

PRELIMINARY STUDY OF USING A GC-FID FOR VOLATILE DETECTION ON STINK BUG INFESTATED 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 labor intensive. The objective of this study was to explore the volatile profile differences between stink bug damaged and undamaged cotton bolls using a GC-FID. 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 result suggests that further separation among treatment levels may be possible, but will be challenging due to the minute differences in volatile profiles under current treatment.

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 desire 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. A gas chromatograph (GC) equipped with a flame ionization detector (GC-FID) usually suffice when individual compounds do

not need to be identified. This information may help develop an alternative sensing approach for stink bug infestation on cotton bolls.

Objective

Based on the rationale above, the objective of this study was to characterize differences in the volatile profile between intact and stink bug damaged cotton bolls using the GC-FID detector.

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 (5th 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	Days on boll	Boll dia (inch)
5	Control	0	1.5
9	Control	0	2.1
1	Control	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

Following insect exposure, bolls were excised from the plant and individually analyzed using chromatography. 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 μ m x 0.25 μ m 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 were encased in the collection bottle prior to VOC collection).

E=60 min (exposure time of SPME fiber to volatiles)

S=5 sec (storage time: amount of time volatiles were 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 (undamaged) 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.

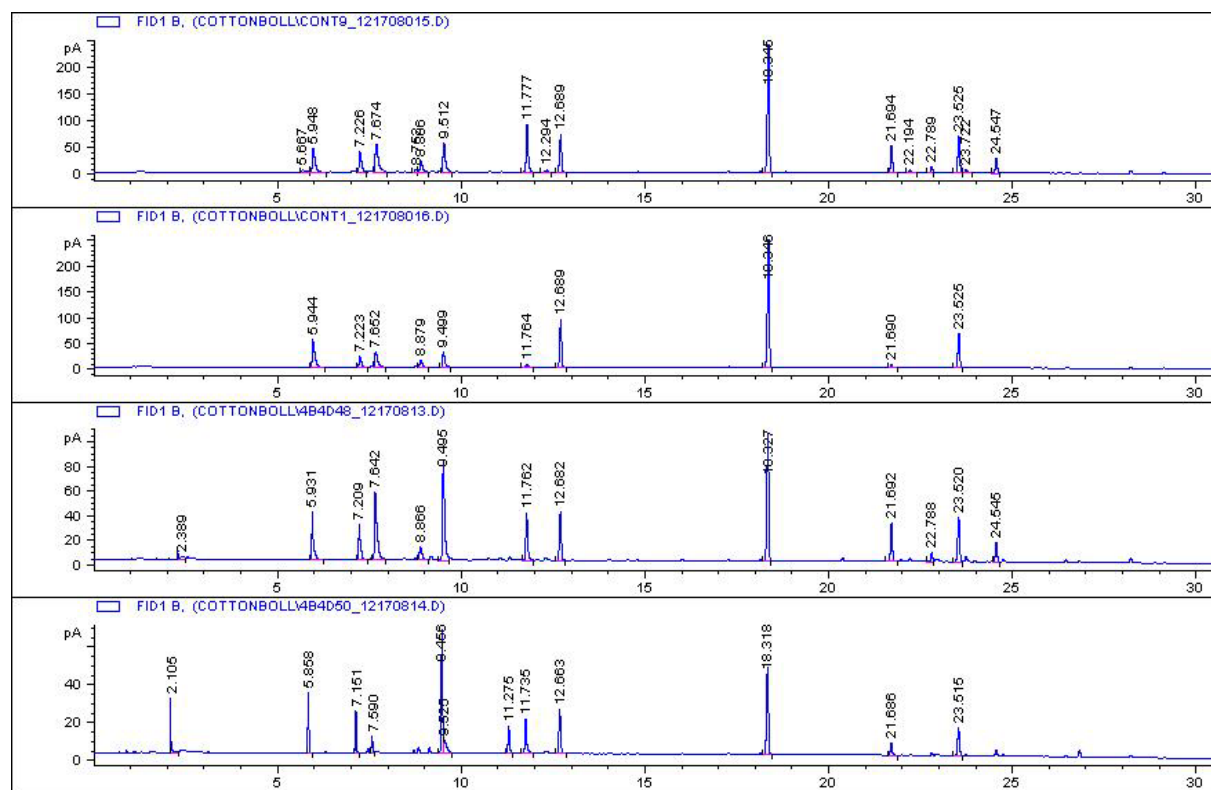


Figure 1. Gas chromatographs of control (top two graphs) and stink bug damaged (bottom two graphs) cotton bolls.

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	16.445	2.105	40	2.941
7.674	318	23.214	5.858	67	4.913
9.512	259	18.907	7.590	22	1.635
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

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). More samples and improved infestation strategies are needed to characterize the volatile profiles from stink bug damaged cotton bolls. This information may shed light on the possibility of developing an alternative sensing approach for stink bug infestation on cotton bolls using a gas sensor.

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