AN INNOVATIVE APPROACH FOR THE ASSESSMENT OF STINK BUG DAMAGED COTTON BOLLS USING ELECTRONIC ODOR-SENSING TECHNOLOGY.

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Abstract

Phytophagous stink bugs are a major pest in B.t. cotton varieties. These bugs feed directly on cotton bolls and cause damage to the fiber resulting in millions of dollars in crop losses annually. Current assessment practices are problematic and there is a need for a more efficient and cost effective method of detection and control of these pests. It is well known plants produce odorous volatile chemicals in response to pest damage. There is potential for a rapid in-field assessment of pest damage using electronic nose technology to detect stink bug induced volatile emissions from cotton bolls. Volatile emissions from stink bug damaged and undamaged bolls were collected and analyzed by gas chromatography, as well as a novel method using a portable electronic nose. Damage by stink bugs induced significantly greater emission of terpenes compared to undamaged controls. Patterns in terpene emissions in response to damage were similar among cotton varieties, and herbivores observed. An Electronic nose was able to discriminate between damaged and undamaged cotton bolls, but not between herbivore species. This technology shows promise for developing a rapid in-field detection system based on odorous chemicals released from damaged bolls.

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important crops in the USA and is produced on 13-14 million acres from California to the Carolinas (USDA-NASS, 2006). The adoption of B.t. cotton varieties (Bollgard, Bollgard II, and WideStrike) has resulted in reduction in the use of broad-spectrum insecticides to control leaf-chewing herbivores such as bollworms and weevils. In the last ten years however, piercing-sucking pests such as stink bugs and plant bugs have flourished in the "low spray" environment of B.t. cotton. Since 1995, insecticide use targeting these pests has risen from 0 to millions of applications, and recently crop losses exceeded 50 million dollars (Williams, 2008). Due to difficulties in assessment and time-consuming scouting practices, there is a need for a more rapid and cost-effective method for determining boll damage from these pests.

One potential method is the use of electronic nose (E-nose) technology to detect plant volatiles induced by Hemipteran pests. It is well known plants emit odorous hydrocarbons (mainly terpenes) into the atmosphere in response to herbivory (Karban and Baldwin, 1997; Paré and Tumlinson, 1999; Arimura 2005). In cotton, herbivory results in the induction of a unique bouquet of volatile compounds not produced in undamaged plants (Rodriguez-Saona *et al*., 2001; Röse and Tumlinson, 2005). Odorous chemicals released from stink bug damaged cotton bolls may be rapidly detected using E-nose technology. In recent years, chemical sensor arrays have been developed for a range of applications from detection of hazardous chemicals in the atmosphere to food spoilage (Fernandes and Gomes, 2008; Röck *et al*., 2008). Electronic sensors have also been used to discriminate among volatiles released from cucumber, tomato, and green pepper plants subject to pests and diseases (Laothawornkitkul et al., 2008).

The objective of this research is to determine the influence of Hemipteran pest damage on volatile emissions from cotton bolls. We determined the similarity in volatile emissions among three cotton cultivars in response to damage by three Hemipteran pests. Furthermore, we conducted preliminary sampling using an E-nose to determine the feasibility of this technology for detecting volatiles from stink bug damaged cotton bolls.

Materials and Methods

Plants and Insects

Cotton cultivars Delta and Pine Land 143 Bollgard II Roundup Ready Flex, 164 Bollgard II Roundup Ready Flex, and Phytogen 370 WideStrike Roundup Ready were used in all experiments. Plants were grown and maintained in a greenhouse under natural light conditions with a daytime temperature of 30°C, and a nighttime temperature of 22°C. A relative humidity no higher than 70% was maintained in the greenhouse. All plants used for experiments were 10-12 weeks old, and all bolls used for volatile collections were 12-14 days post anthesis. Volatiles from a single boll from ten individual plants were sampled from each cultivar to evaluate cultivar-specific differences in constitutive (undamaged) volatile emissions. For all wounding experiments, bolls were enclosed using foam cups with nylon stockings stretched over the outside of the cup and secured using light gauge steel wire.

To tests for species-specific volatile emissions, two adult brown stink bugs, *Euschistus servus* (Say), southern green stinkbugs, *Nezara viridula*, (Linnaeus), or leaf footed bugs, *Leptoglossus phyllopus* (Linnaeus) were placed inside boll enclosures and allowed to feed *ad libitum* for five days.

Volatile collection and analysis

After feeding trials, bugs and enclosures were removed and a nylon polymer gas sampling bag (250 ml volume) was placed over cotton bolls. Collection bags were loosely fastened with a cable tie at the base of the boll to permit airflow through the bag. A volatile collection trap was fastened to the top corner of a 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 with two small plugs of glass wool. A battery operated air-sampling pump (SKC, Inc. Model 224-44XR) fitted with an adjustable low-flow 4-way splitter (SKC, Inc. Model 224-26-04) was used to draw air through the collection bag, across the boll, and directly onto a volatile collection trap at a rate of 300 mL · min^{-1}. Ambient air blanks were collected simultaneously with boll volatile collections to correct for any volatiles contained in the ambient air pulled through a collection bag. In all experiments volatiles were collected for a duration of 1 hour. Immediately following sampling, volatiles were desorbed from collection traps by adding 200 µl of gas chromatography/mass spectrometry (GCMS) grade Hexane, and collected directly into a 2 ml autosampler vial containing a 150 µl insert. An internal standard of n-dodecane was added to each extract to a concentration of 10 ng · $µl^{-1}$ for quantification of volatiles.

Two microliters of all volatile extracts were analyzed by GCMS using a Hewlett Packard 5890 series II gas chromatograph equipped with an HP-5 methyl-silicone capillary column (30 m x 0.25 mm) using helium as a carrier gas at a rate of 1.22 ml · min^{-1}. The injector port temperature was 275°C. The HP-5 column was held at 50°C for 3 min, followed by an increase to a final temperature of 220°C at a rate of 5°C · min^{-1}, followed by a hold at maximum temperature for 20 min. Volatile compounds were identified by mass spectroscopy with a Hewlett Packard 5971 series Mass Selective Detector. Volatile mass spectra were compared to those contained in the National Bureau of Standards essential oil database, as well as the database of essential oil components identified by GC/MS (Adams, 1995). All volatile amounts were log transformed and analyzed by multivariate analysis of variance to test for main treatment differences. If significant differences were found, a Tukey post hoc comparison was used to test for differences among volatile components.

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Electronic nose volatile sampling

In a separate experiment, preliminary analysis of electronic nose (e-nose) (Cyranose 320, Smith's Detection Inc.) was performed. Cotton bolls from DP 164 were exposed to southern green, and green stink bug (*Acrosternum hilare*, Say.) damage for a period of five days using the techniques described above. Stink bug damaged and undamaged bolls were sampled with the e-nose prior to standard method volatile sampling (described above). The Cyranose 320 contains 32 carbon-black polymer composite sensors each with unique properties allowing the sensor matrix to respond to complex odors in an air sample. When volatile compounds are drawn across the sensor array, a change in resistance is measured, and onboard pattern recognition software creates a "smell print" unique to the combination of odors contained in the sample. A typical sampling method for the Cyranose 320 consists of: 1) an initial purge to clear the carbon-black polymer sensors prior to sampling, 2) sample draw in which the analyte to be measured is drawn across the sensors, and 3) a final gas purge in which the odor sampled is evacuated from the sensors. A method was programmed in the Cyranose 320 which consisted of an initial (baseline) purge of 20 seconds, a sample draw of 20 seconds, an air intake purge of 10 seconds, and a final gas purge of 60 seconds.

Damaged and undamaged cotton bolls were enclosed in volatile collection bags used for standard volatile collection. The snout of the Cyranose 320 was inserted into the volatile collection bag and the sampling method was initiated.

Results and Discussion

Undamaged cotton bolls were found to emit a mixture of volatile compounds composed mainly of terpenoids (Figure 1 and 2). There were no significant differences found in the volatiles components emitted among the three cotton cultivars investigated (Figure 1). These results suggest that cotton varieties do not differ significantly in the profile of volatiles released from bolls. A typical gas chromatogram of volatiles released by damaged cotton bolls revealed an increase in the amount and number of compounds released compared to undamaged bolls (Figure 2).

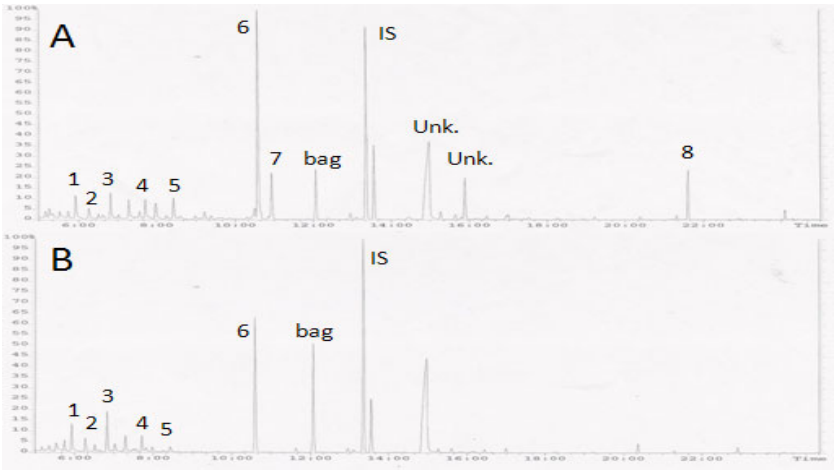

Figure 1. Typical gas chromatograms of volatile compounds emitted from stink bug damaged (A) and undamaged (B) cotton bolls. Peak identities: 1=α-pinene; 2=β-pinene; 3=myrcene; 4=limonene; 5=β-ocimene; 6=nonanal; 7 = (E)-4,8-dimethyl-1,3,7-nonatriene; 8= (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene; bag= peaks originating from sampling bags; IS= internal standard (n-dodecane); Unk.=identity unknown.

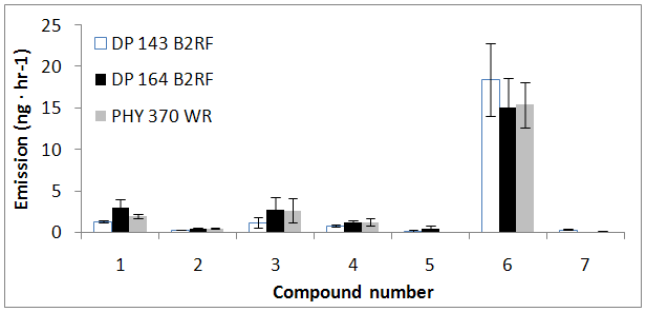

Figure 2: Baseline (constitutive) volatile emissions from three undamaged cotton cultivars. Bars = mean volatile emissions ± s.e. of six plants. Compound numbers correspond to those listed in figure 1.

Several volatile compounds were found to increase in response to damage by the three herbivores investigated (Figure 3). Furthermore, some compounds such as TMTT were released only in response to pest damage (Figure 3). While minor differences were found among the three cultivars, the three herbivores investigated induced a similar pattern in volatile emissions from cotton bolls (Figure 3). This data suggests that Hemipteran pests induce a similar bouquet of volatiles in cotton bolls.

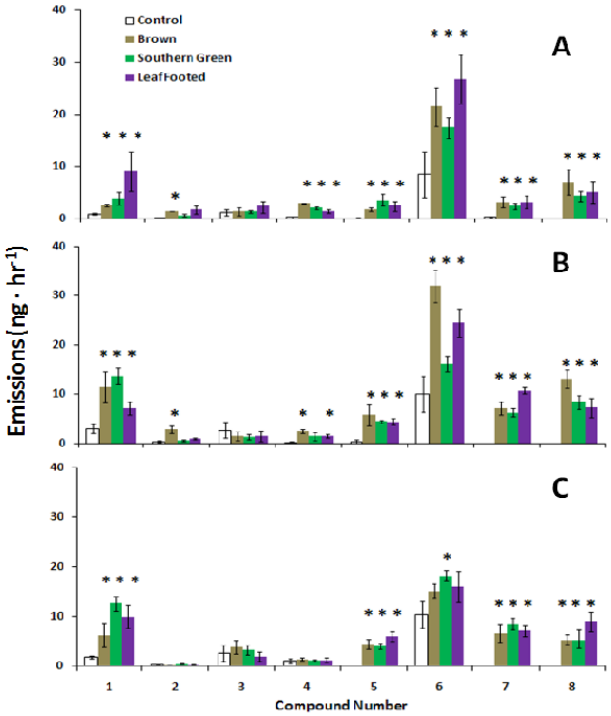

Figure 3. Volatile emissions from cultivar DP 143 (A), DP 164 (B), and PHY 370 (C) in response to damage by brown stink bugs, southern green stink bugs, and leaf footed bugs. Bars = mean volatile emissions ± s.e. of six plants. * = significant difference from control volatile emissions (*Tukey P* < 0.05) Compound numbers correspond to those listed in figure 1.

Sampling cotton bolls using the E-nose resulted in a sensor array response distinct for stink bug damaged bolls compared to undamaged bolls (Figure 4). The sensor array response was similar for green and southern green damaged bolls and these responses were distinct from undamaged bolls (Figure 4).

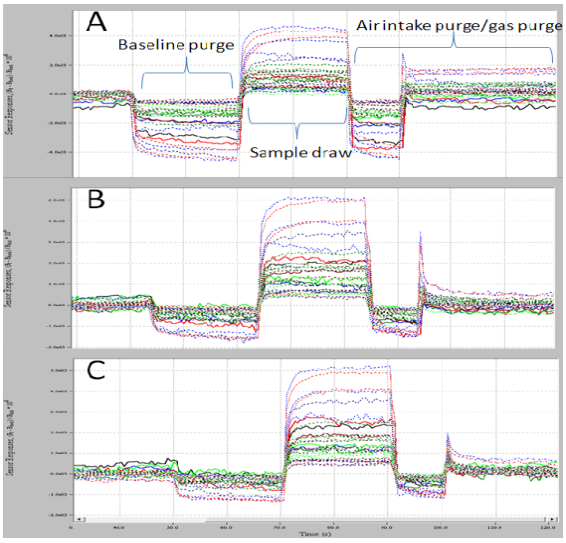

Figure 4. Typical E-nose sensor response to undamaged (A), green stink bug (B), and southern green stink bug damaged (C) cotton bolls.

On-board data analysis of the sensor data from the E-nose verified the differences between damaged and undamaged bolls (Figure 5 and 6). A principal components analysis of sensor data showed separation of stink bug damaged bolls from undamaged bolls (Figure 5). However, upon cross validation of the initial training set, the E-nose was only 76% accurate at discriminating among undamaged, green stink bug damaged, and southern green stink bug damaged bolls (Figure 6). While the E-nose consistently identified undamaged cotton bolls, it was unable to completely distinguish between southern green and green stink bug damaged bolls (Figure 6). All nine of the undamaged bolls sampled in the preliminary training set were identified as undamaged, whereas two of the seven bolls trained on southern green stink bug damage were identified as green stink bug damaged, and three of the five bolls trained on green stink bug damage were identified as southern green stink bug damage (Figure 8). Thus, while the electronic nose could reliably distinguish between damaged and undamaged bolls, it was less reliable in discriminating damage caused by different stink bug species.

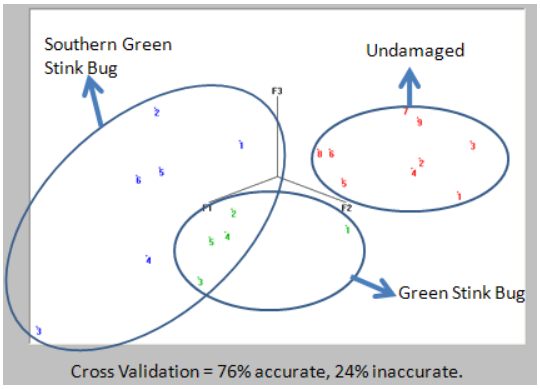

Figure 5. Principal components plot of sensor data from E-nose sampling of green stink bug damaged, southern green stink bug damaged, and undamaged cotton bolls.

Identified As

Trained As	Undamaged	SGSBdamage	GSBdamage	M3Class4	M3Class5	M3Class6
Undamaged	9	0	0	0	0	0
SGSBdamage	0	5	2	0	0	0
GSBdamage	0	3	2	0	0	0
M3Class4	0	0	0	0	0	0
M3Class5	0	0	0	0	0	0
M3Class6	0	0	0	0	0	0

Correct : 76.190 %
Incorrect : 23.810 %

Figure 6. Cross validation of sensor data from E-nose sampling of green stink bug damaged, southern green stink bug damaged, and undamaged cotton bolls.

Summary

We identified the volatiles released from cotton bolls including constitutive (baseline) volatile emissions, as well as volatiles induced in response to stink bug damage. Three cultivars investigated contain a similar mixture of volatiles. Rodriguez-Saona *et al*. (2005) showed that salivary components from Hemipterans (*Lygus* sp.) induce volatile emissions in cotton leaves. Our results suggest that Hemipteran pest damage has the same effect on cotton bolls. Stink bug damage results in a quantitative increase in volatile emissions, as well as a qualitative shift in the profile of volatiles released.

E-nose technology has been shown to be effective at discriminating volatiles from damaged and undamaged plants (Laothawornkitkul *et al*., 2008). Preliminary testing of E-nose in our system indicates this technology is capable of discriminating between damaged and undamaged cotton bolls, but is not 100% effective at discriminating between stink bug species. This suggests that volatiles induced by herbivores with analogous mouth parts, or salivary

components may not be reliably separated by E-nose. E-nose shows promise for development of a rapid in-field assessment tool. Future research will include evaluating the E-nose under field conditions.

References

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

Fernandes, DLA, and Gomes, MTSR. 2008. Development of an electronic nose to identify and quantify volatile hazardous compounds. *Talanta* 77(1): 77-83.

Karban, R., Baldwin, I.T. 1997. Induced responses to herbivory. University of Chicago Press. Chicago.

Laothawornkitkul, J., Moore, J.P., Taylor, J.E., Possell, M., Gibson, T.D., Nicholashewitt, C., and Nigel, P.D. 2008. Discrimination of plant volatile signatures by an electronic nose: A potential technology for plant pest and disease monitoring. *Environ. Sci. Technol.* 42(22): 8433-8439.

National Association of Agricultural Statistics. United States Department of Agriculture. Accessed: 10 Jan. 2009. < http://www.nass.usda.gov/>.

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

Röck, F., Barsan, N. and Weimar, U. 2008. Electronic nose: Current status and future trends. *Chem. Rev.* 108: 705-725.

Rodriguez-Saona, C., Crafts-Brandner, S.J., Williams III, L., and Pare, P.W. 2002. *Lygus Hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *J. Chem. Ecol.* 28(9): 1733-1747.

Röse, U.S.R., and Tumlinson, J.H. 2005. Systemic induction of volatile release in cotton, How specific is the signal to herbivory? *Planta* 222: 327-335.

Williams, M.R. "Cotton crop loss data" Resources – Department of Entomology and Plant Pathology. 19 Mar. 2008. Mississippi State University. 10 Jan. 2009. <http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/>