EXPLOITING VOLATILE EMISSIONS FROM COTTON AS A MANAGEMENT TOOL FOR STINK BUGS

David C. Degenhardt
Jeremy K. Greene
Ahmad Khalilian
Clemson University, Edisto Research and Education Center
Blackville, SC

Abstract

Phytophagous stink bugs are a major pest in B.t. cotton varieties. 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 organic compounds (VOCs) in response to pest damage. There is potential for a rapid in-field assessment of pest damage using electronic nose (E-nose) technology to detect stink bug induced VOC emissions from cotton bolls. In this study, we determined the temporal variation in VOCs released from cotton bolls damaged by brown stink bug, *Euschistus servus* (Say). Emissions from stink bug-exposed bolls increased significantly between 48 and 72 hours compared to bolls not exposed to stink bugs. A preliminary study was also conducted to determine if E-nose could discriminate among VOC profiles from undamaged bolls, and bolls damaged by *E. servus*, or green stink bug, *Nezara viridula* (L.) under field conditions. E-nose was trained to recognize the VOC profile from bolls after three days of exposure to stink bugs. Canonical projection analysis revealed modest separation of treatment groups, and cross validation of the data set showed that E-nose could distinguish among VOCs from healthy bolls, *E. servus* damaged, *N. viridula* damaged bolls with 70% accuracy. The majority of inaccuracy was due to the E-nose inability to discriminate between VOC profiles from bolls damaged by different species of stink bug. The accuracy may be increased if E-nose is used to discriminate between healthy bolls and bolls damaged by stink bugs in general. Despite the modest accuracy of the E-nose in this preliminary field investigation, refinement and continued evaluation of E-nose could provide promising technology for development as a rapid in-field assessment tool for detecting stink bug 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 2009). 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 volatile organic compounds (VOCs) into the atmosphere in response to herbivory (Paré and Tumlinson 1999). 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). We have previously identified the composition of VOCs released from cotton bolls following five days of exposure to several stink bug species (Degenhardt *et al.* 2009). 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 objectives of this research were to determine the temporal variation in VOC from cotton bolls in response to stink bug feeding, and investigate the ability of E-nose to differentiate VOC profiles from healthy bolls, and bolls damage by stink bugs.

Materials and Methods

Temporal variation in Boll VOC emissions in Response to Stink Bug Feeding. Bolls on growth chamber grown plants (Delta and Pine Land 164) were exposed to a single brown stink bug, *Euschistus servus* (Say), 10 days post-anthesis. Enclosures constructed from Styrofoam cups (with the base of the cup removed) with nylon stocking stretched over the cup were placed over bolls and secured with a light-gauge steel wire. A single E. servus was placed inside the enclosure and allowed to feed *ad libitum*. Controls had enclosures placed over bolls with no stink bugs. Boll VOC emissions were sampled from stink bug damaged bolls, and controls in 24 hour intervals over a period of 7 days. At each interval, enclosures and stink bugs were removed and a polyacetate oven bag (300 ml volume) with a VOC collection trap attached to a corner was placed over the boll and loosely secured at the base of the boll permitting air-flow through the bag. Collection traps were constructed from a modified glass Pasteur pipette (10 cm length, 0.5 cm OD) and contained 35 mg Super Q adsorbant (Alltech, Inc., Deerfield, IL) held in place by two small pieces of glass wool. An battery operated air-sampling pump (SKC Inc., Eighy Four, PA) was used to draw air through the collection bag, and on to the trap at a rate of 300 ml/min. At each interval VOCs were collected for 1 hour, and following collection, stink bugs and enclosures were replaced over bolls. This process was repeated for each sampling interval. VOCs were extracted from collection traps by washing with 200 µl of hexane, and collected directly into an autosampler vial. VOC extracts were analyzed by gas chromatography-mass spectrometry. Volatile emissions from bolls were analyzed using repeated measures analysis of variance (repeated ANOVA) to determine differences in total VOC emissions between treatments at each sampling interval.

Field Testing of Electronic Nose Technology. We performed preliminary investigations of field-application of E-nose by enclosing a stink *E. servus*, or southern green stink bug, *Nezara viridula* (L.) on cotton bolls 10 days post anthesis. In order to maintain healthy (undamaged) bolls over the course of development (prior to experimentation), enclosures were placed on white blooms on the day of anthesis. Boll development occurred inside enclosures to ensure pests could not attack bolls prior to experiments. A single stink bug was placed inside enclosures on bolls 10 days post-anthesis in a randomized complete block design. A single row of cotton was considered a single block, and each block was separated by at least two rows. Within each block, each of 3 treatments (control, *E. servus*, and *N. viridula* damage) were randomly assigned to a single boll on individual plants. A total of ten blocks were sampled, and based on the temporal dynamics data (Fig. 1) bolls were sampled following three-day of stink bug exposure. Prior to E-nose sampling enclosures, and stink bugs were removed, and a polyacetate oven bag (300 ml) was placed over the bolls and secured at the base of the boll. Head space VOCs from bolls were allowed to accumulate inside collections bags for 30 minutes prior to E-nose sampling. The snout of a commercially available E-nose (Cyranose 320, Smiths Detection, Pasadena, CA), was inserted into the top corner of a bag, and VOCs were sampled using the sample collection, and data processing parameters described below (Fig. 2). Ten bolls from each treatment group were sampled to train the E-nose, followed by canonical projection analysis, and cross validation by on-board data analysis software.

Results and Discussion

Temporal variation in Boll VOC emissions in Response to Stink Bug Feeding. Total VOC emissions from bolls damaged by *E. servus*, were similar to control bolls following 24, or 48 hours of feeding damage (Fig. 1). Total VOC emissions from bolls damaged by *E. servus* increased significantly compared to healthy bolls between 48- and 72-hours of exposure (Fig. 1). VOC emissions increased by 2.1-fold over control VOC emission following 4-days of exposure, and a 2.2-fold increase was detected following 5-days of exposure (Fig. 1). These data indicate that stink bug feeding results in a significant increase in VOC emissions between two- and three-days of feeding injury, and that emissions remain elevated during exposure to stink bugs. Cotton bolls exposed to stink bug feeding damage require at least two days of *ad libitum* feeding damage before significant increases in VOC emissions can be detected using standard VOC sampling techniques.

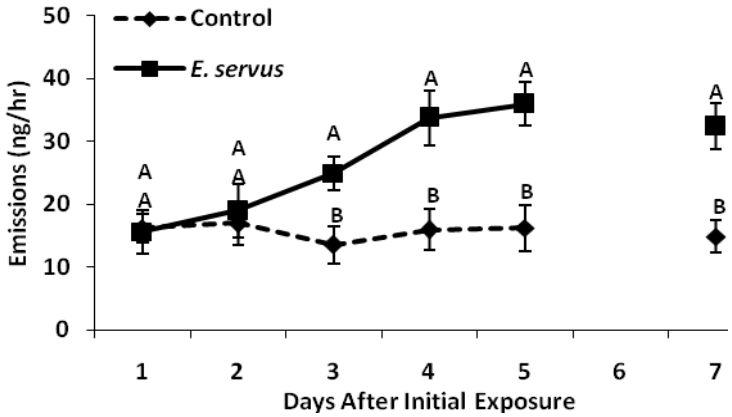

Figure 1. Temporal variation in total volatile organic compound (VOC) emissions from healthy (Control) cotton bolls and bolls damaged by *Euschistus servus* (*E. servus*). Data points represent mean VOC emissions (± 1 SE); n=6 bolls. Different letters represent significant difference in total VOC emissions between treatments at each sampling interval (repeated measures ANOVA; $P < 0.05$).

Field Testing of Electronic Nose Technology. Following three days of exposure to either *E. servus*, or *N. viridula* under field conditions, VOCs from cotton bolls were sampled by E-nose using a short, ten-second sampling sequence (Fig. 2). Each sample involves an initial baseline purge of the sensors, and sample draw, followed by a sensor purge to remove the sampled odors from the sensor module (Figure 2).

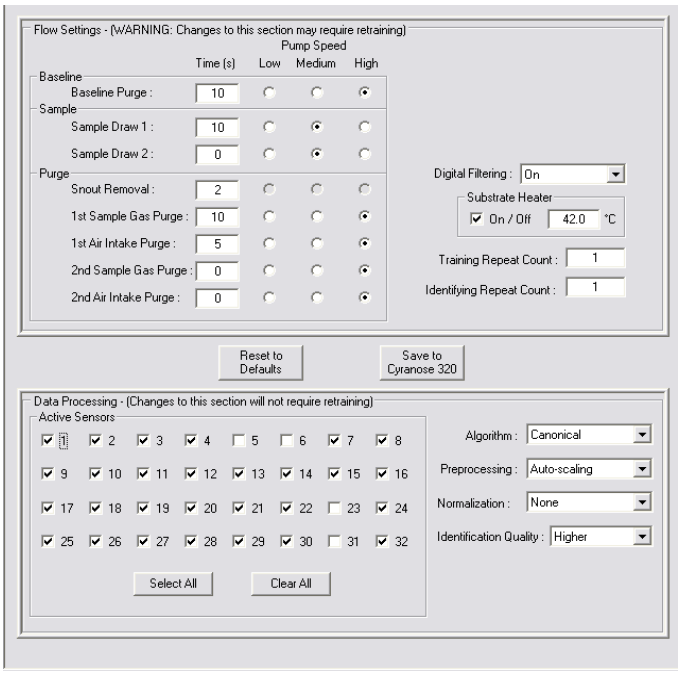

Figure 2. Sampling and data processing settings for the Cyranose 320, used during in-field sampling of cotton boll VOC emissions in response to stink bug feeding damage.

Based on canonical projection analysis, E-nose sampling of healthy cotton bolls, and bolls damaged by *E. servus*, or *N. viridula* resulted in modest separation of treatment groups (Figure 3). A large amount of variation was detected within treatment groups, suggesting that strong variation in VOC emissions may occur under field conditions. Furthermore, greater overlap between *E. servus* and *N. viridula* treatment groups was observed compared to the control group indicating that E-nose may not be capable of accurately distinguishing between VOC profiles released in response to feeding damage by different stink bug species (Figure 3).

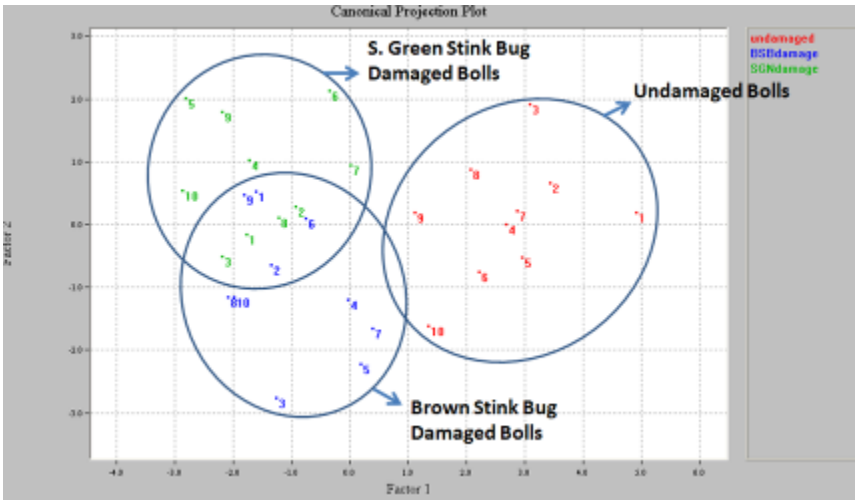

Figure 3. Canonical projection plot based on smell prints generated by the sensor resistance data from E-nose sampling of undamaged cotton bolls, and bolls damaged by *Euschistus servus* (brown stink bug damage), and *Nezara viridula* (southern green stink bug damage) after three days of exposure under field conditions.

Following canonical projection analysis, cross-validation of the data set was performed to determine the predictive accuracy of the model (Figure 4). Cross-validation of the sensor data indicated that E-nose was 70% accurate at discriminating among the three treatment groups (Figure 4). The majority of the E-nose inaccuracy was attributed to the inability to reliably distinguish between bolls damaged by *E. servus*, or *N. viridula*. For example, of the ten samples drawn from *E. servus* damaged bolls, eight were correctly identified as *E. servus* damage, while two were identified as *N. viridula* damage (Figure 4). Furthermore, of the ten samples drawn from *N. viridula* damaged bolls, only five were correctly identified as *N. viridula* damage, while four were identified as *E. servus* damage, and 1 was classified as a healthy boll (Figure 4). When considering the ten samples drawn from control bolls, eight were correctly identified as undamaged while two were identified as *E. servus* damage (Figure 4). These data indicate that E-nose is less capable at differentiating between VOC profiles from cotton bolls released in response to different species of stink bugs causing injury.

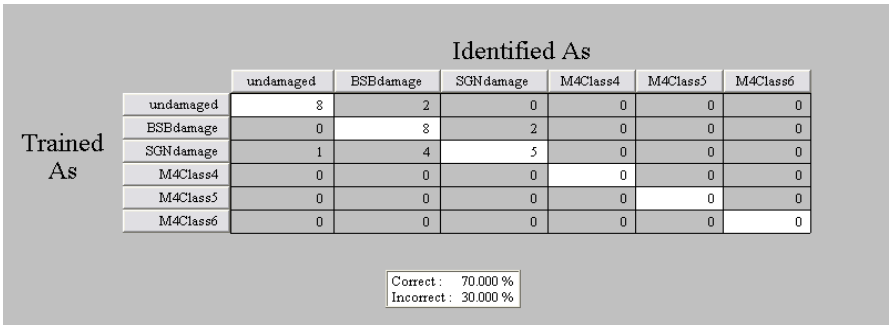

Figure 4. Cross validation of the E-nose sensor data showing the predictive accuracy of the E-nose ability to discriminate among healthy bolls (undamaged), *E. servus* damaged bolls (BSBdamage) and *N. viridula* damaged bolls (SGNdamage) under field conditions.

Summary

Our results indicate that VOC emissions from cotton bolls damaged by stink bugs show strong temporal variation compared to healthy, undamaged bolls, and emissions from damaged bolls increase significantly compared to undamaged bolls between two and three days of exposure. Results from our preliminary field investigation indicate that after three days of exposure to stink bugs, E-nose is only modestly accurate at discriminating among VOC profiles from healthy bolls, and bolls damaged by *E. servus*, and *N. viridula*. Nonetheless, E-nose technology shows

significant promise as a rapid in-field assessment tool for determining stink bug injury to cotton bolls. With continued refinement and testing of E-nose, the predictive accuracy of this technology could be increased. For example, identifying a subset of sensors that response specifically to VOC emissions from damaged bolls may reduce the variation within treatment groups. Furthermore, understanding how the profile in VOC emissions may change after stink bug injury, may provide a more accurate cue for E-nose detection. For example, if the VOC profile from a cotton boll shifts in response to callous wart formation several days after stink bug feeding, then E-nose may be more accurate at discriminating healthy and undamaged bolls based on these VOC emissions. These questions will be the subject of further investigations.

Acknowledgements

The authors would like to thank the staff at the Edisto Research and Education Center for raising plants for growth chamber experiments, as well as technical assistance with field experiments. This project was partially funded by a Cotton Inc. grant (09-630).

References

Degenhardt, DC, Greene, JK, Khalilian, A, and Reeves, RB. 2009. An innovative approach for the assessment of stink bug damaged bolls using electronic odor-sensing technology. *In* Proceedings Beltwide Cotton Production Conferences, National Cotton Council of America, San Antonio, TX.

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

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: 8433-8439.

National Association of Agricultural Statistics. United States Department of Agriculture. Accessed: 10 Jan. 2010. < 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: 1733-1747.

Röse, USR, and Tumlinson, JH. 2005. Systemic induction of volatile release in cotton, How specific is the signal to herbivory? Planta 222: 327-335.

Williams, MR. 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.