GC-FID AND E-NOSE FOR DETECTION OF STINK BUG INFESTATION ON COTTON BOLLS

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Abstract

Sucking insect pests, such as stink bugs, have become one of the most important pest complexes of southeastern cotton production. Stink bug feeding can cause young bolls to fall off the plant, lint staining, uniformity issues, reduced lint quality, and reduced yields. Currently, manual boll collection and internal evaluation is the most effective method to identify and quantify the boll damage; however, this procedure is excessively labor intensive. The objective of this study was to develop a quick method to detect stink bug damaged cotton bolls using a portable electronic nose on excised bolls. Results show that the volatile profiles emitted by undamaged and stink bug damaged cotton bolls were similar, but most volatiles were identified in decreased quantity in the stink bug damaged bolls. The electronic nose showed potential to separate bolls infested over a 2-4 day period from undamaged bolls. These results suggest that further separation among treatment levels may be possible, but will be challenging due to the minute differences in volatile profiles.

Introduction

Piercing/sucking insect pests including stink bugs and plant bugs are quickly replacing the budworm/bollworm complex as the most important insect complex of Georgia cotton production. Stink bug feeding can cause young bolls to fall off the plant, lint staining, uniformity issues, reduced lint quality, and reduced yields. Toews et al. (2008) recently showed that quantifying internal boll damage is by far the most sensitive sampling technique for this pest. Unfortunately, manually dissecting individual bolls was also the most time consuming sampling method tested. Growers and scouts are in need of a quicker method to ascertain the level of internal damage.

A common plant defense to insect attack is the synthesis of volatile compounds that repel herbivores and attract the natural enemies. Lewis (1990) reported that plant volatiles induced by herbivore feeding are often used as olfactory signals by foraging herbivores and their natural enemies. Keen scientists view these intricate ecological relationships as an opportunity to exploit the system for pest management purposes. In pioneering work with cotton and southern green stink bugs, Williams et al. (2005) found that (1) female southern green stink bug feeding induced volatile production in plants, (2) feeding injury by female southern green stink bug increased volatile emissions in intact maize by approximately 2-fold compared to control plants, and (3) volatile production was affected by gender and life stage of the bug. Traditionally, chemical ecologists have used gas chromatography and mass spectrometry (GC-MS) for detecting individual components in an odor profile. However, identification of individual compounds in a volatile mixture is not necessary when only differentiation of two overall smell patterns is needed. A breakthrough in odor detection was the development of the electronic nose sensors, which consist of an array of cross-responsive sensors inspired by mammalian olfactory systems (Gardner and Bartlett, 1994). The electronic nose can be trained and used to distinguish different "smellprints". These devices have been successfully used in the food quality and safety detection arena (Li et al., 2007). Henderson et al. (2006) explored the feasibility of using the electronic nose for the detection of stink bug damaged cotton bolls; they showed that the E-nose could be used to detect heavily damaged bolls or the presence of stink bugs themselves.

Objectives

Based on the rationale above, the objectives of this study were to:

1. Characterize differences in the volatile profile between intact and stink bug damaged cotton bolls using the GC-FID detector.
2. Determine whether the electronic nose is able to detect stink bug damaged cotton bolls under different treatment conditions.

Materials and Methods

Prior to analyses, cotton bolls were systematically damaged by caging stink bugs on the developing bolls for fixed periods of time. Cotton plants (FM 9063 B2RR) were grown in 11.3 liter pots housed in a greenhouse at the Coastal Plain Experiment Station at Tifton. When the bolls reached 7-10 days past anthesis, lab-reared southern green stink bugs (5^{th} instars) were caged on the bolls for a duration of 72 h. Boll circumference was measured with a veneer calipers following the stink bug exposure to assure similar bolls. In total, 6 treatments were made. Negative control bolls were completely undamaged while the positive control was mechanically damaged using a number 00 insect pin. The pin was inserted five times in each boll to a depth of 3 mm. Stink bug damaged bolls were treated in four different ways: 2 bugs for 2 days, 2 bugs for 4 days, 4 bugs for 2 days, and 4 bugs for 4 days (Table 1).

Table 1. Cotton boll samples and treatment

Bag	Trt	d on boll	boll dia
5	Contol	0	1.5
9	Contol	0	2.1
1	Contol	0	1.7
16	Pin	0	2
15	Pin	0	2.4
35	2 bugs	2	1.4
54	2 bugs	2	2.5
57	2 bugs	2	1.4
42	2 bugs	4	1.8
40	2 bugs	4	1.9
51	2 bugs	4	1.7
28	4-bugs	2	2.6
37	4-bugs	2	3.1
44	4-bugs	2	1
59	4-bugs	4	1.4
48	4-bugs	4	1.7
50	4-bugs	4	1.7

The Cyranose 320 electronic nose (Smith Detection Inc., Pasadena, CA) were used in this study. This conducting polymer electronic nose has 32 internal sensors that swell like the sponge while they are exposed to the volatile compounds. Immediately prior to analysis, each individual cotton boll was excised from the plant and placed in a 200 ml glass jar sealed by a Teflon septum. The electronic nose sampling needle was inserted through the septum when collecting the volatile samples of cotton bolls. Each sample was measured two times. Data from the E-nose was analyzed by principal component analysis (PCA) using MATLAB (Mathworks Inc, Natick, MA) software.

A gas chromatograph (GC) coupled with a flame ionization detector (GC-FID) (Agilent 6890) was used in this study to characterize and quantify volatiles produced by the treated bolls. Procedures for GC analyses included setting the initial oven temperature at 40°C with a 4°C/min ramp until the temperature reached 180°C. The temperature of detector was set to a static 250°C. Helium was used as the carrier gas with a flow rate of 3 ml/min. Volatiles were separated on a 30 m x 250 um x 0.25 um capillary column. The solid phase micro extraction fiber (SPME) was used due to its ease of use.

The following parameters were taken when sampling using the SPME:
P=5 min (permeation time, amount of time bolls are encased in the collection bottle prior to VOC collection).
E=60 min (exposure time of SPME fiber to volatiles)
S=5 sec (storage time. volatiles are stored on the fiber prior to injection)
T=15 min (thermal desorption time of SPME fiber in the GC-FID injection port)

Results

As shown in Figure 1, chromatographs from intact and damaged bolls were very similar overall except for a few compounds that were observed in different quantities, such as those observed at RT 9.5 min and RT 2.1 min. Although the compound at RT 9.5 min showed up in chromatographs of both control and damaged bolls, its relative abundance in the stink bug damaged bolls was greater compared to the undamaged or mechanically damaged cotton bolls. The abundance of this compound was relatively small in both chromatographs of control bolls. A second compound, found at RT 2.1, was found in small amounts in the damaged bolls but was absent in the spectra of undamaged bolls. These two compounds need to be identified by their mass spectra.

We observed on the chromatographs that the overall concentration of volatile compounds from intact bolls was greater than observed from the damaged bolls. This was further proven by the integrated peak areas in Table 2. The external standard was established to quantify the mass of the volatile compound from the integrated peak area. For instance, the integrated peak area of volatile compound RT 11.735 min in undamaged bolls was 335.6 pA*s (corresponding to 24.5 ng), while the integrated area and concentration of the same compound (RT 9.46 min in chromatograph of damaged bolls) was only 42 pA*s (3.1 ng). It was observed that most volatile compounds emitted by both intact bolls and damaged bolls were in the ng range, which is obviously a very low concentration.

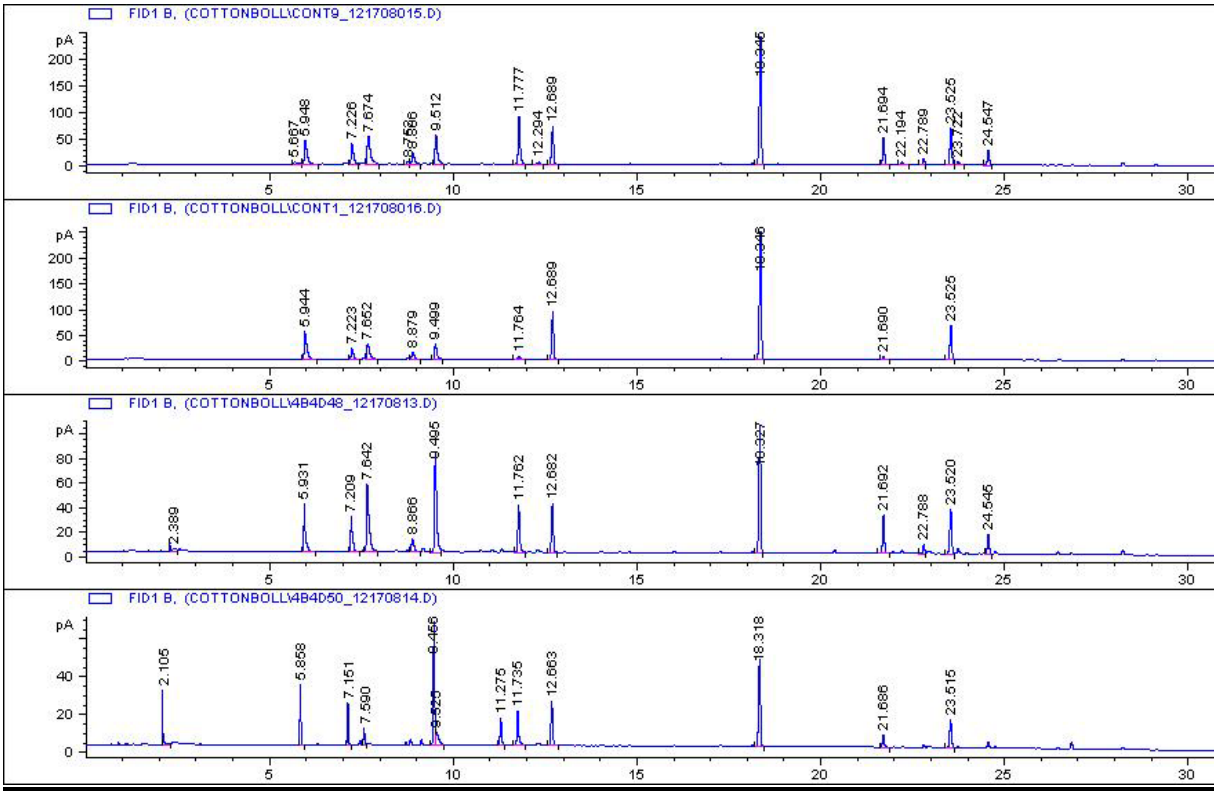

Figure 1. Gas chromatographs of control (top two graphs) and stink bug damaged (bottom two graphs) cotton bolls.

Table 2. Chromatograph area integration and quantification of major volatile compounds

Intact bolls			Damaged bolls		
RT	Area (pA*s)	Conc. (ng)	RT	Area (pA*s)	Conc. (ng)
5.948	225.28	16.44544	2.105	40.29	2.94117
7.674	318	23.214	5.858	67.3	4.9129
9.512	259	18.907	7.59	22.4	1.6352
11.77	335.6	24.4988	9.456	174	12.702
21.694	175.5	12.8115	11.735	42	3.066
24.547	94.67	6.91091	21.685	22.3	1.6279

E-nose data from the same bolls were evaluated using principal component analysis. Due to a conflict with the GC-FID measurement, some boll samples could not be sampled using the E-nose. In total, 26 data samples were obtained using the E-nose as shown in Figure 2.

These 26 samples could be divided into two large groups: minor damage and more aggressive damage as separated by the black line (Figure 2). In the minor damage grouping, 2 samples from control group and 4 samples from pin treatment could be separated from other stink bug damaged treatment. Two of the "2b4d" samples (numbers 15 and 16) were erroneously grouped in the minor damage category. In the group of more aggressive damage, almost all of these samples were from the two most severe insect damaged treatment groups. Some of this variability in perceptible damage may reflect differences in feeding variability, regardless of treatment.

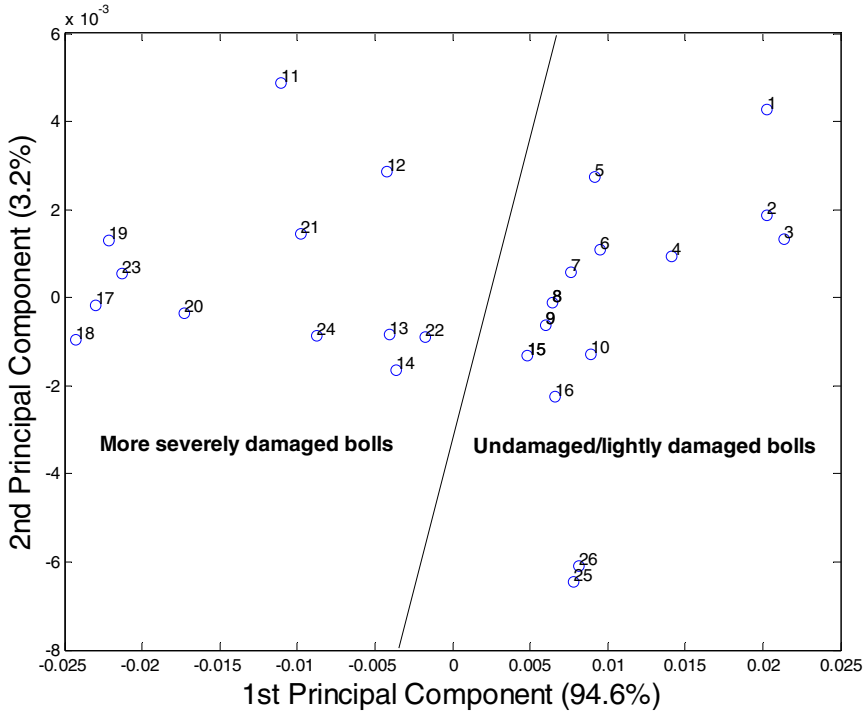

Figure 2. Principal component score plot of E-nose response (number 1-4: pin treatment; 5-10: 2b2d; 11-16: 2b4d; 17-22: 4b2d; 23-24: 4b4d; 25-26: control)

Summary

Based on chromatographs obtained from the GC-FID, it was observed that volatile profiles detected from intact and damaged bolls were similar except for the relative abundance of a few volatiles. The concentration of these volatile compounds was very low (6-24 ng for control group and 1-12.7 ng for damaged group). Although the E-nose showed the promise to separate bolls with no damage/light damage from heavily damaged bolls, the limits of detection may prevent being able to separate lightly damaged bolls from undamaged bolls. More samples and improved infestation strategies are needed to characterize the efficacy of the E-nose for stink bug damaged cotton boll detection.

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References

Gardner, J. W. and P. N. Bartlett (1994). A brief history of electronic noses. Sensors and Actuators B: Chemical 18(1-3): 210-211.

Henderson, W., A. Khalilian and Y. Han (2006). Detecting Stink Bugs/Damage in Cotton Utilizing a Portable Electronic Nose. 2006 ASABE Annual International Meeting. Portland, Oregon.

Lewis, W.J. 1990. Use of learned odours by a parasitic wasp in accordance with host and food needs. Nature. 348(6302): 635-636.

Li, C., P. Heinemann and J. Irudayaraj (2007). Detection of apple defects using an electronic nose and zNose. Transactions of the ASABE 50(4): 1417-1425.

Toews, M.D., J. Greene, F.P.F. Reay-Jones, and R.B. Reeves. 2008. A comparison of sampling techniques for stink bugs in cotton. *In* Proceedings of the Beltwide Cotton Conferences, January 8-11, Nashville, TN, National Cotton Council of America. *In Press*.

Williams III, L., C. Rodriguez-Saona, P. W. Pare and S. J. Crafts-Brandner (2005). The Piercing-Sucking Herbivores *Lygus hesperus* and *Nezara viridula* Induce Volatile Emissions in Plants. Archives of Insect Biochemistry and Physiology 58: 84-96.