Volatile Emissions from Developing Cotton Bolls in Response to Hemipteran Feeding Damage[1]

D.C. Degenhardt[2], J.K. Greene, A. Khalilian[3], and R.B. Reeves[4]

Department of Entomology, Soils, and Plant Sciences, Clemson University, Edisto Research and Education Center, 64 Research Road, Blackville, South Carolina 29817 USA

J. Entomol. Sci. 46(3): 177-190 (July 2011)

Abstract Hemipteran pests feed directly on cotton fruiting structures (bolls) causing damage to fiber and yield. Herbivore-induced volatile emissions have been well studied with regard to leaf-chewing insects, but no research has examined the release of volatiles from developing cotton bolls in response to damage from piercing-sucking insects. We compared volatile emissions from bolls in response to feeding damage by brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula*, (L.), and the leaf footed bug, *Leptoglossus phyllopus* (L.) under laboratory conditions. Volatile emissions from bolls in response to *N. viridula* and mechanical damage were investigated under field conditions. Volatiles were collected using dynamic headspace sampling and analyzed by gas chromatography/mass spectrometry. Under laboratory conditions, feeding by hemipterans resulted in a significant increase in volatile emissions from bolls compared with undamaged bolls. Damaged bolls released significantly greater amounts of acyclic terpenes and methyl ketones compared with undamaged bolls. Feeding by different hemipteran species elicited a similar quantitative increase in emissions, but significant differences were detected in the emissions of some individual compounds. Under field conditions, feeding damage by *N. viridula* resulted in significantly greater volatile emissions compared with undamaged and mechanically-damaged bolls indicating that physical damage alone did not account for the complete blend of volatiles released in response to biotic injury. During feeding, hemipterans inject a complex blend of salivary and digestive enzymes, and some of these compounds may activate volatile induction from bolls. The implication for piercing-sucking damage on biochemical pathways mediating volatile synthesis is discussed.

Key Words *Gossypium*, Pentatomidae, Coreidae, herbivory, volatiles

Herbivore feeding induces plants to release volatile emissions that act as semiochemicals influencing numerous interactions between plants, herbivores, and natural enemies (Dicke et al. 1990, Karban and Baldwin 1997, De Moraes et al. 1998, Arimura et al. 2005). Volatile hydrocarbons are produced in virtually all plants and consist mainly of mono and sesquiterpenes, as well as low-molecular weight aromatic and aliphatic saturated and unsaturated alcohols, aldehydes, fatty acids, and ketones (Dudareva et al. 2006). In cotton, *Gossypium hirsutum* (L.), many volatiles are stored

[1]Received 24 August 2010; accepted for publication 20 November 2010.

[2]Address inquiries (email:ddegenh@clemson.edu).

[3]Department of Agricultural and Biological Engineering, Clemson University, Edisto Research and Education Center, 64 Research Road, Blackville, South Carolina 29817

[4]Department of Entomology, North Carolina State University, Campus box 7630, Raleigh, North Carolina 27695-7630 Email: ddegenh@clemson.edu

in lysigenous glands and contribute to the production of heliocides and gossypol (Loughrin et al. 1994, Opitz et al. 2008). In response to herbivore feeding, volatiles are synthesized *de novo* both at the site of wounding and systemically in undamaged leaves of damaged plants (Paré and Tumlinson 1997, 1999). Plants wounded by herbivores release a different compositional blend of volatiles compared with healthy (undamaged) plants and plants damaged mechanically, indicating that physical damage from mouthparts does not account for the inclusive blend produced in response to herbivory (Paré and Tumlinson 1997). Induced volatile emissions may vary depending on the herbivore species or feeding mode (Turlings et al. 1997) and may convey species-specific information to foraging natural enemies (De Moraes et al. 1998). Volatile-inducing elicitors such as β-glucosidase (Mattiacci et al. 1995), volicitin (Alborn et al. 1997), and inceptin (Schmelz et al. 2007) have been isolated from the oral secretions of different leaf-chewing caterpillars and have been shown to induce the synthesis of volatiles when applied to mechanical wound sites (Alborn et al. 1997). Whereas much of the knowledge on the biosynthesis and regulation of herbivore-induced emission of volatiles is derived from studies on leaf-chewing herbivore damage (Mattiacci et al. 1995, Paré and Tumlinson 1997, Turlings et al. 1997), few studies have investigated induced volatile emissions from plants in response to feeding by hemipterans that use piercing-sucking mouthparts.

Hemipterans feed by repeatedly piercing/probing host tissue with modified mandibular and maxillary stylets and secreting salivary components and digestive enzymes to liquefy host tissue (Panizzi et al. 2000). Feeding damage from hemipterans is known to induce volatile emissions from cotton and corn leaves (Rodriquez-Saona et al. 2002). Maize seedlings injured by southern green stink bug, *Nezara viridula* (L.), released greater amounts of terpenes, and this was due mainly to both mechanical damage from stylets and salvary gland extracts (Williams et al. 2005). Similarly, Rodriguez-Saona et al. (2002) demonstrated that oral secretions from western tarnished plant bug, *Lygus hesperus* (Knight), were capable of inducing volatile emissions from cotton leaves similar to blends produced by actual feeding damage, or treatment with volicitin. Although no specific elicitors of plant volatiles have been isolated from hemipterans, their salivary and digestive enzymes are a complex mixture of proteinases, peroxidases, pectinases, and lipases, some of which may serve as bioactive compounds that induce plant defenses (Wolfson and Murdoch 1990, Miles, 1999, Liu et al. 2009).

This study was initiated to investigate the induction of volatile emissions from developing cotton fruiting structures in response to feeding by hemipteran pests. In cotton, hemipterans (especially stink bugs) feed directly on developing fruiting structures (bolls) and are an increasing threat to cotton production in the US (Williams 2009). Prior to 1996, phytophagous hemipterans were of little importance in cotton. The success of eradication efforts for boll weevil, *Anthonomus grandis grandis* Boheman, in the Southeast (Haney et al. 1996) and the commercial release of transgenic cotton cultivars expressing toxins from *Bacillus thuringiensis* to control bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), have resulted in a decrease in the number of insecticide applications. Consequently, infestations of hemipteran pests, including the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), brown stink bug, *Euschistus servus* (Say), green stink bug, *Acrosternum hilare* (Say), and *N. viridula*, have increased in midsouthern and southeastern cotton production (Greene et al. 2001a). No research has examined piercing-sucking insects and induced volatile emissions from cotton bolls despite these structures being the

primary host plant material for foraging hemitperans. Here, we addressed the following specific questions: (1) Does hemipteran feeding damage induce volatile emissions from cotton bolls? (2) Is volatile induction influenced by different species of hemipteran pests? (3) Do induced volatile emissions differ between herbivore-damaged and mechanically-damaged bolls?

Materials and Methods

Plants and insects. Cotton variety Phytogen 370 WideStrike® Roundup Ready® (Dow AgroScience, Indianpolis, IN) was used in all experiments. Plants were sown on 5 March 2008 in a plastic germination tray and individually transplanted into 12-L pots containing a 3:1 soil:sand mixture (Baccto Lite™: play sand) 2 wk after germination. Plants were fertilized with Osmocote® and maintained in a greenhouse at the Clemson Univ. Edisto Research and Education Center (EREC) in Blackville, SC, under natural light conditions with a daytime temperature of 30°C and a nighttime temperature of 22°C. A relative humidity from 60 - 80% was maintained in the greenhouse. All plants used for experiments were 10 - 12 wk old, and all bolls used for volatile collections were 10 - 12 d postanthesis.

Adults and nymphs of *E. servus*, *N. viridula*, and leaf footed bug, *Leptoglossus phyllopus* (L.), were initially collected from field populations in soybean, and species were maintained separately in cages in the insect rearing facility at EREC. Insects were fed on a source of fresh green beans and provided with water on moistened cotton pads until initiation of experiments.

Laboratory experiments. Two 2 - 4-d-old adults of either *E. servus*, *N. viridula*, or *L. phyllopus* were placed inside enclosures covering a single cotton boll. Six replications of each species and unexposed controls were conducted on individual plants. Enclosures were constructed from polystyrene foam cups with the base of the cup removed and a nylon stocking stretched over the outside of the cup (Greene et al. 1999). Enclosures were placed over a boll and secured using light gauge steel wire twisted around the nylon stocking at the peduncle. Bugs were placed inside an enclosure which was subsequently sealed with a light gauge steel wire twisted around the nylon stocking. Bugs were allowed to feed *ad libitum* for 5 d prior to collection of volatiles. Enclosures were checked every 24 h, and dead individuals were replaced as needed. Control plants had enclosures placed over bolls but contained no insects. After 5 d, enclosures and bugs were removed from bolls, and volatile collection bags (described below) were placed over bolls 30 min after removal of bugs. Following collection of volatiles from bolls, seed and lint were examined for evidence of feeding injury and staining indicative of boll-rotting bacteria (data not shown).

Field experiments. The effect of stink bug injury and mechanical injury on volatile emissions from cotton bolls were compared using a randomized complete-block design in a 1.5-ha field at EREC containing *G. hirsutum* var. Delta and Pine Land 555. Enclosures, as described previously, were placed over white blooms, and bolls developed inside enclosures to prevent damage prior to experimentation. In each block, volatile emissions were collected from 12 - 14-d-old cotton bolls exposed to an adult of *N. viridula* for 3 d, mechanically damaged, or undamaged using the procedures described below. Each treatment was assigned to a single boll from individual plants separated by 2 - 3 plants in 4 blocks. Each block was randomly assigned to a single row separated by 2 - 4 rows. A single adult of *N. viridula* was placed inside an enclosure and allowed to feed *ad libitum* for 3 d. After insects were placed on bolls in the

field, bolls from the mechanical damage treatment were wounded by inserting a 0.25-mm diam insect pin in the carpel wall of each locule to a depth of 7 mm. The pin was mounted to the eraser of a pencil to ensure consistency of depth of puncture wounds on bolls. Bolls from all treatments (bug damaged, mechanically damaged, and undamaged) remained in enclosures for the duration of the experiment.

Volatile collection and analysis. In both experiments volatiles were sampled using a dynamic head-space collection method. A polyacetate oven bag (Reynolds, Inc., Richmond, VA) modified to a volume of 300 ml was placed over a boll and loosely fastened with a small cable tie at the base of the boll to permit airflow through the bag. A volatile trap was fastened to the top corner of a collection bag using a cable tie. Volatile collection traps were constructed from glass Pasteur pipettes (10 cm long, 0.5 cm OD) and contained 35 mg of Super Q adsorbant polymer (Alltech Assoc., Deerfield, IL, USA) held in place by two small plugs of glass wool. A battery-operated air-sampling pump (SKC, Inc. Eighty Four, PA, Model 224 - 44XR) fitted with an independently controlled, adjustable, 4-way splitter (SKC, Inc. Model 224 - 26 - 04) was used to draw ambient air through the collection bag across the boll and directly onto the trap at a rate of 300 ml/min. Volatiles were collected for a duration of 1 h. Ambient air blanks were collected simultaneously with boll volatile collections to correct for any volatile contaminants contained in the air pulled through a collection bag.

Volatiles were extracted from adsorbant traps by washing with 300 µL of analytical grade hexane. An internal standard of n-dodecane was added to the extract to a final concentration of 10 ng/µl, and 2 µL of each sample was analyzed by gas chromatography (GC) on a Hewlett-Packard 6890 gas chromatograph equipped with a RTX-5 30 m × 0.25 mm (i.d.) fused silica column with a 0.25 µm-thick dimethylpolysiloxane stationary phase (Restek, Bellefonte, PA). Injections were made in the splitless mode for 0.5 min. The GC injector temperature was set at 250°C with the column oven at 50°C for 10 min followed by an increase to 150°C at 5°C/min followed by an increase to 250°C at a rate of 15°C/min followed by a final increase to 300°C at a rate of 10°C/min and held for 5 min. Helium was used as a carrier gas at a flow rate of 1 ml/min. Samples were subsequently analyzed by mass spectrometry (MS) using a Varian VG-70S (Waters Corp., Milford, MA) operated in electron impact mode. The amount of volatiles was calculated by conversion of peak area units to mass (ng) based on the total peak area of the internal standard in each sample extract. Compounds were identified by comparison of retention time and mass spectra with those in the Environmental Protection Agency-National Institutes of Health data base, spectra from the library of essential oil components identified by GCMS (Adams 1995), and spectra obtained from known standards (Sigma-Aldrich, Inc., Milwaukee, WI), as well as solvent extracts of boll material. Several peaks were not identified due to low quality of match (< 90%) to mass spectra and analytic standards and thus were not included in the analysis.

Statistical analyses. For the laboratory experiment, a one-way analysis of variance (ANOVA) (Proc GLM) was used to test for differences in total amounts of volatiles among the treatments (bolls unexposed, or exposed to *E. servus*, *N. viridula*, or *L. phyllopus*). Treatment means were separated using Tukey's HSD test following a significant ($P < 0.05$) F test (SAS Institute 2008). Data were $\log_e(x + 1)$ transformed prior to analysis to satisfy the assumptions of normality and homogeneity of variance among treatments (Zar 1999). Volatile compounds were also compared across treatments using principal components analysis (PCA) (Proc Princomp), a technique used for reducing complex multivariate data to a smaller set of orthogonal, uncorrelated components that account for the maximum amount of variation (SAS Institute 2008).

The first principal component accounts for the greatest amount of variation in the data, whereas additional components account for successively smaller amounts of variation. Using PCA, the importance of each compound to the separation of treatments can be assessed by a plot of vector loadings for each compound. All volatile compounds identified in this experiment were used in the analysis.

For the field experiment, volatile emissions from bug-damaged, mechanically-damaged, and undamaged cotton bolls were compared using a two-factor ANOVA using treatment as main effect and block as a random effect in the model. Data were $\log_e(x + 1)$ transformed prior to analysis to satisfy the assumptions of normality and homogeneity of variance among treatments (Zar 1999). Treatment means were separated using Tukey's HSD test following a significant ($P < 0.05$) F test (SAS Institute 2008).

Results

Laboratory experiment. Ten compounds were identified in gas chromatograms of head-space volatiles collected from herbivore damaged bolls (Fig. 1). The majority of compounds identified were terpenoid in origin (mono, sesqui-, and homoterpenes), and 2 compounds were identified as the aliphatic ketone, 6-methyl-hepten-2-one (hereafter referred to as methyl-heptenone) (compound 3), and the aldehyde, nonanal (compound 7). Nonanal was the predominant compound detected in cotton boll emissions, regardless of treatment (Fig. 1). In addition to nonanal, cyclic terpenes were the primary compounds released from undamaged bolls, whereas several acyclic compounds, including β-ocimene (compound 6), 4,8-dimethyl-1,3,7-nonatriene (DMNT) (compound 8), β-farnesene (compound 9), and 4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)(compound 10), were detected in head space of bolls damaged by all three hemipteran species (Fig. 1).

Total volatile emissions from bolls damaged by *E. servus*, *N. viridula*, and *L. phyllopus* were significantly greater than those from undamaged bolls ($F = 9.41$; df = 3, 20; $P < 0.001$) (Fig. 2A). A 2.2-fold increase in volatile emissions was detected in response to feeding by *E. servus*, a 1.7-fold increase in response to *N. viridula* damage, and a 1.8-fold increase in response to *L. phyllopus* feeding damage (Fig. 2A). Hemipteran damage also influenced the blend of volatile compounds released from bolls (Fig. 2B). Three cyclic monoterpenes, α-pinene (compound 1), β-pinene (compound 2), and limonene (compound 5), as well as nonanal (compound 7), were detected in undamaged bolls, and these compounds did not increase significantly in response to damage from hemipteran pests (Fig. 2B). However, damage by hemipterans caused a qualitative shift to a blend dominated by acyclic terpenes, including β-ocimene (compound 6), DMNT (compound 8), β-farnesene (compound 9), and TMTT (compound 10), as well as methyl-heptenone (compound 3) (Fig. 2B). Many of the acyclic volatiles detected in damaged bolls increased significantly in response to at least one of the hemipterans investigated (Fig. 2B). Emissions of β-farnesene (compound 9) and methyl-heptenone (compound 3) exhibited the largest increases in response to damage with a 6- and 3-fold increase, respectively, in emissions compared with undamaged bolls (Fig. 2B). Emission of two compounds, β-ocimene (6) and myrcene (4), was dependent on the species of hemipteran causing damage (Fig. 2B). Significantly greater emissions of myrcene (4) were detected only in response to *E. servus* feeding damage, and emission of β-ocimene (6) was significantly greater in response to *E. servus* and *N. viridula* feeding, compared with the undamaged control and bolls damaged by *L. phyllopus* (Fig. 2B).

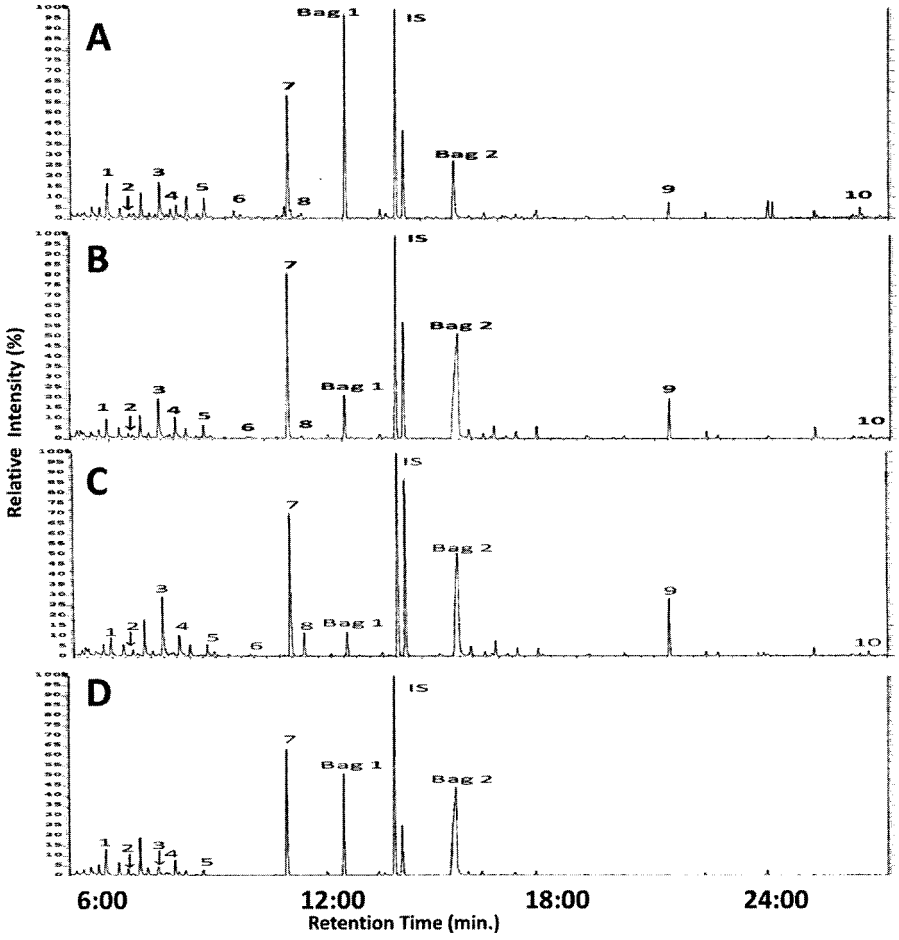

Fig. 1. Gas chromatograms of volatile organic compounds emitted from cotton bolls damaged by (A) brown stink bug, *Euschistus servus*, (B) southern green stink bug, *Nezara viridula*, (C) leaffooted bug, *Leptoglossus phyllopus*, and (D) undamaged bolls. Peak identities: 1, α-pinene; 2, β-pinene; 3, 6-methyl-5-heptene-2-one; 4, myrcene; 5, limonene; 6, β-ocimene; 7, nonanal; 8, 4,8-dimethyl -1,3,7-nonatriene; 9, β-farnesene; 10, 4,8,12-trimethyl-1,3,7,11-tridecatetraene. IS = internal standard (n-dodecane); Bag 1 = artifact 1 from sampling bag (vinyl ester); Bag 2 = artifact 2 from sampling bag (caprolactam).

Field experiment. Under field conditions, herbivory by *N. viridula* caused a significant ($F = 20.94$, df = 2, $P = 0.002$) increase in volatile emissions when compared with emissions from mechanically-damaged and undamaged bolls, with no significant difference among blocks ($F = 3.31$, df = 3, $P = 0.09$) (Fig. 3A). Total volatile emissions from cotton bolls damaged by *N. viridula* increased 2-to 3-fold compared with those from control and

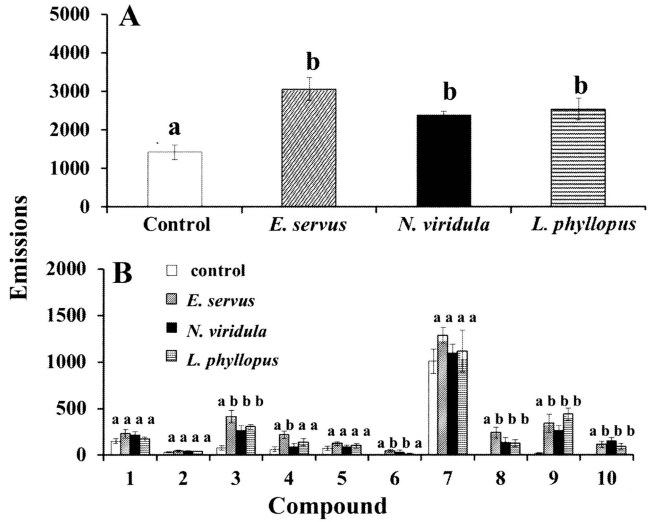

Fig. 2. (A) Total volatile emissions recovered from the head-space of undamaged cotton bolls (control) and bolls enclosed with brown stink bug, *Euschistus servus*, southern green stink bug, *Nezara viridula*, or leaffooted bug, *Leptoglossus phyllopus*, under laboratory conditions. Bars represent mean total emissions ± 1 SE (n = 6 bolls). Bars with the same letter are not significantly different (Tukey $P > 0.05$). (B) Profile of volatile compounds detected from undamaged cotton bolls and bolls damaged by brown stink bug, *Euschistus servus*, southern green stink bug, *Nezara viridula*, and leaffooted bug, *Leptoglossus phyllopus*. Bars represent mean volatile emissions ± 1 SE (n = 6). Bars with the same letter are not significantly different (Tukey $P > 0.05$). Compound numbers correspond to those listed in Fig. 1.

mechanically-damaged bolls, respectively (Fig. 3A). The blend of volatiles released from bolls under field conditions were similar to the blend released under laboratory conditions, but the quantities of individual compounds released in response to hemipteran feeding damage were different between the two studies (Fig. 2; Fig. 3). Limonene, β-ocimene, and DMNT were the predominant compounds released in response to *N. viridula* damage under field conditions (Fig. 3B). Damage by *N. viridula* resulted in significant increases in acyclic terpene emissions, including an 8-fold increase in β-ocimene and DMNT compared with undamaged bolls (Fig. 3B). Furthermore, *N. viridula* feeding did not result in an increase in emission of α-pinene, or β-farnesene under field conditions (Fig. 3B). Emission of individual volatiles from mechanically wounded bolls did not differ from volatiles released from undamaged bolls (Fig. 3B).

The influence of hemipteran feeding damage on volatile emissions from cotton bolls is displayed in principal component space (Fig. 4). Mean principal component scores are plotted for damaged and undamaged bolls, and describe the influence of hemipteran feeding damage on the variation in volatile emissions from cotton bolls (Fig. 4A). Both PC1 and PC2 accounted for 74% of the variation (61% and 13%, respectively) in volatile

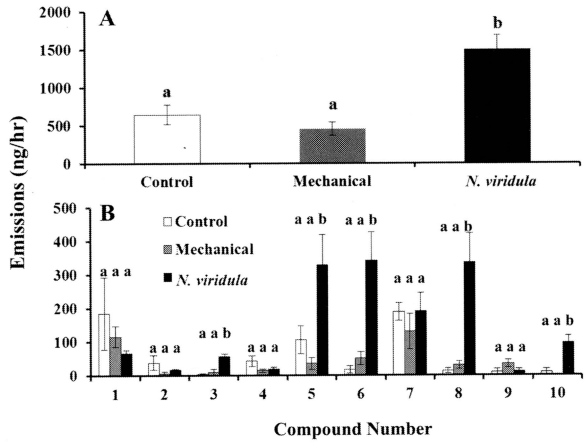

Fig. 3. (A) Total volatile emissions recovered from the head-space of undamaged cotton bolls, bolls damaged mechanically with an insect pin, and bolls enclosed with southern green stink bug, *Nezara viridula* under field conditions. Bars represent mean total emissions ± 1 SE (n = 4 bolls). Bars with the same letter are not significantly different (Tukey $P > 0.05$). (B) Profile of volatile compounds released from undamaged cotton bolls, mechanically damaged, and bolls damaged by *N. viridula*, under field conditions. Bars represent mean volatile emissions ± 1 SE (n = 4). Bars with the same letter are not significantly different (Tukey $P > 0.05$). Compound numbers correspond to those listed in Fig. 1.

profiles. Symbols that are closer together have a similar profile of volatile emissions (Fig. 4A). Volatile profiles from undamaged bolls varied mainly along PC2 and clustered along negative values of PC1, whereas profiles from damaged bolls, especially bolls damaged by *E. servus*, varied strongly along positive values of PC1 (Fig. 4A). The effect of acyclic terpenes on the separation of treatments in Fig. 4A is suggested by a vector correlation plot of volatile loadings (Fig. 4B). The length of vectors represents the magnitude of importance of each compound on the separation of treatments in Fig. 4A. Difference between damaged and undamaged bolls are due to compounds strongly positive for PC1, mainly methyl-heptenone (compound 3) and acyclic terpenes β-ocimene (compound 6), DMNT (compound 8), and β-farnesene (compound 9) (Fig. 4B). In contrast, cyclic terpenes which are positive for PC2, including α-pinene (compound 1), limonene (compound 5) and nonanal (compound 7), accounted mainly for the variation in profiles of undamaged bolls along PC2 (Figs. 4A,B).

Discussion

Plants respond to herbivore damage by releasing volatile emissions that influence numerous ecological interactions including host-location by herbivores, and indirect defense by attraction of natural enemies (Karban and Baldwin 1997, Dudareva et al.

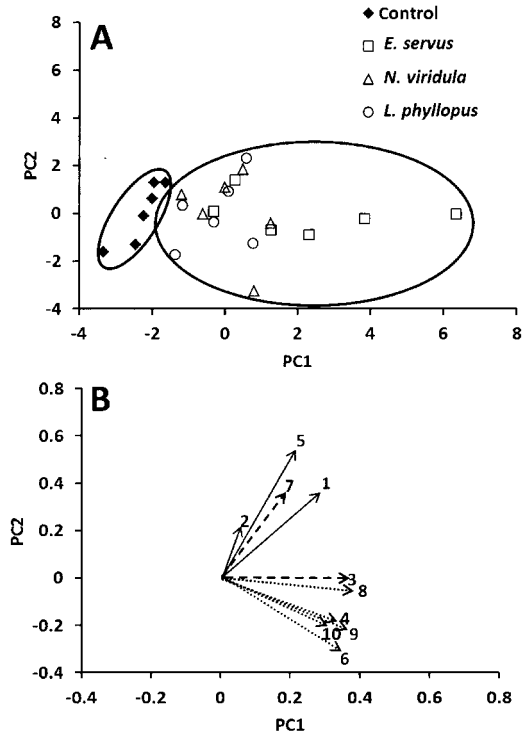

Fig. 4. (A) Volatile profiles from undamaged cotton bolls and bolls damaged by brown stink bug, *Euschistus servus*, southern green stink bug, *Nezara viridula*, or leaffooted bug, *Leptoglossus phyllopus*, under laboratory conditions, summarized as principal component scores based on the first two principal component axes. (B) Vector correlation plot of volatile loading factors showing PC1 (x-axis) plotted against PC2 (y-axis) for each compound with vectors originating from (0,0). Numbers correspond to volatile compounds listed in Fig. 1. Solid vectors point to cyclic terpenoids; long-dashed vectors point to the aliphatic aldehyde, nonanal, and ketone, methyl heptenone; short-dashed vectors point to acyclic terpenoids. Compound numbers correspond to those listed in Fig. 1.

2006). Studies investigating volatile emissions in response to piercing-sucking insect damage (especially stink bugs) have focused on emissions from leaves (Rodriguez-Saona et al. 2002, 2003, Moraes et al. 2005, Williams et al. 2005), however, stink bugs and other hemipterans such as tarnished plant bug, *L. lineolaris*, are increasingly important and destructive pests to cotton production, primarily targeting developing fruit (e.g. cotton bolls). In this study, we demonstrated that feeding by 3 species of hemipteran pests in cotton resulted in quantitative and qualitative changes in volatile emissions from bolls. Furthermore, feeding damage caused by stink bugs resulted in a quantitative increase in several volatiles compared with undamaged bolls or

mechanical injury. The release of acyclic volatiles from herbivore-damaged bolls, but not mechanically-damaged bolls, suggests that these compounds are released specifically in response to herbivore feeding, possibly due to activation by elicitors in hemipteran saliva (Rodriguez-Saona et al. 2002, Williams et al. 2005). According to the PCA analysis, the release of acyclic terpenes from cotton bolls damaged by stink bugs partially accounts for the separation of damaged bolls from undamaged bolls, indicating that acyclic terpenes and methyl –heptenone account for the majority of the variation in volatile profiles from damaged bolls. Acyclic terpenes are known to be induced in cotton leaves in response to leaf-chewing caterpillar damage as well as piercing-sucking bug damage (Loughrin et al. 1994, Paré and Tumlinson 1997, Williams et al. 2005), and our results suggest a similar response in cotton bolls exposed to herbivore feeding.

In some cases, closely related herbivore species can induce different blends of volatiles from damaged plants suggesting the presence of species-specific elicitors (De Moraes et al. 1998). In our study, some components of the volatile blend were found to differ slightly in response to damage by different hemipteran species which may reflect species–specific differences in the composition and/or structure of elicitors in salivary fluids (Felton and Korth 2000). Whereas herbivore-induced blends are elicited by components of herbivore oral secretions, a recent study has questioned whether sufficient amounts of elicitors are regurgitated during actual feeding (Peiffer and Felton 2009). Repeated mechanical injury has been shown to induce volatile emissions similar to herbivory, suggesting that the effect of physical damage from feeding mouthparts to induced volatiles emissions may be somewhat underestimated (Mithöfer et al. 2005). Although we did not assay oral secretions specifically, the difference in volatile emissions between herbivore and mechanically-damaged bolls in our study suggest that hemipteran-induced volatile emissions are more likely due to biotic factors associated with herbivore damage rather than mechanical/physical damage.

We found that experimental conditions also had a strong impact on the volatile emissions from cotton bolls. The induction of volatiles in response to herbivory is strongly influenced by changes in temperature, light, soil humidity, and nutrient availability (Gouinguené and Turlings 2002). The production and emissions of terpenes is known to differ among cotton varieties (Loughrin et al. 1995). Kigathi et al. (2009) demonstrated that emission of β-ocimene from red clover, *Trifolium pretense* (L.), increased in response to herbivory in the greenhouse, but decreased under field conditions. Differences in volatile emissions detected in the field and in greenhouse experiments in our study are likely the result of differences in abiotic and biotic factors that may affect the accumulation and/or induction of volatiles. Furthermore, the level of damage sustained by other plant pests (e.g., herbivores and/or pathogens) prior to or during a wounding event may also influence the overall volatile profile (Rodriguez-Saona et al. 2003, Rostas and Turlings 2008). Whereas the cotton bolls sampled in our field experiment were protected from damage prior to the experiment, the remainder of the plant was exposed to the elements and may have sustained some additional injury due to a combination of biotic and abiotic factors (wind, rain, etc.) that could have strained the fruiting structures as the enclosures exerted stress.

The *de novo* production of volatiles in response to herbivory and/or elicitors is influenced by the action of ethylene, jasmonic acid (JA) and salicylic acid (SA)-mediated biochemical pathways (Walling 2000, Schmelz et al. 2003a, 2003b).

Evidence suggests that the influence of JA and SA pathways on induction of volatile emissions may depend on the particular organism inflicting damage (i.e., leaf-chewers versus piercing-sucking bugs) (Walling 2000, Leitner et al. 2005). This may be a mechanism allowing plants to "fine-tune" defense responses against different attackers (Reymond and Farmer 1998, Thaler et al. 2002, Rostás and Turlings 2008). Whereas, hemipterans are thought to induce defenses mainly through SA-mediated responses (Walling 2000), both JA and SA pathways have been implicated in the induction of volatiles in response to piercing-sucking herbivores (Ozawa et al. 2000, Leitner et al. 2005; Ament et al. 2006). Interestingly, plant responses (including induced volatile emissions) to hemipteran pests are suggested to have strong overlap with responses to pathogens which primarily induce SA-mediated pathways (Kaloshian and Walling 2005), and stink bugs, particularly *N. viridula*, have been reported as vectors of boll-rotting pathogens (Medrano et al. 2007). Following volatile sampling, bolls examined for internal symptoms of damage exhibited early signs of lint staining, indicative of boll rotting pathogenesis. Stink bugs and boll rot are increasing problems to cotton production, and it is not known how these pathogens and stink bugs may interact to influence volatile emissions from these structures. The interaction between hemipteran-feeding and boll-rotting pathogenesis is likely to impact the relative levels of JA and SA and subsequently, influence the volatile blend produced. Future investigations will determine the influence of boll rotting pathogens on volatile emissions, and isolation of stink bug oral secretions to specifically evaluate the effect of stink bug salivary and digestive enzymes on the elicitation of volatiles from cotton bolls.

Changes in volatile emissions following herbivore damage are known to influence indirect defenses by attracting foraging natural enemies of herbivores (Karban and Baldwin 1997, Paré and Tumlinson 1999). In most cases, indirect defenses have been linked to natural enemies that target the larval life-stage of herbivores. Adults of *N. viridula* (L.) are parasitized by six species of *Trichopoda* (Diptera: Tachinidae) within their respective geographic ranges of the world (Todd and Lewis 1976, Jones 1988). Whereas it remains to be determined if induced volatiles influence parasitoid attraction in this system, many of the volatiles induced by adult hemipterans in this study including methyl heptenone, β-ocimene, DMNT, β-farnesene, and TMTT, are known to attract foraging parasitoids in many tritrophic systems (Turlings et al. 1995, Du et al. 1998, Ishiwari et al. 2007). Further knowledge of potential tritrophic interactions in this system may benefit integrate pest management.

The significance of stink bugs, especially in the southeastern USA, has necessitated the development of targeted management strategies for the pest group. Treatment thresholds have been developed based on field-sampling with a beat cloth to determine population levels (Greene et al. 2001a) and/or hand-picking of bolls to assess internal damage in the form of punctures, warts, and seed/lint staining (Greene et al. 2001b, Greene et al. 2009). Unfortunately, these scouting practices are perceived as problematic, time-consuming, and costly. As a result, insecticides are often aggressively or inadequately applied for control. Electronic gas sensor arrays can discriminate among volatiles released from cucumber, *Cucuminus sativa* (L.), tomato, *Lycopersicon esculentum* (Mill.), and green pepper, *Capsicum annum* (L.), subjected to pests and diseases (Laothawornkitkul et al. 2008). Use of electronic sensing technology to discriminate among stink bugs or bug-induced damage to cotton bolls is technically feasible (Henderson et al. 2010) and would likely be welcomed by stakeholders involved with the production of cotton.

Acknowledgments

The authors thank Mike Walla at the University of South Carolina Mass Spectrometry facility for technical assistance with GC/MS sample analysis, Wesley Porter and the staff at the Edisto Research and Education Center for assistance with field experiments, and Wittko Francke from University of Hamburg, and Jocelyn Millar from University of California Riverside for kindly providing samples of DMNT and TMTT. This research was supported by funding from cotton incorporated (CI # 09-630), and CSREES/USDA, under project number SC-1700317. Technical Contribution No. 5734 of the Clemson University Experiment Station.

References Cited

Adams, R. P. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publ, Carol Stream, IL.

Alborn, H.T., T. C. J. Turlings, T. H. Jones, G. Stenhagen, J. H. Loughrin and J. H. Tumlinson. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. Science 276: 945-949.

Ament, K., C. C. Van Schie, H. J. Bouwmeester, M. A. Haring and R. C. Schuurink. 2006. Induction of a leaf specific geranylgeranyl pyrophosphate synthase and emission of E.E.-4,8,12-trimethyltrideca-1,3,7,11-tetraene in tomato are dependent on both jasmonic acid and salicylic acid signaling pathways. Planta 224: 1197-1208.

Arimura, G., C. Kost and W. Boland. 2005. Herbivore-induced, indirect plant defences. Biochim. Biophys. Acta 1734: 87-98.

DeMoraes, C. M., W. J. Lewis, P. W. Paré, H. T. Alborn and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. Nature 393: 570-573.

Dicke, M., T. A. Vanbeek, M. A. Posthumus, N. Bendom, H. Vanbokhoven and A. E. Degroot. 1990. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions-involvement of host plant in its production. J. Chem. Ecol. 16: 381-396.

Du, Y., G. M. Poppy, W. Powell, J. A. Pickett, L. J. Wadhams and C. M. Woodcock. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. J. Chem. Ecol. 24: 1355-1368.

Dudareva, N., F. Negre, D. A. Nagegowda and I. Orlova. 2006. Plant volatiles, Recent advances and future perspectives. Crit. Rev. Plant Sci. 25: 417-440.

Felton, G. W. and K. L. Korth. 2000. Trade-offs between pathogen and herbivore resistance. Curr. Opin. Plant Biol. 3: 309-314.

Gouinguené, S. P. and T. C. J. Turlings. 2002. The effect of abiotic factors on induced volatile emissions from corn plants. Plant Physiol. 129: 1296-1307.

Greene, J. K., S. G. Turnipseed, M. J. Sullivan and G. A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic Bacillus thuringiensis cotton. J. Econ. Entomol. 92: 941-944.

Greene, J. K., S. G. Turnipseed, M. J. Sullivan and O. L. May. 2001a. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403-409.

Greene, J. K., G. A. Herzog and P. M. Roberts. 2001b. Management decisions for stink bugs, pp. 913-917. *In* C. P. Dugger and D. A. Richter [eds.], Proceedings, Beltwide Cotton Production Research Conferences, 9-13 January 2001, Anaheim, CA, National Cotton Council of America, Memphis, TN.

Greene, J., J. Bacheler, P. Roberts, M. Toews, J. Ruberson, F. Reay-Jones, D. Robinson, D. Mott, D. Morrison, T. Pegram, T. Walker and C. Davis. 2009. Continued evaluations of internal boll-injury treatment thresholds for stink bugs in the Southeast, pp. 1091-1100. *In* Proceedings Beltwide Cotton Production Conferences, National Cotton Council of America, Memphis, TN.

Haney, P.B., W.J. Lewis and W.R. Lambert. 1996. Cotton production and the boll weevil in Georgia: history, cost of control, and benefits of eradication. Univ. Georgia Agric. Exp. Stn. Res. Bull. 428.

Henderson, W. G., A. Khalilian, Y. J. Han, J. K. Greene and D. C. Degenhardt. 2010. Detecting stink bugs/damage in cotton utilizing a portable electronic nose. Comput. Electron. Agric. 70: 157-162.

Ishiwari, H., T. Suzuki and T. Maeda. 2007. Essential compounds in herbivore-induced plant volatiles that attract the predatory mite, *Neoseiulus womersleyi.* J. Chem. Ecol. 33: 1670-1681.

Jones, W. A. 1988. World review of the parasitoids of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 81: 262-273.

Kaloshian, I. and L. L. Walling. 2005. Hemipterans as plant pathogens. Annu. Rev. Phytopathol. 43: 491-521.

Karban, R. and I. T. Baldwin. 1997. Induced Responses to Herbivory. Univ. Chicago Press, Chicago, IL.

Kigathi, R. N., S. B. Unsicker, M. Reichelt, J. Kesselmeier, J. Gershenzon and W. W. Weisser. 2009. Emission of volatile organic compounds after herbivory from *Trifolium pretense* (L.) under laboratory and field conditions. J. Chem. Ecol. 35: 1335-1348.

Laothawornkitkul, J., J. P. Moore, J. E. Taylor, M. Possell, T. D. Gibson, C. Nicholashewitt and N. D. Paul. 2008. Discrimination of plant volatile signatures by an electronic nose: A potential technology for plant pest and disease monitoring. Environ. Sci. Technol. 42: 8433-8439.

Leitner, M., W. Boland and A. Mithöfer. 2005. Direct and indirect defenses induced by piercing-sucking and chewing herbivores in *Medicago truncatula.* New Phytol. 167: 597-606.

Liu, Y., W. L. Wang, G. X. Guo and X. L. Ji. 2009. Volatile emission in wheat and parasitism by *Aphidius avenae* after exogenous application of salivary enzymes of *Sitobion avenae.* Entomol. Exp. Appl. 130: 215-221.

Loughrin, J. H., A. Manukian, R. R. Heath, T. C. J. Turlings and J. H. Tumlinson. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. Proc. Natl. Acad. Sci. USA 91: 11836-11840.

Loughrin, J. H., A. Manukian, R. R. Heath and J. H. Tumlinson. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. J. Chem. Ecol. 21: 1217-1227.

Mattiacci, L., M. Dicke and M. A. Posthumus. 1995. β-glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. Proc. Natl. Acad. Sci. USA 92: 2036-2040.

Medrano, E.G., J.F. Esquivel and A.A. Bell. 2007. Transmission of cotton seed and boll rotting bacteria by the southern green stink bug (*Nezara viridula* L.). J. Appl. Microbiol. 103: 436-444.

Miles, P. W. 1999. Aphid saliva. Biol. Rev. Camb. Philos. Soc. 74: 41-85.

Mithöfer, A., G. Wanner and W. Boland. 2005. Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. Plant Physiol. 137: 1160-1168.

Moraes, M. C. B., R. Laumann, E. R. Suji, C. Pires and M. Borges. 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi.* Entomol. Exp. Appl. 115: 227-237.

Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoids accumulation in cotton (*Gossypium hirsutum*) foliage is a general wound response. J. Chem. Ecol. 34: 508-522.

Ozawa, R., G. Arimura, J. Takabayaski, T. Shimoda and T. Nishioka. 2000. Involvement of jasmonate- and salicylate-signaling pathways for the production of specific herbivore-induced volatiles in plants. Plant Cell Physiol. 41: 391-398.

Panizzi, A. R., J. E. McPherson, D. G. James, M. Javahery and R. M. McPherson. 2000. Stink bugs (Pentatomidae), pp. 421-474. *In* C.W. Shaefer and A.R. Panizzi [eds.], Heteroptera of economic importance. CRC, Boca Raton, FL.

Paré, P. W. and J. H. Tumlinson. 1997. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol. 114: 1161-1167.

Paré, P. W. and J. H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. Plant Physiol. 121: 325-331.

Peiffer, M. and G. W. Felton. 2009. Do caterpillars secrete "oral secretions"? J. Chem. Ecol. 35: 326-335.

Reymond, P. and E. E. Farmer. 1998. Jasmonate and salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol. 1: 404-411.

Rodriguez-Saona, C., S. Crafts-Brandner, L. Williams III and P. W. Paré. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. J. Chem. Ecol. 28: 1733-1747.

Rodriguez-Saona, C., S. J. Crafts-Brandner and L. A. Canas. 2003. Volatile emissions triggered by multiple herbivore damage: Beet armyworm and whitefly feeding on cotton plants. J. Chem. Ecol. 29: 2539-2550.

Rostas, M. and T. C. J. Turlings. 2008. Induction of systemic acquired resistance in *Zea mays* also enhances the plant's attractiveness to parasitoids. Biol. Control 46: 178-186.

SAS Institute. 2008. User's guide (version 9.2). SAS Institute, Cary, NC.

Schmelz, E. A., H. T. Alborn, E. Banchio and J. H. Tumlinson. 2003a. Quantitative relationships between induced jasmonic acid levels and volatile emissions in *Zea mays* during *Spodoptera exigua* herbivory. Planta 216: 665-673.

Schmelz, E. A., H. T. Alborn and J. H. Tumlinson. 2003b. Synergistic interactions between volicitin, jasmonic acid, and ethylene mediated insect-induced volatile emission in *Zea mays*. Physiol. Plant. 117: 403-412.

Schmelz, E. A., S. LeClere, M. J. Carroll, H. T. Alborn and P. E. A. Teal. 2007. Cowpea chloroplastic ATP synthase is the source of multiple plant defense elicitors during insect herbivory. Plant Physiol. 144: 793-805.

Thaler, J. S., R. Karban, D. E. Ullman, K. Boege and R. M. Bostock. 2002. Cross-talk between jasmonate and salicylate plant defense pathways, effects on several plant parasites. Oecologia 131: 227-235.

Todd, J. W. and W. J. Lewis. 1976. Incidence and oviposition patterns of *Trichopoda pennipes* (F.), a parasite of the southern green stink bug, *Nezara viridula* (L.). J. Georgia Entomol. Soc. 11: 50-54.

Turlings, T. C. J., J. H. Loughrin, P. J. McCall, U. S. R. Rose, W. J. Lewis and J. H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. USA 92: 4169-4174.

Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz and S. Dorn. 1997. The induction of volatile emissions in maize by three herbivore species with different feeding habits: Possible consequences for their natural enemies. Biol. Control 11: 122-129.

Walling, L. 2000. The myriad of plant responses to herbivores. J. Plant Growth Regul. 19: 195-216.

Williams, L., C. Rodriquez-Saona, P. W. Paré and S. J. Crafts-Brandner. 2005. The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. Arch. Insect Biochem. 58: 84-96.

Williams, M. R. 2009. Cotton insect losses-2008. *In* Proceedings of the Beltwide Cotton Conferences, San Antonio, TX, 5-8 January 2009. National Cotton Council of America, Memphis, TN.

Wolfson, J. L. and L. L. Murdock. 1990. Diversity in digestive proteinase activity among insects. J. Chem. Ecol. 16: 1089-1102.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.