



American Society of  
Agricultural and Biological Engineers

*An ASABE Meeting Presentation*

*Paper Number: 061103*

## **Detecting Stink Bugs/Damage in Cotton Utilizing a Portable Electronic Nose**

**Will Henderson, Extension Associate**

Edisto Research & Education Center, Clemson University

**Ahmad Khalilian, Professor**

Agricultural & Biological Engineering, Clemson University

**Young Han, Professor**

Agricultural & Biological Engineering, Clemson University

**Written for presentation at the  
2006 ASABE Annual International Meeting  
Sponsored by ASABE  
Oregon Convention Center  
Portland, Oregon  
9 - 12 July 2006**

**Abstract.** *The goal of this study was to develop effective and affordable tools for detecting stink bugs and stink bug induced damage in cotton production. A commercially available electronic nose (Cyranose 320) was used for this purpose and its performance was evaluated under laboratory and field conditions. The volatile compounds given off by stink bugs were identified to be trans-2-decenal and trans-2-octenal. The E-nose was trained to identify stink bugs' (presence) smell prints. Only four sensors, out of 32 available, responded to volatile chemicals produced by bugs. The same sensors showed identical responses (smell prints) to trans-2-decenal as compared to those obtained from stink bugs. Also, under laboratory conditions, the Cyranose accurately predicted damaged bolls, interior walls of bolls and locks with lint and seed approximately 95 percent of the time. Under laboratory conditions, the E-nose identified presence of stink bugs 100 percent of the time. There was a strong correlation ( $R^2 = 0.95$ ) between the number of stink bugs in a sample and the Cyranose sensors response. Under field conditions, the E-nose was able to identify stink bug damaged bolls 67% of the time.*

**Keywords:** *Electronic nose, Stink bugs, Cotton, Plant volatiles, Precision Agriculture*

---

The authors are solely responsible for the content of this technical presentation. The technical presentation does not necessarily reflect the official position of the American Society of Agricultural and Biological Engineers (ASABE), and its printing and distribution does not constitute an endorsement of views which may be expressed. Technical presentations are not subject to the formal peer review process by ASABE editorial committees; therefore, they are not to be presented as refereed publications. Citation of this work should state that it is from an ASABE meeting paper. EXAMPLE: Author's Last Name, Initials. 2006. Title of Presentation. ASABE Paper No. 06xxxx. St. Joseph, Mich.: ASABE. For information about securing permission to reprint or reproduce a technical presentation, please contact ASABE at [rutter@asabe.org](mailto:rutter@asabe.org) or 269-429-0300 (2950 Niles Road, St. Joseph, MI 49085-9659 USA).

---

## Introduction

Tremendous progress has been made in reducing reliance on highly hazardous insecticides in cotton production by the relatively recent introduction of genetically engineered varieties (*Bacillus thuringiensis* -- B.t.). However, this progress is imminently threatened by a complex of sucking bugs insects (e.g. stink bugs) that were previously controlled under the “high-insecticide-input” system of conventional varieties. No insecticides were reported for stink bugs control on U.S. cotton in 1995 but, after 6 years of B.t. production, crop losses from these pests exceeded \$50 million (Williams, 2002).

Current scouting and monitoring techniques for stink bugs are time-consuming and unreliable. In addition, growers and consultants lack ready access to effective, efficient and affordable decision-making and management tools, making them particularly susceptible to unneeded use of insecticides. Intervention thresholds for stink bugs in cotton are based on field-sampling with a beat cloth (Greene et al. 2001) to determine population levels and/or hand-picking of ½ grown bolls to assess internal damage in the form of punctures, warts and seed/lint staining. Farmers and consultants find these methods to be difficult and cumbersome. As a consequence, applications of insecticides are often made before bugs are present or after economic damage has occurred. Unless more accurate and effective diagnostic and management strategies are developed for stink bugs, cotton production may once again enter an era of dependence on broad-spectrum, highly-toxic chemicals.

Cotton plants and bolls release chemicals as part of a defensive mechanism against pests (Paré and Tumlinson 1997), or as a by-product of chemical reactions in bolls damaged by sucking bugs (Drees and Jackman 1999, Williams and Tugwell 2000). These chemicals are molecularly specific, normally volatile, and potentially detectible as a characteristic of stink bug presence and/or boll damage in cotton. Commercially available electronic nose detectors that employ several sensing elements and pattern recognition software to detect volatile compounds at the parts-per-million (ppm) to parts-per-billion (ppb) threshold levels have been developed (Zeiger 2003). Several chemical-specific sensors have been used to detect a broad range of alcohols, aldehydes, esters, hydrocarbons, and volatile sulfur compounds, as well as individual volatiles associated with fruit ripening and food safety (Simon et al. 1997, Norton 1982, Dodd et al. 2004, Balasubramanian et al. 2004). There is no published information on the use of electronic nose technology for detecting the presence of stink bugs and internal plant damage.

## Objectives:

The objectives of this study were to:

1. Determine the feasibility of detecting stink bug presence/damage utilizing an electronic nose.
2. Identify chemical volatiles released by stink bugs utilizing standard laboratory methods and relate chemical intensity to insect population densities using E-nose technology.

## Materials and Methods

### *Cyranose 320 (E-nose)*

The Cyranose 320 (Smiths Detection Inc., Pasadena, California) is a commercially available, portable electronic nose made of an array of carbon polymers and is capable of simulating the human olfactory sense. With this system, an air stream is drawn across the array of 32 sensors

and the change in resistance to the sensors is measured. The resistance change creates a “smellprint” for the compounds. Four sensors, out of 32 available (5, 6, 23, and 32) were sensitive to water vapor and were not used for data collection in this study.

Before the Cyranose 320 can be used to identify the presence of stink bugs and/or plant damage it has to be trained. In addition it is important to develop a method specific to the application that the C-320 will be used for. Seven factors were determined in order to create an accurate method: 1) system set-up, 2) substrate temperature, 3) pump speeds, 4) purge conditioning, 5) choosing times of baseline, sample draw(s), and purges, 6) digital filtering, and 7) collecting data and reviewing the C-320 output.

1. The system set up was accomplished by connecting the C-320 to a PC using a serial (RS-232) cable.
2. The substrate temperature was typically set as low as possible, but at least 7<sup>0</sup>C higher than the highest expected ambient temperature during normal operation.
3. The pump speeds for baseline and sample draw(s) were set to medium; however, a high setting was used for all purges.
4. The charcoal filter was used for all tests in the lab and in the field to eliminate the variability from organic contaminants.
5. Baseline purge, sample draw(s), and purge(s) times were 30 second, 60 seconds for most test, and 180 seconds, respectively. Snout removal was set to 5 sec in all methods to allow for complete removal of needle from sampling equipment.
6. Digital filtering smoothes the resistance reading from the sensors. Digital filtering was set to “ON” to improve the signal to noise ratio.

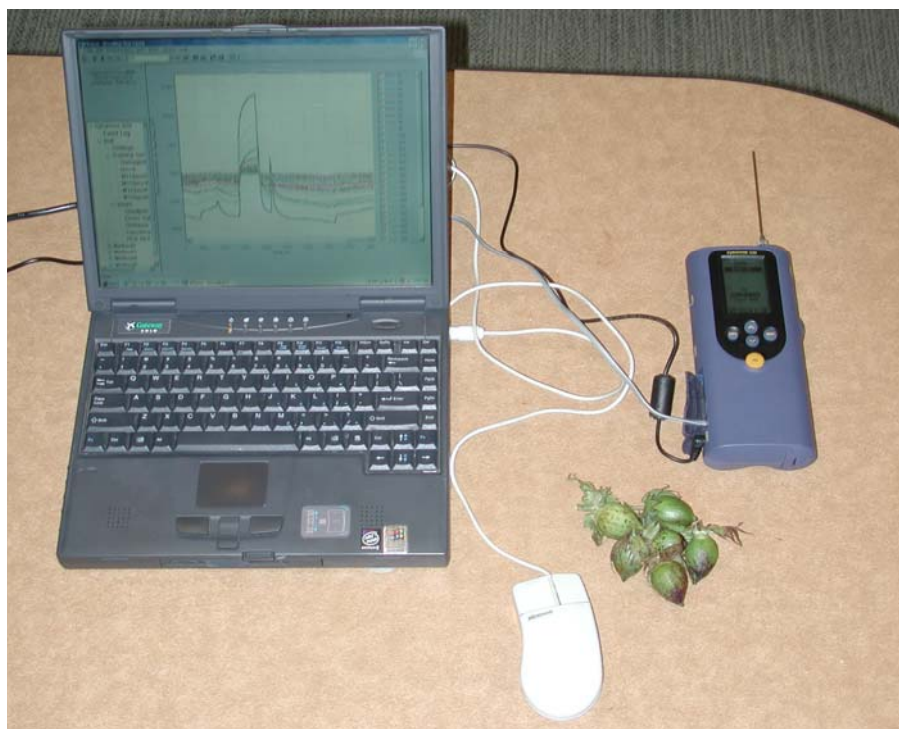


Figure 1: Cyranose 320 Connected to a PC Using RS-232 Cable.

### ***Rearing of Stink Bugs***

Stink bugs were collected in soybeans and cotton using a beat cloth or sweep net and were taken into a controlled rearing room (27° C and 70% relative humidity). The insects were exposed to 16 hours of light and 8 hours of darkness to simulate outside conditions. The insects were caged in plastic food containers with a plastic mesh over the top for air circulation and were fed a diet of fresh green beans and green peanuts.

### ***Insect Volatile Collection***

Insect volatiles were collected using a 250 mL Erlenmeyer flask. The inlet air was connected to a cylinder of zero grade medical air through a flow control valve set at 250 mL/min and a charcoal filter (Analytical Research Services, Gainesville, FL). The outlet air was attached to a collection trap (Super Q). At the end of the sampling period the trapped volatiles were removed from the collection trap with 0.5 ml of hexane into a 1.1 ml auto sampler vial with a crimp top cap. Volatile chemicals produced by stink bug were analyzed using a Gas Chromatography/Mass Spectrometry (GC/MS) machine.

### ***Verification of Stink Bug Compounds Using Cyranose 320***

Known compounds from stink bugs (trans-2-decenal and trans-2-octenal) were purchased and were compared to the volatiles released by stink bugs. Each compound was diluted in water at concentrations of 0.67, 1, 1.33, 2, and 4 mg/L. A 0.5-mL sample from each concentration was put into a 250-mL Erlenmeyer flask for testing with the C-320. These corresponded to 1.3, 2, 2.65, 4, and 8 µg/L. The similar techniques were used to identify smellprints of the stink bugs.

### ***Stink Bug Population Densities***

The C-320 was used to determine the stink bug population densities. Different numbers of stink bugs (1 to 10) were placed into the 250-mL flask and the “smellprints” for each group were identified using the E-nose. The test was replicated three times. The C-320 was also put through a series of caged field tests to see if it could determine the number of stink bugs present in a contained outdoor environment. Sixteen field cages (1.2 m wide X 1.2 m deep X 1.5 m high) covered with an insect mesh were placed in a cotton field. These cages were used to isolate the bugs and cotton plants in the field. The following insect density treatments were applied using a randomization complete block arrangement with four replications: 1 stink bug per cage, 3 stink bugs per cage, 6 stink bugs per cage, and control (no stink bugs). Before placing the stink bugs in the cage, Bidrin (insecticide) was sprayed in the cage to remove any insects or pests that may have been on the cotton.

### ***Assessment of Boll Damage***

Cotton bolls were caged in the field using 470-mL Styrofoam cups with nylon stockings stretched over the outside of the cup. Rubber bands were used to hold the cups to the plant as well as securing the nylon stockings over the top of the cup. 50% of the caged bolls were marked undamaged (red) and the other half was marked damaged (black). The bolls were approximately the size of a quarter which is the same size that a cotton scout will look at when determining damage. In the cups marked ‘damaged’ one stink bug was added to each cup. The stink bugs were allowed to feed for four to five days to ensure that the volatile chemicals were released by the plant. The bolls were removed from the plant and 10 random samples from each group were used for training of the C-320. The remaining bolls were used to see if

the C-320 could correctly identify the difference between damaged and undamaged bolls. Also, the system was used to identify damaged locks with lint and seed and interior walls of bolls.

## Results and Discussion

### *Stink Bug Presence*

The C-320 was trained to differentiate between two species of the stink bugs (green and southern green). The PCA plot gave discrete groupings (Figure 2) from the training exposures with a cross validation of 96% correct and 4% incorrect. However, in validation mode the C-320 was giving responses of 'confused' or 'unknown'. It was hypothesized that although the PCA plot showed very distinct groupings for two species of stink bugs, there was not enough distinction chemically for the C-320 to determine which type of the stink bug is present.

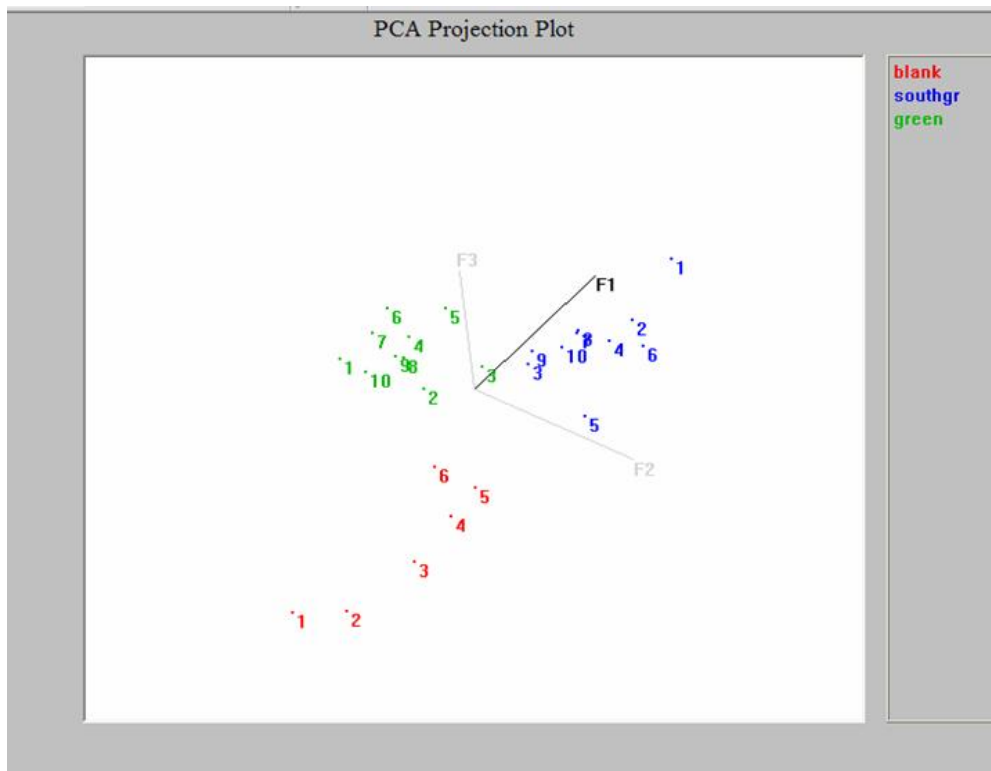


Figure 1: The PCA Plot for two species of stink bugs and control (no stink bug)

To overcome the C-320's inability to determine the presence of stink bugs from the above method with two different species of stink bugs, a new method was developed based on the difference between stink bugs (one variety) and a control. This method gave two very distinct groupings in the PCA plot with a cross validation of 100% correct (Figure 3). This method allowed the C-320 to detect the presence of stink bugs 100% of the time. Only four sensors (sensor numbers 20, 26, 28, and 29) out of 32 available, showed responses to stink bug presence.

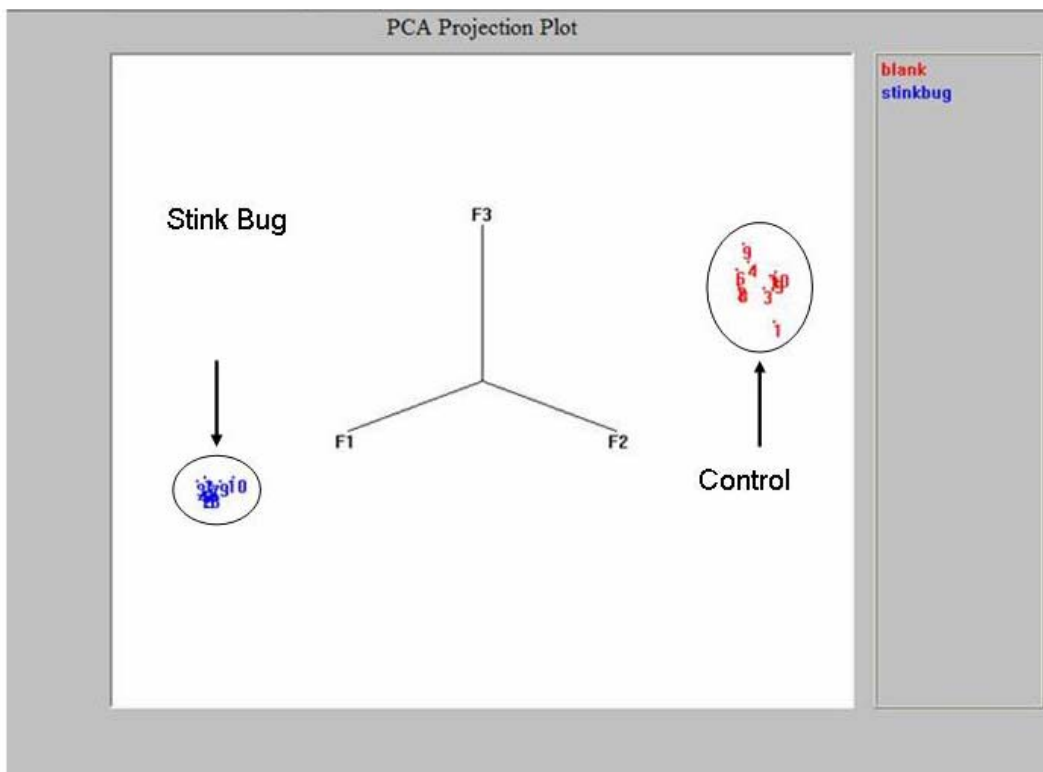


Figure 3: PCA plot showing discrimination between stink bugs and control (no bugs)

### ***Stink bug population densities***

Another important discovery was the development of a curve that correlated the sensor response of the C-320 to the number of stink bugs present. Only the responses from the four major sensors (sensor numbers 20, 26, 28, and 29) were used in the development of the curve. To develop this curve stink bugs were placed in a 250 mL Erlenmeyer flask from one to ten. Three samples were taken with the C-320 for each number of insects. The maximum response for each of the four sensors was recorded. The three samples were average to give the average sensor response for the specified number of insects. Figure 4 shows the correlation between the stink bugs population densities vs. C-320 sensor responses. The magnitude of the C-320 sensors response increased with the number of stink bugs. There was a high correlation ( $R^2 > 0.94$ ) between the sensor response and the number of stink bugs. The process of determining the number of stink bugs present under field conditions did not show promise under field conditions.

### ***Analysis of Stink Bug Compounds Using Cyranose 320***

The C-320 was able to verify the results of Gilby and Waterhouse (1964) that trans-2-decenal and trans-2-octenal are present in the volatile chemicals associated with stink bugs. The best correlation to stink bug response and trans-2-decenal was at a concentration of 1.3 $\mu$ g/L (Figure 5). The best correlation to stink bug response and trans-2-octenal was at a concentration of 2 $\mu$ g/L. The sensor response of the C-320 showed similar magnitude and identity to trans-2-decenal and trans-2-octenal at these concentrations. Only five sensors (#18, 20, 26, 28, and 29) responded to both chemicals and stink bugs with peak magnitude of 5000  $\Delta R/R$ . However, there were numerous cases that the response from sensor number 18 was not significant.

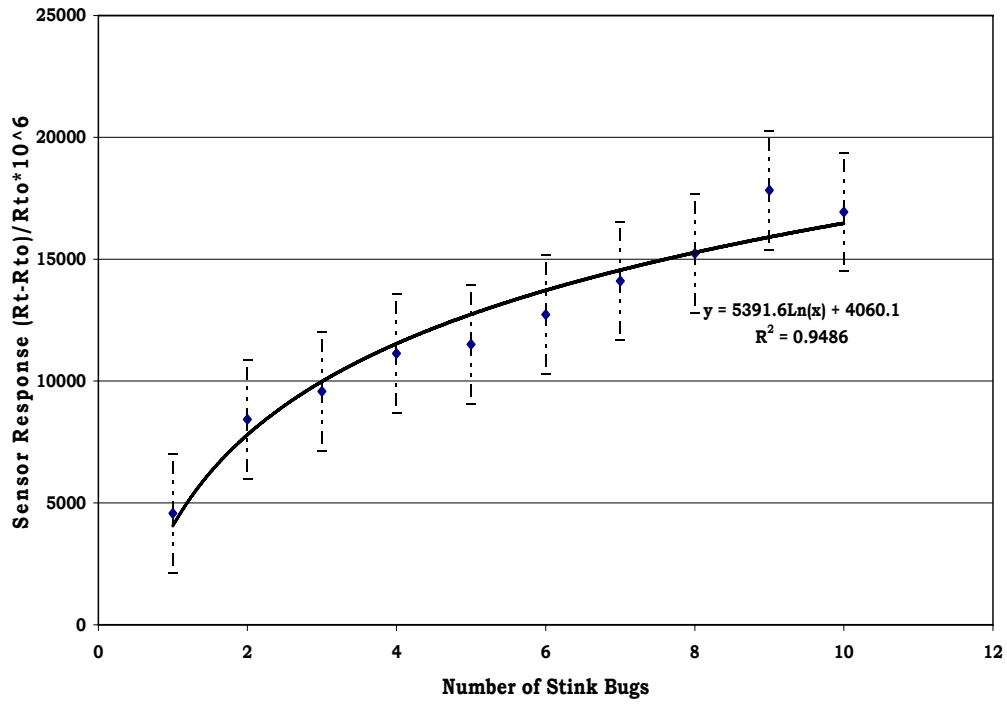
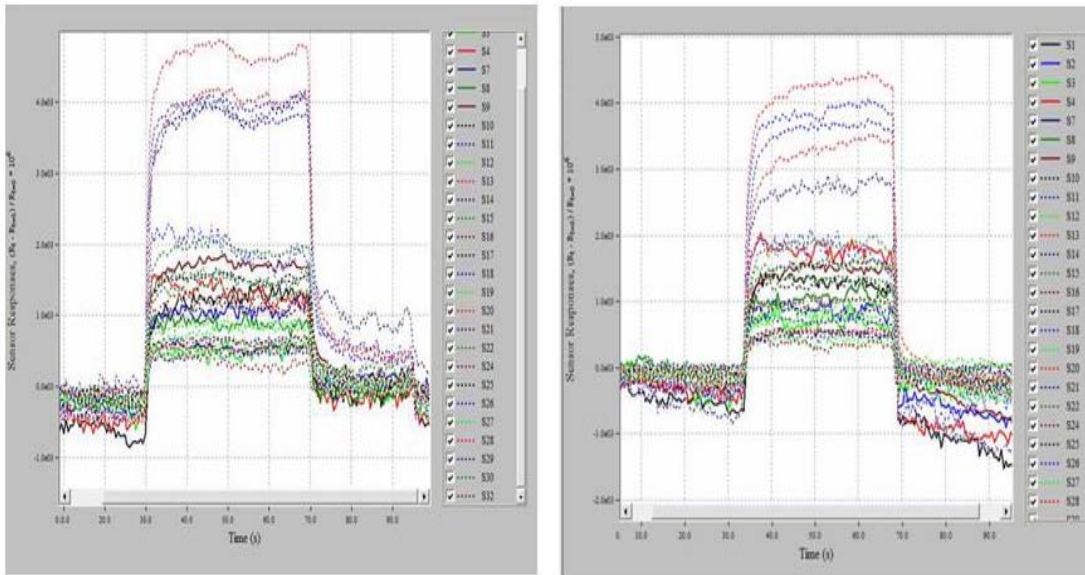


Figure 4: 95% Confidence Interval for Stink Bug Curve



Stink Bug

Trans-2-Decenal

Figure 5: C-320 Response of Stink Bug Compared to Trans-2-Decenal

**Sensitivity Analysis of the Cyranose 320**

The C-320 showed great sensitivity to the presence of the volatile chemicals elicited from stink bugs. Three scenarios were created to see how fast the C-320 could respond to being exposed to these volatile chemicals. Figure 6 show an example of C-320 being exposed to stink bug volatiles for 10 second cycles. All sensors were active for this process. This response shows that the C-320 could be used in a site-and-time specific spraying regime where the presence of these volatile chemicals is only sporadically present.

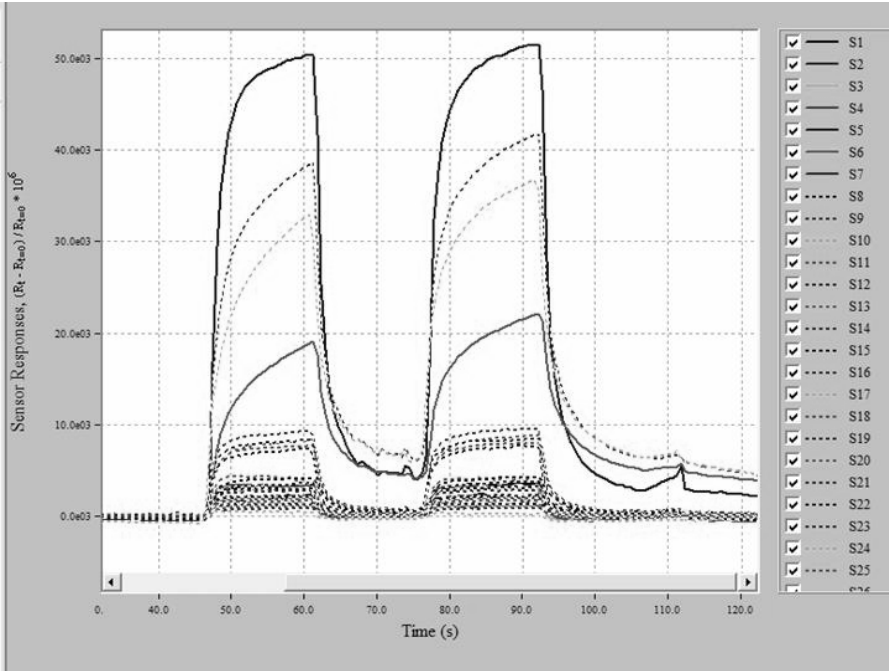


Figure 6: An example of the C-320 sensitivity test

**Analysis of Boll Damage Using the Cyranose 320**

Figure 7 shows the healthy and stink bug-damaged bolls and their effects on open bolls during harvest. Table 1 shows the prediction percentages of the C-320 compared to the actual boll condition looking at the whole boll, boll wall, and lint. The E-nose accurately predicted damaged bolls, interior walls of bolls and locks with lint and seed approximately 95 percent of the time. Under field conditions, the E-nose was able to identify stink bug damaged bolls 67% of the time.

Table 1: Prediction of damaged and healthy boll materials using C-320

Boll Material	Actual Condition	E-Nose Prediction		
		Right	Wrong	Unknown
Whole Boll	Good	80%	15%	5%
	Damaged	90%	5%	5%
Boll Wall	Good	95%	0%	5%
	Damaged	95%	0%	5%
Lint	Good	80%	5%	15%
	Damaged	100%	0%	0%

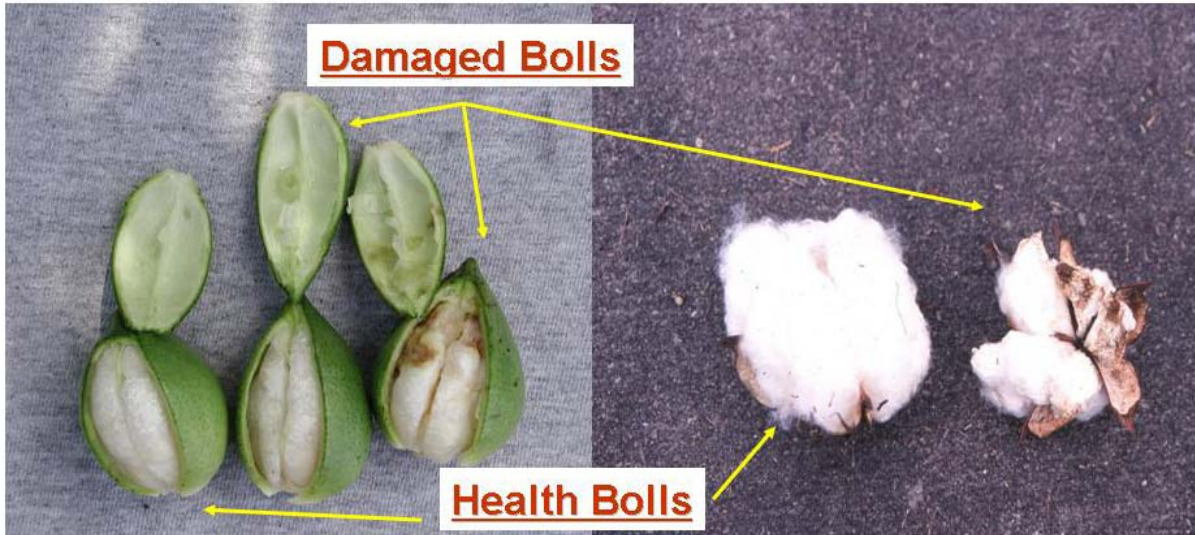


Figure 7: Healthy and stink bug-damaged cotton bolls

## Conclusion

- The Cyranose 320 showed extreme promise as an instrument in determining stink bug damage by external properties.
- The E-nose accurately predicted damaged bolls, interior walls of bolls and locks with lint and seed approximately 95 percent of the time.
- Under field conditions, the E-nose was able to identify stink bug damaged bolls 67% of the time.
- Under laboratory conditions, the E-nose identified presence of stink bugs 100 percent of the time.
- There was a strong correlation ( $R^2 = 0.95$ ) between the number of sting bugs in a sample and the Cyranose sensors response.
- Using C-320 the volatile compounds given off by stink bugs were verified to be trans-2-decenal and trans-2-octenal.
- Only four sensors, out of 32 available, responded to volatile chemicals produced by bugs. The same sensors showed identical responses (smell prints) to trans-2-decenal and trans-2-octenal as compared to those obtained from sting bugs.

## Acknowledgements

The authors acknowledge the support of the South Carolina Cotton Board, EPA, and Southern IPM.

## Disclaimer

Mention of a trade name does not imply endorsement of the product by Clemson University to the exclusion of others that might be available.

## References

- Balasubramanian, S., S. Panigrahi, C. M. Logue, M. Marchello, C. Doetkott, H. Gu, J. Sherwood, and L. Nolan. 2004. Spoilage Identification of beef using an electronic nose system. *Transactions of the ASAE* Vol. 47(1): 135-140.
- Dodd, T. H., S. A. Hale, S. M. Blanchard. 2004. Electronic nose analysis of tilapia storage. *Transactions of the ASAE* Vol. 47(5): 1625-1633.
- Drees, B. M. and J. Jackman. 1999. Southern Green Stink Bug. *Field Guide to Texas Insects*. <http://insects.tamu.edu/images/insects/fieldguide/aimg73.html>
- Gilby, A. R., D. F. Waterhouse. 1964. The composition of the scent of the green vegetable bug, *Nezara viridula*. Division of Entomology, C.S.I.R.O., Canberra, Australia. June 16, 1964.
- Greene, J. K., G. A. Herzog, and P. M. Roberts. 2001. Management decisions for stink bugs, pp. 913-917. In C. P. Dugger and D. A. Richter [eds.], *Proceedings, Beltwide Cotton Production Research Conferences, January 2001, Anaheim, CA, National Cotton Council of America, Memphis, TN*.
- Norton, H.N. 1982. *Biomedical sensors: fundamentals and applications*. Noyes Publications, Park Ridge, NJ, USA; ISBN 0-8155-0890-5.
- Paré, PW, and JH Tumlinson. 1997. De Novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology*, 114:1161-1167.
- Simon E.J. D. J. Charles, G. E. Miles, U. M. Peiper, A. Mizrach, A. Hetzroni, Y. Grinspun, P. Angers, N. Ozer, L. Yu1. 1997. Electronic Sensing of Fruit Ripeness Based on Volatile Gas Emissions.
- Williams, L., III & N. P. Tugwell. 2000. Histological description of tarnished plant bug, (Heteroptera: Miridae) feeding on small cotton floral buds. *Journal of Entomological Science* 35: 187-195.
- Williams, M. R. 2002. Cotton insect losses - 2000, [web page]. In J. McRae and D. A. Richter [eds.], *Proceedings, Beltwide Cotton Production Research Conferences, January 2002, Atlanta, GA, National Cotton Council of America, Memphis, TN*.
- Zeiger, Ken. 2003. Electronic Sniffer. *Electronic Sensor Technology of Newbury Park, California*. <http://www.globaltechnoscan.com/27thDec-2ndJan/sniffer.htm>