treatment		
	LT	MT	HT
(*E*)-β-Ocimene	433±259a	494±114a	1100±141a
Linalool	0±0a	26.5±14.2a	46.9±5.78b
Nicotine	0±0a	0±0a	78±50.4a
β-Caryophyllene	100.9±62.8a	125.3±29.3a	195.9±54.4a
Unidentified sesquiterpene	6.8±6.8a	0±0a	5.9±5.9a
(*E,E*)-α-Farnesene	14.7±14.7a	5.11±5.11a	20.4±13.5a
Total volatiles	555±342a	651±157a	1447±232a

Numbers in each row followed by the same letter indicate no significant differences (Tukey's HSD test, $P<0.05$)
Low levels of thrips feeding damage (LT), medium levels of thrips feeding damage (MT), and high levels of thrips feeding damage (HT) ($N=3$).

compounds (an unidentified sesquiterpene and (*E*,*E*)-α-farnesene) were also emitted in response to low levels of thrips feeding, but were only observed in one replicate (Table 1). In response to medium and high levels of thrips feeding damage, (*E*)-β-ocimene and β-caryophyllene remained the dominant compounds and were released in increasing amounts, although not significantly so ($P>0.05$; Table 1). In addition to releasing (*E*)-β-ocimene and β-caryophyllene, plants responded to medium and high levels of thrips feeding damage by releasing linalool (Table 1). Significantly more linalool was released by plants receiving high levels of thrips feeding damage compared to those with low levels of thrips damage (ANOVA: $F_{2,\ 7}=9.55$, $P=0.010$; Table 1). Nicotine was also released by plants in response to high levels of thrips damage, although the difference was not significant due to large variance (ANOVA: $F_{2,\ 7}=2.93$, $P=0.119$; Table 1). The compound (*E*,*E*)-α-farnesene was released in response to all three levels of thrips feeding damage, but it was only consistently released by plants receiving high levels of thrips damage (Table 1).

The highest level of thrips feeding induced the consistent release of five compounds (Table 1). In comparison, feeding by *H. virescens* induced the release of 11 compounds (Fig. 2; Table 2). Of the five shared compounds released by plants in response to thrips and caterpillar feeding, (*E*)-β-ocimene, linalool, β-caryophyllene, and (*E*,*E*)-α-farnesene were all released in greater amounts in response to caterpillar feeding ($P<0.05$). Because several of the compounds induced by thrips feeding were also among the most dominant compounds released in response to caterpillar feeding, it is conceivable that the observed differences are due solely to the differences in feeding damage. Additionally, because the feeding habits of thrips and caterpillars are so dissimilar, standardizing the amount of damage among treatments is problematic. However, we did observe consistent differences in plant volatile responses to caterpillars vs thrips that persisted over a range of thrips damage levels. Moreover, when we look at the relative proportions of individual compounds in each of the blends, we see that the ratios of (*E*)-β-ocimene to β-caryophyllene (i.e., the two dominant compounds released in response to thrips and caterpillars) are dissimilar (Fig. 1b). The relative proportion of (*E*)-β-ocimene to β-caryophyllene in the caterpillar blend is almost 1:1 (46 to 39%) vs 4.3:1 (78 to 18%), 4:1 (76 to 19%), and 5.8:1 (76 to 13%) in the low, medium, and high thrips feeding blends, respectively, which suggests that plants are responding differently to the two herbivores irrespective of damage (Fig. 1b).

Conceivably, these differences might still reflect differences in damage levels, as the observable leaf damage caused by caterpillar feeding is considerably more extensive than

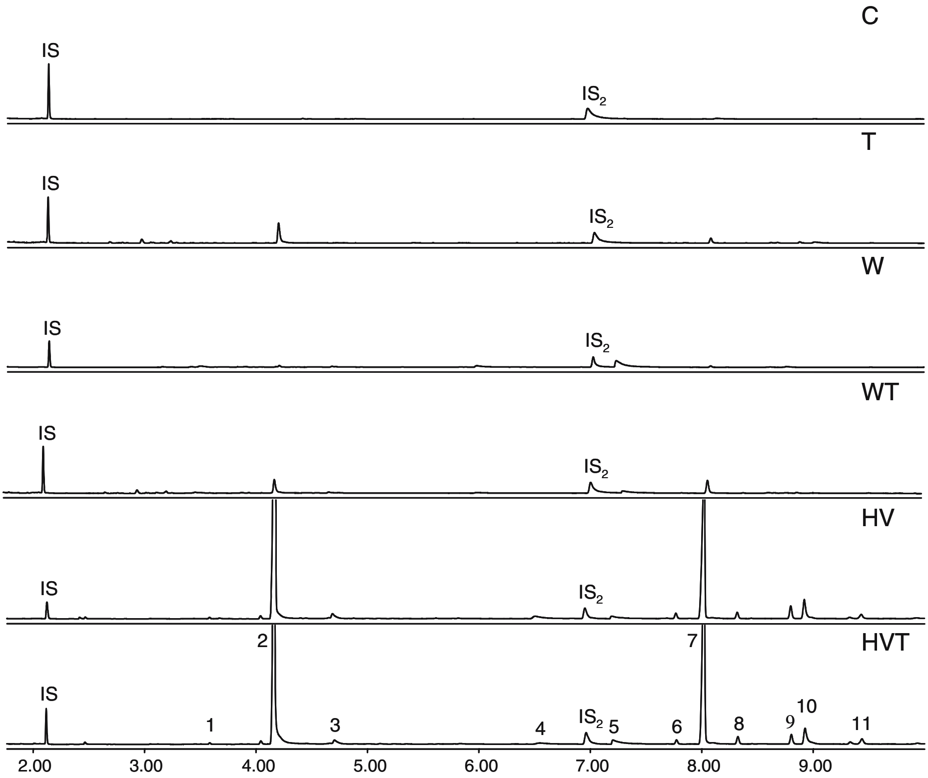

Fig. 2 Chromatograms showing typical daytime emission profiles of volatiles released by tobacco plants receiving the following treatments: unwounded control plants (C), thrips feeding (T), mechanical wounding (W), mechanical wounding plus thrips feeding (WT), *H. virescens* feeding (HV), or *H. virescens* plus thrips feeding (HVT). Compounds are labeled as follows: *1*, myrcene; *2*, (*E*)-β-ocimene; *3*, linalool; *4*, indole; *5*, nicotine; *6*, β-elemene; *7*, β-caryophyllene; *8*, α-humulene; *9*, unidentified sesquiterpene; *10*, (*E*,*E*)-α-farnesene; *11*, caryophyllene oxide; *IS* and *IS*$_2$, internal standards (*n*-octane and *n*-nonyl-acetate)

that caused by even high levels of thrips feeding ($P<0.001$), although leaf loss is probably a poor indicator of the damage inflicted by thrips feeding. Another approach to standardizing the treatments is to compare overall responses at damage levels where the amounts of the dominant compounds are similar. The level of (*E*)-β-ocimene emitted by plants on the second day of caterpillar feeding (data not shown) was similar to that released in response to high levels of thrips damage on d 4 ($P>0.05$; Table 1). Yet, the overall blends were notably different: all 11 compounds typically released in response to caterpillar feeding were induced by d 2 compared to only five compounds released in response to high levels of thrips feeding. Of the shared compounds, β-caryophyllene and (*E*,*E*)-α-farnesene were released in greater amounts in response to caterpillar feeding ($P<0.05$). There were no significant differences in the amounts of linalool or nicotine released by plants that were damaged by thrips or caterpillars ($P>0.05$). These results suggest that there are differences in plant responses to thrips and caterpillar feeding that cannot be entirely explained by differences in leaf-damage area.

Plants that were mechanically wounded or those that received mechanical wounding plus thrips feeding released three compounds [(*E*)-β-ocimene, nicotine, and β-caryophyllene;

Table 2 Amounts (nanogram per day; mean±SE) of volatiles released on d 4 by tobacco plants receiving varying treatments

Compound	Treatment					
	C	T	W	WT	HV	HVT
Myrcene	0±0a	0±0a	0±0a	0±0a	17.2±6.1b	22.25±3.3b
(*E*)-β-Ocimene	0±0a	433±259b	15.2±10.1a	121.1±44.7b	4,299±982c	5,315±1100c
Linalool	0±0a	0±0a	0±0a	0±0a	125.1±29.6b	178.3±34.3b
Indole	0±0a	0±0a	0±0a	0±0a	74.1±52.4b	141.7±66.8b
Nicotine	0±0a	0±0a	233±97.1b	160.1±76.4b	390±201b	538±217b
β-Elemene	0±0a	0±0a	0±0a	0±0a	89.6±45.2b	101.5±35.1b
β-Caryophyllene	0±0a	100.9±62.8b	39.5±8.5b	124.3±33.3b	3,704±684c	6,166±964c
α-Humulene	0±0a	0±0a	0±0a	0±0a	122.8±23.6b	208.6±34.7c
Unidentified sesquiterpene	0±0a	6.8±6.8a	0±0a	0±0a	219.2±87.7b	267.8±93.8b
(*E,E*)-α-Farnesene	0±0a	14.7±14.7a	0±0a	0±0a	293±106b	457±171b
Caryophyllene oxide	0±0a	0±0a	0±0a	0±0a	89.4±14.1b	166.3±33.6c
Total volatiles	0±0a	555±342b	288.2±94.9b	405.5±90.1b	9,423±2006c	13,562±1978c

Numbers in each row followed by the same letter indicate no significant differences (Tukey's HSD test on log-transformed data, $P<0.05$).

Unwounded control plants (C), thrips feeding (T), mechanical wounding (W), mechanical wounding plus thrips feeding (WT), *H. virescens* feeding (HV), or *H. virescens* plus thrips feeding (HVT) ($N=4$).

Table 2]. Nicotine was the most abundant compound released by mechanically wounded plants and by mechanically wounded plants that also received thrips feeding (Table 2).

Simultaneous Herbivory

Simultaneous feeding by thrips and *H. virescens* induced the release of the same 11 compounds emitted in response to *H. virescens* feeding alone (Fig. 2; Table 2). However, simultaneous herbivory induced the release of more α-humulene ($P<0.05$) and caryophyllene oxide ($P<0.01$; Table 2). There were no significant differences in the amount of leaf area removed by simultaneous herbivory (1,678±348 mm^2 damage/day) compared to caterpillar feeding alone (1,691±417 mm^2 damage/day), although this measure likely fails to capture the intensity of thrips feeding damage.

Thrips Distribution and Ovipositional Preferences

Effects of Mechanical Wounding

More adult thrips were found on unwounded plants (22.3±2.8 thrips/plant) than wounded plants (4.6±0.7 thrips/plant) over four sampling days (ANOVA: $F_{1,\ 40}=102.12$, $P<0.001$; Fig. 3). Thrips also displayed an apparent ovipositional preference for unwounded plants, with more larvae recovered on control plants (45±11.7 larvae/plant) compared to wounded plants (6.5±2.5 larvae/plant; $P<0.01$).

Effects of Caterpillar Feeding and Oral Secretions

We observed more adult thrips on unwounded plants than on any other treatment over six sampling days (ANOVA: $F_{4,\ 165}=15.74$, $P<0.001$; Fig. 4a). Additionally, more thrips were

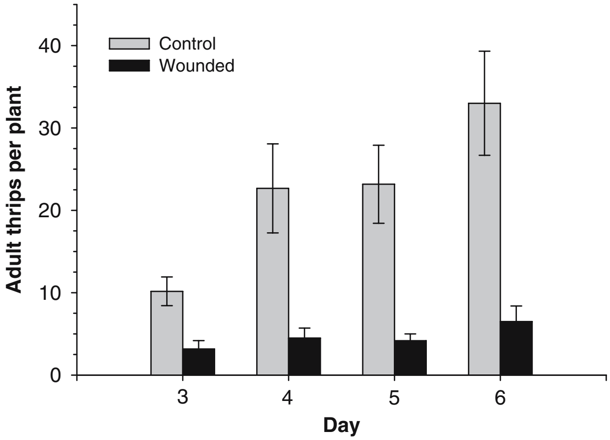

Fig. 3 Mean (±SE) number of adult WFT on control and mechanically wounded plants over four sampling days ($N=6$)

found on plants damaged by *H. virescens* and plants treated with *H. virescens* saliva than on plants treated with *H. virescens* regurgitant ($P<0.01$; Fig. 4a). Female thrips preferred to oviposit into unwounded control plants compared to mechanically wounded plants or plants treated with *H. virescens* regurgitant ($P<0.05$; Fig. 4b). Although there were no statistical differences, thrips appeared to prefer control plants as oviposition sites compared to plants damaged by *H. virescens* and plants treated with *H. virescens* saliva (Fig. 4b). They also appeared to prefer plants damaged by *H. virescens* and plants treated with *H. virescens* saliva compared to mechanically wounded plants and plants treated with *H. virescens* regurgitant, although, again, the observed differences were not statistically significant (Fig. 4b).

Effects of Volatile Nicotine

More adult thrips were found on plants encircled by blank (control) septa (19.78±3.34 thrips/plant) than on plants encircled by septa releasing nicotine (7.56±1.42 thrips/plant) over three sampling days (ANOVA: $F_{1,\ 14}=33.3$, $P<0.001$; Fig. 5). More larvae were also recovered on plants encircled by control septa (97.7±17 larvae/plant) compared to plants encircled by the nicotine septa (37±8.08 larvae/plant; $P=0.032$).

Discussion

Induced Plant Volatiles

Simultaneous feeding by multiple herbivores is common in natural ecosystems (Strauss 1991; Vos et al. 2001). Previous research has demonstrated that plant responses to feeding by a single herbivore species differ from response to attack by multiple herbivores (e.g., Shiojiri et al. 2001; Rodriguez-Saona et al. 2003) and that attack by multiple species can have both positive and negative effects on preference and performance of subsequent herbivores and herbivore natural enemies (Cardoza et al. 2002; Shiojiri et al. 2002; Rodriguez-Saona et al. 2005). In our study, simultaneous herbivory by thrips and *H. virescens* induced the same 11 compounds as caterpillar feeding alone, although there were significant differences in the relative amounts of two individual compounds, α-humulene and caryophyllene oxide. Total

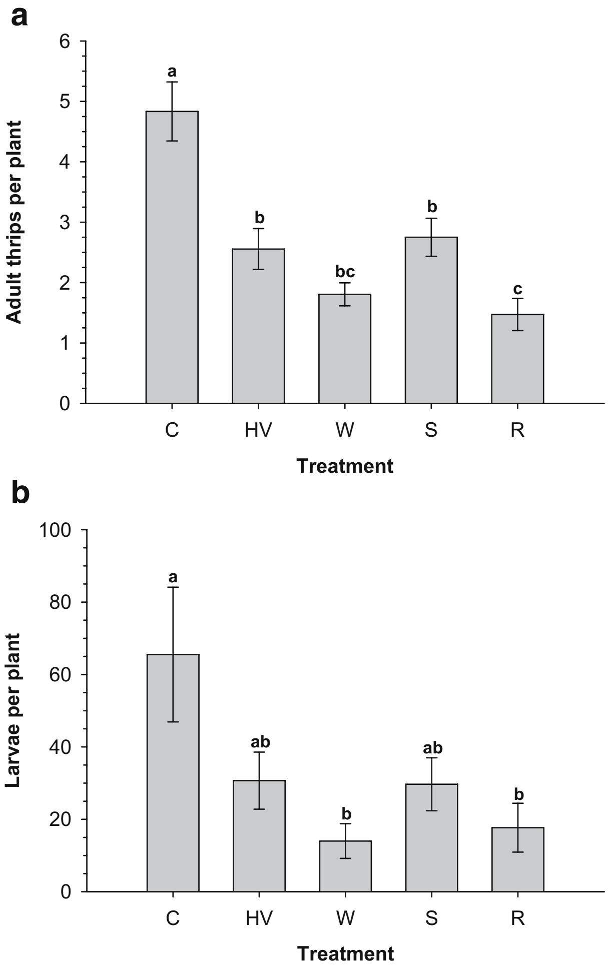

Fig. 4 Mean (±SE) number of adult WFT (**a**) and larvae (**b**) on plants receiving the following treatments: unwounded control plants (C), *H. virescens* feeding (HV), mechanical wounding (W), mechanical wounding plus *H. virescens* saliva (S), or mechanical wounding plus *H. virescens* regurgitant (R) (N=6). Letters above each bar indicate the significant differences as indicated by Tukey's HSD test, $P<0.05$

volatile production in our study was not reduced in response to simultaneous herbivory, in contrast to the 60% reduction observed in cotton plants when exposed to simultaneous feeding by silverleaf whitefly and beet armyworm compared to beet armyworm feeding alone (Rodriguez-Saona et al. 2003). However, our findings are similar to those reported by Cardoza et al. (2002) who found that simultaneous pathogen infection and herbivore feeding in peanut plants, *Arachis hypogaea*, induced the release of some compounds in higher quantities compared to herbivore feeding alone. Dual-damaged plants were also shown to be more attractive to the parasitoid *Cotesia marginiventris* (Cardoza et al. 2003). Similarly, Shiojiri et al. (2002) demonstrated an increase in parasitism by *Cotesia glomerata* on plants infested by two caterpillar species compared to plants infested with their host caterpillar alone. It remains to be determined whether the changes in volatile emissions that we observed in response to simultaneous infestation by thrips and caterpillars similarly alter the behavior of herbivore natural enemies.

Our results provide the first direct evidence that thrips induce volatiles and support previous research that provided evidence for the use of thrips-induced plant volatiles in

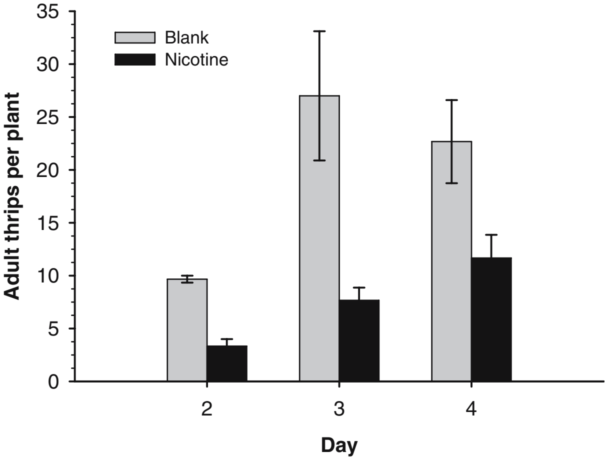

Fig. 5 Mean (±SE) number of adult WFT on unwounded plants encircled by blank rubber septa (control) and rubber septa containing nicotine over three sampling days (N=3)

both avoidance (Pallini et al. 1997) and attraction (Venzon et al. 1999) of heterospecifics to thrips-infested plants. Plants responded to feeding by the piercing–sucking insect WFT by emitting a volatile blend that was qualitatively and quantitatively distinct from that released in response to caterpillar feeding. While the very different feeding modes of these two herbivores make it difficult to standardize damage levels across treatments, the observed differences in volatile blends persisted across a wide range of thrips damage levels. Moreover, the overall volatile responses to thrips vs caterpillar feeding were different at damage levels where the amount of the most dominant compound released was similar. These results suggest that there are systematic differences in the volatile response to thrips vs caterpillar feeding that cannot be wholly explained by differences in the amount of damage inflicted. This contrasts with the previous finding by Turlings et al. (1998) that feeding by the stemborer *Ostrinia nubilalis* on maize induced a volatile blend qualitatively similar to that induced by caterpillar feeding but released in much lower quantities. The phloem-feeding aphid *Rhopalosiphum maidis*, which causes limited cell damage, did not induce measurable volatile emissions at all, although other aphid species elicit plant volatile responses (Du et al. 1996, 1998).

Thrips Distribution and Ovipositional Preferences

The ability of herbivores to distinguish among plants as suitable oviposition sites is important for the fitness of their offspring (e.g., Ohsaki and Sato 1994). Our results indicate that thrips are able to distinguish among plants as suitable hosts and that induced plant responses influence this preference. Thrips exhibited a consistent distributional preference for unwounded plants over all other treatments and preferred plants fed on by *H. virescens* and those treated with *H. virescens* saliva to those treated with *H. virescens* regurgitant (Figs. 3 and 4a). A similar pattern was observed for thrips ovipositional preferences, although significant differences were observed only between unwounded controls and plants that were mechanically wounded or treated with regurgitant (Fig. 4b). However, the fact that control plants had double the number of larvae compared to plants damaged by *H. virescens* and those treated with saliva suggests that adult thrips may be able to distinguish among these treatments as suitable oviposition sites for larval development

(Fig. 4b). While it is possible that poor egg hatch or increased larval mortality may have contributed to the low numbers of larvae recovered on wounded plants, we did not observe any dead adults or larvae throughout the choice experiments. Moreover, Maris et al. (2004) reported a direct correlation between the number of eggs laid and the larvae that emerged even on a resistant pepper variety.

The observed thrips preference for unwounded plants is consistent with the observation that the application of jasmonic acid, a natural elicitor of plant responses similar to those elicited by chewing herbivores, resulted in a decrease in the abundance of thrips on tomato (Thaler et al. 2001). Herbivore-induced plant responses were also found to reduce thrips feeding compared to un-induced control plants in the absence of prey (Agrawal et al. 1999). In contrast, Rodriguez-Saona and Thaler (2005) investigated the effects of herbivore-induced responses on the abundance of arthropods on tomato and found that patch type (i.e., induced vs un-induced) did not affect the abundance of thrips. However, because thrips are omnivores, prey availability may explain why patch type did not affect abundance (Agrawal et al. 1999).

We previously reported that tobacco plants treated with *H. virescens* regurgitant release significantly higher levels of volatile nicotine compared to those treated with *H. virescens* saliva or induced by caterpillar feeding (Delphia et al. 2006). Thus, we suspected that the similar pattern of thrips distribution and ovipositional preferences observed in this study might be explained, in part, by differential nicotine induction in plants treated with *H. virescens* saliva and regurgitant. In our choice assays, thrips preferred plants encircled by blank (control) septa compared to those encircled by septa releasing nicotine (Fig. 5), and more larvae were also found on the control plants, suggesting that nicotine has a significant effect on thrips behavior. This finding is consistent with studies demonstrating that induced plant volatiles can repel herbivores, such as ovipositing moths and aphids (Bernasconi et al. 1998; De Moraes et al. 2001).

Other authors have previously suggested a role for caterpillar saliva in suppressing induced plant defense mechanisms (Musser et al. 2002; Na and Chenzhu 2004; Bede et al. 2006). For example, glucose oxidase, an enzyme first identified from the saliva of the corn earworm, *Helicoverpa zea*, and subsequently found in other caterpillar species, suppresses the wound-inducible production of foliar nicotine in tobacco, *N. tabacum* (Musser et al. 2002). It is possible that foliar nicotine, which increases upon mechanical wounding (McCloud and Baldwin 1997; Musser et al. 2002), as well as other non-volatile metabolites (Duffey and Stout 1996), likely contributed to thrips distribution and ovipositional preferences observed in our experiments.

In summary, our results offer insights into plant responses to feeding by piercing–sucking insects, simultaneous feeding by herbivores with different feeding habits, and the influence of induced plant responses on subsequent herbivore behavior. Tobacco plants responded to all three levels of thrips feeding damage by releasing a volatile blend distinct from that released in response to caterpillar feeding. Simultaneous feeding induced the release of the same 11 compounds as caterpillar feeding alone; however, two compounds were produced in greater quantities. Induction of plant defenses had significant impacts on the distribution and ovipositional preferences of WFT: significantly more adults were found on unwounded control plants in both thrips choice tests, and more thrips were found on plants damaged by caterpillars and plants treated with caterpillar saliva compared to plants treated with caterpillar regurgitant. Furthermore, thrips preferred to oviposit into unwounded control vs wounded plants or plants treated with caterpillar regurgitant. These differences may be explained, at least in part, by the apparent repellant effect of nicotine, as significantly fewer adult and larval thrips were found on plants encircled by septa releasing volatile nicotine compared to those encircled by blank septa.

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