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Research Article

Temporal Dynamics and Electronic Nose Detection of Stink Bug-Induced Volatile Emissions from Cotton Bolls

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Management decisions for stink bugs (Pentatomidae) in *Bt* cotton are complicated by time-consuming sampling methods, and there is a need for more efficient detection tools. Volatile compounds are released from cotton bolls in response to feeding by stink bugs, and electronic nose (E-nose) technology may be useful for detecting boll damage. In this study, we investigated the temporal dynamics of volatile emissions in response to feeding by stink bugs and tested the ability of E-nose to discriminate between odors from healthy and injured bolls. Feeding by stink bugs led to an approximate 2.4-fold increase in volatile organic compound (VOC) emissions. Principal components analysis of E-nose sensor data showed distinct (100%) separation between stink bug-injured and healthy bolls after two days of feeding. However, when E-nose was used to randomly identify samples, results were less accurate (80–90%). These results suggest that E-nose is a promising technology for rapid detection of stink bug injury to cotton.

1. Introduction

Phytophagous stink bugs (Heteroptera : Pentatomidae) are major pests of food and fiber crops worldwide [1]. In the USA, stink bugs are increasingly destructive in cotton, *Gossypium hirsutum* (L.). Prior to the introduction of transgenic cotton varieties expressing insecticidal proteins from *Bacillus thuringiensis* (*Bt*), growers were afforded coincidental control of stink bugs through the use of broad-spectrum insecticides for major pests such as bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.). With the widespread adoption of *Bt* varieties, eradication of the boll weevil, *Anthonomus grandis grandis* (L.), and the subsequent reduction in the use of broadspectrum insecticides, stink bugs have emerged as important pests threatening cotton production, especially in the southeastern USA [2]. Since 1995, insecticide use targeting these pests has risen from zero to millions of applications, and crop losses recently exceeded 50 million dollars [3]. Stink bugs feed directly on cotton bolls resulting in boll abscission and lint staining and reduced fiber quality and yield [2, 4–6]. Scouting techniques for stink bugs require destructive sampling of cotton bolls with visual inspection for internal lint staining and callus warts on internal carpal walls [7]. Due to difficulties in assessment and time-consuming scouting practices, there is a need for a more reliable, rapid, and nondestructive method for determining boll injury from these pests.

It is well known that herbivory results in the induced synthesis of volatile organic compounds (VOCs) from plant tissues including a variety of terpenoids, phenylpropanoids, and fatty-acid derivatives [8, 9]. In cotton, terpenoids are stored in lysigenous glands, and herbivore feeding causes the release of stored terpenes, as well as the induction of novel compounds through a combination of physical damage and elicitors from salivary components of the attacking herbivore [10–13]. Stink bug feeding has been shown to induce VOC emissions from cotton [14], corn [15], and soybean [16]. Recently, it was shown that stink bug feeding on cotton bolls led to a 2-3-fold increase in VOC emissions compared with healthy bolls and that induced emissions were similar in response to feeding by different hemipteran species, including southern green stink bug, *Nezara viridula* (L.), and brown stink bug, *Euschistus servus* (Say) [17].

In the last decade, electronic nose (E-nose) technology has been developed to detect VOC emissions at the level of parts-per-million to parts-per-billion [18]. These devices characterize the overall profile of an odor as a digital "smellprint" generated by the change in resistance of several nonspecific gas sensors when exposed to components of the VOC mixture [18]. E-noses have been used for a range of applications, including detection of diseases [19], microbes [20], hazardous chemicals [21], and changes in food quality [22]. E-nose technology has also been successfully applied to monitoring pest damage in several systems. Based on brief (7 to 12 sec) samples from the headspace of tomato plants, E-nose was capable of discriminating pathogen infection, herbivore damage, and mechanical damage based on changes in VOC emissions among treatments [23]. In a laboratory study, it was demonstrated that an E-nose was 90% accurate at differentiating excised healthy boll from those injured by stink bugs [24].

Because stink bugs are known to induce VOC emissions from cotton bolls [17], E-nose technology could potentially serve as a rapid, nondestructive tool for monitoring stink bug injury to cotton bolls; however, this technology has yet to be tested for this purpose under field conditions. The overall goal of this study was to determine the feasibility of using an E-nose to detect and differentiate between VOCs from healthy (undamaged) bolls and those exposed to stink bug feeding (damaged) under field conditions. The specific objectives were to (1) determine the temporal dynamics in VOC emissions from cotton bolls in response to feeding by stink bugs, (2) determine the ability of an E-nose to differentiate between undamaged bolls and those damaged by stink bugs, and (3) determine if an E-nose can discriminate between VOCs induced in response to feeding by *E. servus* and *N. viridula*.

2. Materials and Methods

2.1. Plants and Insects. Experiments were conducted during August 2010 at the Edisto Research and Education Center (EREC) in Blackville, SC, USA. Field trials were carried out in a 1.5-hectare field planted with *G. hirsutum* var. Delta and Pine Land 161 B2RF.

Adults and nymphs of *E. servus* and *N. viridula* were initially collected from field populations in soybean and maintained separately in an insect rearing chamber at EREC. Insects were fed on a source of fresh green beans and provided with water on moistened cotton pads until initiation of experiments. Male and female adult stink bugs (5–7 d in stage) were used in all experiments.

2.2. E-Nose Instrumentation. The E-nose used in all experiments was the Cyranose 320 (Smiths Detection, Inc., Pasadena, CA, USA). The Cyranose 320 is a conductimetric, chemiresistive E-nose containing 32 sensors constructed of an aluminum substrate coated with a thin film of variable carbon composite polymers. Preliminary tests indicated that four sensors were sensitive to water vapor, and these sensors were deactivated for all experiments to improve discrimination capability. A typical sample cycle for the E-nose consisted of a sensor baseline purge, sample collection, and a sample purge. In this study, a high pump speed ($180\,\text{mL}\cdot\text{min}^{-1}$) was used for baseline and sample purges, and a medium pump speed ($120\,\text{mL}\cdot\text{min}^{-1}$) was used for all sample collections. When exposed to chemical vapors during sample collection, the film on each sensor swells causing an increase in resistance. The change in resistance for each sensor ($\Delta R/R$) is represented as the difference between electrical resistances (ΔR) recorded during sample collection and baseline purge during the sample cycle and the resistance (R) recorded at the end of the baseline purge. The resistance from all 32 sensors is measured, and the overall composition of individual sensor responses represents a "smellprint" for an odor. The resistance data from all sensors is analyzed by on-board pattern recognition algorithms including K-nearest neighbor, K-means clustering, and canonical discriminant analysis. The canonical algorithm was used for all analyses in this study.

2.3. VOC Collection and Analysis. A randomized complete block design was used to evaluate the temporal dynamics in VOC emissions and E-nose detection (described below) of undamaged and damaged bolls. In each block, three white blooms at the same node on individual plants were enclosed in cages to protect developing bolls from insect damage prior to experiments. Cages were constructed from polystyrene foam cups with the base of the cup removed and nylon stocking stretched over the cup. Cages were placed over blooms, and the nylon stocking was secured around the peduncle using a 24-gauge steel wire. The nylon stocking was also secured around the top of each cage using another piece of 24-gauge steel wire. For analysis of stink bug-induced VOC emissions, a single adult *N. viridula* or *E. servus* was placed in cages on bolls 10–12 d postanthesis and allowed to feed *ad libitum*. Stink bugs were removed in 24 hr intervals to collect VOC emissions from damaged and undamaged bolls. Volatiles were sampled using a dynamic headspace sampling method. A polyacetate oven bag (Reynolds, Inc., Richmond, VA) modified to a volume of 300 mL was placed over a boll and loosely fastened with a small cable tie at the base of the boll to permit airflow through the bag. A volatile trap was connected to the top corner of each bag using a small cable tie. Volatile collection traps were constructed from glass Pasteur pipettes (10 cm long, 0.5 cm [o.d.]) and contained 35 mg of Porapak Q adsorbent polymer (Alltech Assoc., Deerfield, IL, USA) held in place by two small plugs of glass wool. A battery-operated air-sampling pump (SKC, Inc. Eighty Four, PA, Model 224-44XR) fitted with an independently controlled, adjustable, 4-way splitter (SKC, Inc. Model 224-26-04) was used to draw ambient air through the bag across the boll and directly onto the trap at a rate of $300\,\text{mL}\cdot\text{min}^{-1}$. Volatiles were collected for 3 hr during each sampling interval. Ambient air blanks were collected simultaneously with boll volatile collections to correct for any volatile contaminants contained in the air pulled through the collection bag. Emissions from damaged and undamaged bolls were collected in 24 hr intervals over a 4 d period.

Volatiles were extracted from adsorbent traps by washing with 150 μL of analytical grade hexane and collecting the extract directly into a 2 mL autosampler vial containing a 150 μL insert. Two μL of each sample were analyzed by gas chromatography (GC) on a Hewlett-Packard 6890 gas chromatograph equipped with a RTX-5 column (30 m $\times$ 0.25 mm [i.d.], 0.25 μm film thickness) (Restek, Bellefonte, PA). Injections were made in the splitless mode for 0.5 min with an inlet temperature of 250°C. The oven was held at 50°C for 10 minutes and then increased to 150°C at 5°C·min^{-1}, followed by an increase to 250°C at a rate of 15°C·min^{-1}, and a final increase to 300°C at a rate of 10°C·min^{-1}. Helium was used as a carrier gas at a flow rate of 1 mL·min^{-1}. Samples were subsequently analyzed by mass spectrometry (MS) using a Varian VG-70S (Waters Corp., Milford, MA) operated in electron impact mode. Compound identities were confirmed by comparison with mass spectra and retention indices of the library of essential oil components identified by GCMS [25], with spectra obtained from known standards (Sigma-Aldrich, Inc., Milwaukee, WI), and from high-resolution mass spectra from solvent extracts of boll material. Quantification of compounds was based on external standard curves of α-pinene for monoterpenes and β-caryophyllene for sesquiterpenes. The C11 homoterpene, DMNT, and the C16 homoterpene, TMTT, were quantified based on the standard curve of α-pinene and β-caryophyllene, respectively.

2.4. E-Nose Training on Damaged and Undamaged Bolls. Before an E-nose can be used for discrimination between treatments, it must be trained to recognize the odor profile among replicates of treatment groups. The temporal dynamics in E-nose detection of stink bug feeding damage were examined by training the E-nose on VOC emissions from bolls damaged by *N. viridula*, as well as undamaged bolls, in 24 hr intervals over a 4 d feeding period. During each 24 hr training period, cages and stink bugs were removed, and bags were placed over bolls (described previously) prior to E-nose sampling. Headspace VOCs were allowed to accumulate inside the bag for 30 minutes prior to E-nose training. To train the E-nose, the snout of the E-nose was inserted into the top corner of a bag, and VOCs were sampled using a 10 sec baseline purge, a 15 sec sample collection, and a 30 sec postsample purge. VOCs were sampled from 8 damaged and 8 undamaged bolls during each training period, and the same bolls were sampled on consecutive training periods. Following E-nose training, bugs and cages were placed back over bolls. This process was repeated for each 24 hr training set. Smellprint data from each temporal training set were analyzed separately by canonical discriminant analysis followed by cross-validation to assess the accuracy of each training set.

2.5. Identification Accuracy of E-Nose Temporal Training Sets. To evaluate the accuracy of E-nose training data, each temporal training set was used to randomly identify a separate set of 10 bolls damaged by *N. viridula*, and 10 undamaged bolls immediately following collection of training data. Bolls used for E-nose identification were exposed to stink bugs for an equivalent amount of time as those used for training. The same bolls were used for consecutive training set evaluations. The identification quality of the E-nose was set to "highest," which included a range of potential responses including correct identifications (undamaged or damaged), as well as incorrect identifications (incorrect treatment, confused, or unknown). The number of correct and incorrect identifications was recorded after each sample exposure, and the identification accuracy was determined based on the percentage of correct identifications.

2.6. E-Nose Discrimination of Species-Specific Feeding Damage. To examine the ability of E-nose to discriminate between feeding damage by different species of stink bugs, a single adult of *E. servus* or *N. viridula* (5–7 d in stage) was placed on individual 10–12 d old bolls inside cages (described previously). Stink bugs were allowed to feed *ad libitum* for 3 d, after which cages and stink bugs were removed and a polyacetate oven bag was placed over the bolls. E-nose was used to randomly sample VOCs from undamaged bolls and those damaged by *E. servus* and *N. viridula* using the sampling protocol described previously.

2.7. Statistical Analysis. A mixed model, repeated measures analysis of variance (PROC MIXED) [26] was used to test for differences in total and individual volatile emissions using plant treatment (control, *N. viridula*-damaged, and *E. servus*-damaged) and time (0, 24, 48, 72, and 96 hr) as main effects in the model statement, with plant assigned as the repeated subject. Volatile emissions data were $\log(x + 1)$ transformed prior to analysis to satisfy assumptions of normality. Differences in the emissions of individual compounds among treatments were analyzed by Tukey post hoc comparisons. On-board data analysis software was used for canonical discriminant analysis and cross-validation analysis of E-nose training sets. The proportions of correct identifications of damaged and undamaged bolls following each temporal training session were statistically analyzed using *chi*-square analysis (PROC FREQ) [26].

3. Results

3.1. Volatile Emissions from Cotton Bolls in Response to Stink Bug Feeding. Herbivory by *N. viridula* and *E. servus* had a significant effect on the emission of VOCs over time (Table 1; Figure 1). A significant interaction between treatment and time (sampling period) indicated that the effect of stink bug feeding on VOC emissions varied significantly among sampling periods (Table 1). Prior to placement of stink bugs in cages (time 0), total VOC emissions were not significantly different among treatment (Figure 1). Significantly greater emissions were detected from bolls exposed to *N. viridula* and *E. servus* compared with controls 48 hr after feeding (Figure 1). Total emissions showed the strongest increase between the 48 and 72 hr sampling period, and emissions remained significantly elevated 96 hr after initial exposure (Figure 1). Feeding by stink bugs resulted in approximately a 1.3-, 1.9-, 2.2-, and 2.4-fold increase in total VOC emissions

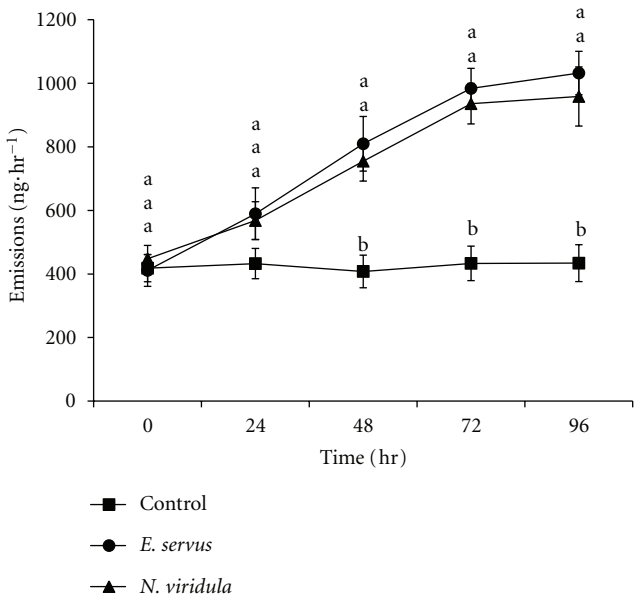

Figure 1: Temporal dynamics of total VOC emissions from cotton bolls exposed to *Euschistus servus and Nezara viridula* and unexposed (control) bolls under field conditions. Data points represent mean VOC emissions (±1 SE; $n = 5$). Different letters indicate a significant difference in total VOC emissions between treatments at each sampling interval (repeated measures ANOVA $P < 0.05$).

Table 1: Repeated measures analysis of variance on total volatile emissions released from cotton bolls exposed to *Euschistus servus* and *Nezara viridula* and unexposed bolls collected over a 4 d feeding period.

Effect	Num DF	Den DF	*F* value	*P* value
Treatment	2	12	41.54	<0.0001
Time	4	48	16.1	<0.0001
Treatment ∗ Time	8	48	3.98	0.0011

24, 48, 72, and 96 hr, respectively, from initial exposure to bolls (Figure 1). No significant difference was detected in total VOC emissions between *E. servus*- and *N. viridula*-damaged bolls at any sampling period (Figure 1).

Herbivory by *E. servus* and *N. viridula* resulted in a strong increase in the emission of several acyclic terpenes compared with controls, including the C10 monoterpene β-ocimene, the C15 sesquiterpene β-farnesene, and the C11 and C16 homoterpenes 4,8-dimethyl-1,3,7-nonatriene (DMNT) and 4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), respectively (Figure 2). Emissions of β-ocimene, β-farnesene, and DMNT increased significantly in bolls after 48 hr of exposure to stink bugs and remained elevated 72 and 96 hr after introduction of bugs (Figure 2). A significant increase in emissions of TMTT was not detected until 72 hr after exposure to stink bugs (Figure 2). Furthermore, while small quantities of β-ocimene, β-farnesene, and DMNT were detected in controls, TMTT was not detected in the headspace emissions of controls during any sampling interval (Figure 2).

3.2. E-Nose Training on Damaged and Undamaged Bolls. According to principal components analysis and cross-validation of training data, smellprints of damaged bolls were separated from smellprints of undamaged bolls with 81.25% accuracy after 24 hr of exposure under field conditions (Figure 3(a); Table 2). Smellprints of bolls exposed to *N. viridula* for 48, 72, and 96 hr were separated with 100% accuracy from smellprints of undamaged bolls (Figures 3(b), 3(c), and 3(d); Table 2). Interclass Mahalanobis distances (*M*-distance) provided an indication of the degree of separation between classes following each training session (Table 2). Longer durations of exposure led to greater separation of smellprints from damaged and undamaged bolls according to the interclass *M*-distance values generated from the cross-validation analysis (Table 2).

3.3. Identification Accuracy of E-Nose Temporal Training Sets. Regardless of the length of feeding exposure, identification accuracy was always lower compared with training set accuracy (Tables 2 and 3). When using the smellprints from training sets collected after 24 and 48 hr of feeding, the E-nose correctly identified bolls (damaged or undamaged) 60 and 65% of the time, respectively (24 hr: *chi*-square $P = 0.37$; 48 hr: *chi*-square $P = 0.18$; Table 3). Identification accuracy was markedly improved when using the smellprints from training sets collected after 72 and 96 hr of exposure to stink bugs. The E-nose correctly identified bolls (damaged or undamaged) with 95% accuracy after 72 hr of feeding exposure (*chi*-square $P < 0.0001$; Table 3) and 90% accuracy after 96 hr of exposure (*chi*-square $P = 0.0003$; Table 3).

3.4. E-Nose Detection of Stink Bug Species-Specific Feeding Injury. Overall, nine of the 28 active sensors showed a strong change in resistance in response to headspace emissions from cotton bolls (Figure 4). Smellprints collected from the headspace of unexposed bolls (Figure 4(a)) were distinct compared with smellprints from bolls exposed to *E. servus* and *N. viridula* (Figures 4(b) and 4(c)). Differences among smellprints were analyzed by canonical discriminant analysis followed by cross-validation (Figure 5; Table 4). Treatment groups were separated mainly along the first canonical component, with smellprints from unexposed bolls located along positive values of the first component and exposed bolls along negative values (Figure 5). A greater separation was observed between control and exposed bolls than between bolls exposed to *E. servus* or *N. viridula* (Figure 5). Cross-validation of the dataset indicated that all treatment groups were separated with 70% accuracy, but smellprints from bolls exposed to either stink bug species were separated with much less accuracy (65%) than control and damaged bolls (87.5%) (Table 4).

4. Discussion

In this study, it was demonstrated that E-nose technology can readily detect and distinguish VOCs emitted from cotton bolls damaged by stink bugs under field conditions. Herbivory by *N. viridula* and *E. servus* resulted in a significant

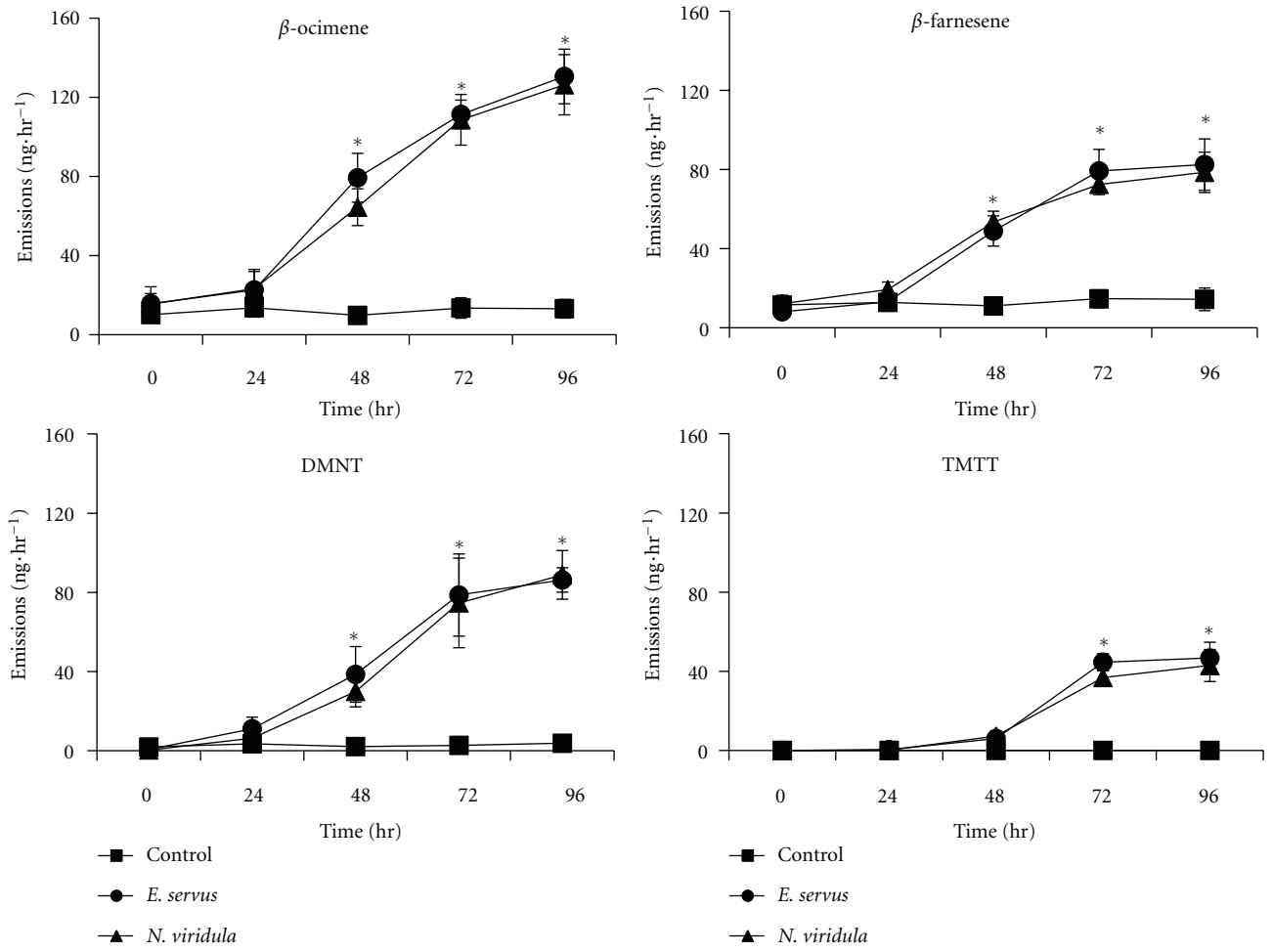

Figure 2: Temporal dynamics of induced VOC emissions from cotton bolls exposed to *Euschistus servus* and *Nezara viridula* and unexposed (control) bolls under field conditions. Data points represent mean VOC emissions (±1 SE; $n = 5$). Asterisks indicate significant difference ($P < 0.05$) between control and infested bolls.

Table 2: Cross-validation, training accuracy, and interclass Mahalanobis distance of training sets based on E-nose sensor response to headspace VOC emissions from 8 unexposed (control) bolls and 8 bolls exposed to *Nezara viridula*.

	Time		Identified as		Training accuracy	Interclass M-distance
			N. viridula	Control		
Trained as	24 hours	*N. viridula*	7	1	81.25%	2.339
		Control	2	6		
			N. viridula	Control		
	48 hours	*N. viridula*	8	0	100%	5.117
		Control	0	8		
			N. viridula	Control		
	72 hours	*N. viridula*	8	0	100%	5.125
		Control	0	8		
			N. viridula	Control		
	96 hours	*N. viridula*	8	0	100%	5.837
		Control	0	8		

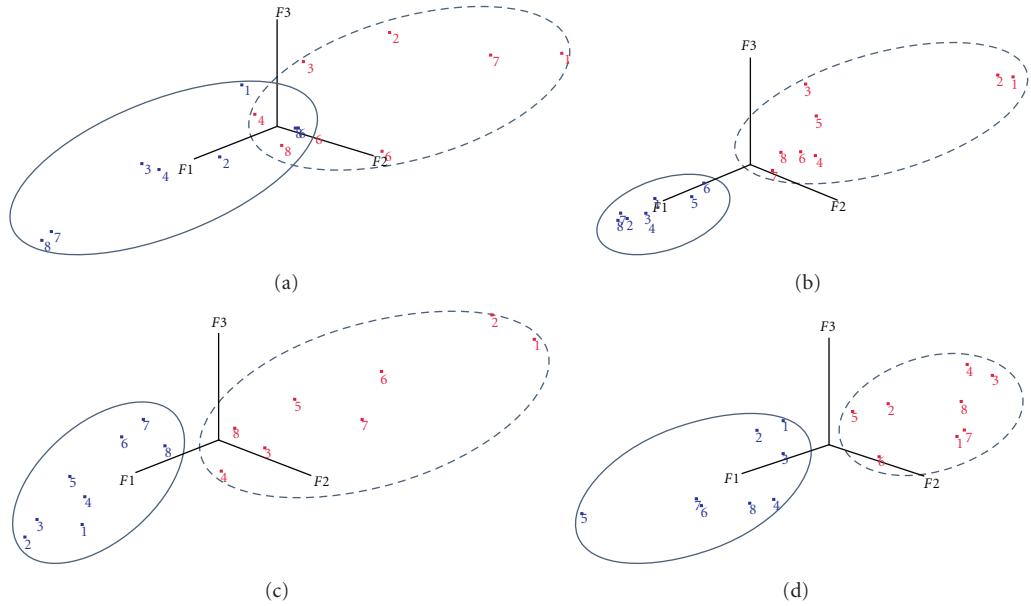

Figure 3: Principal components plot of E-nose sensor responses to headspace VOC emissions from unexposed cotton bolls (solid ellipsoids) and bolls exposed to *Nezara viridula* (dashed ellipsoids) after 24 (a), 48 (b), 72 (c), and 96 (d) hr of feeding.

Table 3: Accuracy of E-nose training sets used to identify 10 unexposed bolls (control) and 10 bolls exposed to *Nezara viridula* collected during a 96 hr feeding trial.

Time	Treatment	Correct[a]	Incorrect[b]	Identification accuracy
24 hr	*N. viridula*	5	5	50%
	Control	7	3	70%
48 hr	*N. viridula*	4	6	40%
	Control	9	1	90%
72 hr	*N. viridula*	9	1	90%
	Control	10	0	100%
96 hr	*N. viridula*	8	2	80%
	Control	10	0	100%

[a]Classification includes total number of bolls correctly identified by E-nose (damaged or control).
[b]Classification includes total number of bolls incorrectly identified by E-nose (false, confused, unknown).

Table 4: Cross-validation of the training set used to train the E-nose to recognize headspace VOC emissions from undamaged (control) bolls and bolls damaged by *Euschistus servus* and *Nezara viridula* following 3 days of feeding damage.

		Identified as		
		Control	*E. servus*	*N. viridula*
Trained as	Control	8	2	0
	E. servus	0	8	2
	N. viridula	1	4	5

increase in VOC emissions 24–48 hr after initial exposure, and temporal dynamics in VOC emissions were similar between the two species. Furthermore, E-nose was capable of accurately (90% success) identifying bolls damaged by stink bugs from undamaged bolls, and the degree of separation between treatments increased with increasing exposure of stink bugs. Finally, E-nose was much less accurate (65% success) at differentiating VOC emissions induced by different species of stink bugs.

In response to stink bug feeding injury, cotton bolls released VOC emissions in significantly greater quantities compared with controls between 24–48 hr of exposure. Herbivory by stink bugs has been shown to induce VOC emission from leaves of several plant species [14–16], as well as from cotton bolls [17]. This study provides a more detailed analysis of the timing of induced VOC emissions in response to stink bug feeding damage. Similar quantitative changes were observed in overall emissions as well as the emissions of specific volatiles in response to feeding by both *N. viridula* and *E. servus*, suggesting that induced VOCs were not species-specific for stink bugs, at least for the level of sensitivity in this experiment. The specificity of induced VOC emissions is determined in part by the feeding mode of herbivores [27, 28] but also by the presence of elicitors in the oral secretion of insects [29]. Oral secretions from stink bugs applied to leaves in the absence of physical injury caused a 2-fold increase in sesquiterpene emissions in corn seedlings [3], indicating that stink bugs contain bioactive compounds in their regurgitant that induces VOCs. The similarities of induced VOC emissions between bolls damaged by *N. viridula* and *E. servus* suggest that, in addition to similar types of physical damage from piercing-sucking mouthparts, these species may also contain bioactively similar elicitors.

E-nose was not capable of accurately discriminating between VOC profiles induced in response to feeding damage from closely related stink bug species. In a previous study, it was shown that E-nose could differentiate between the

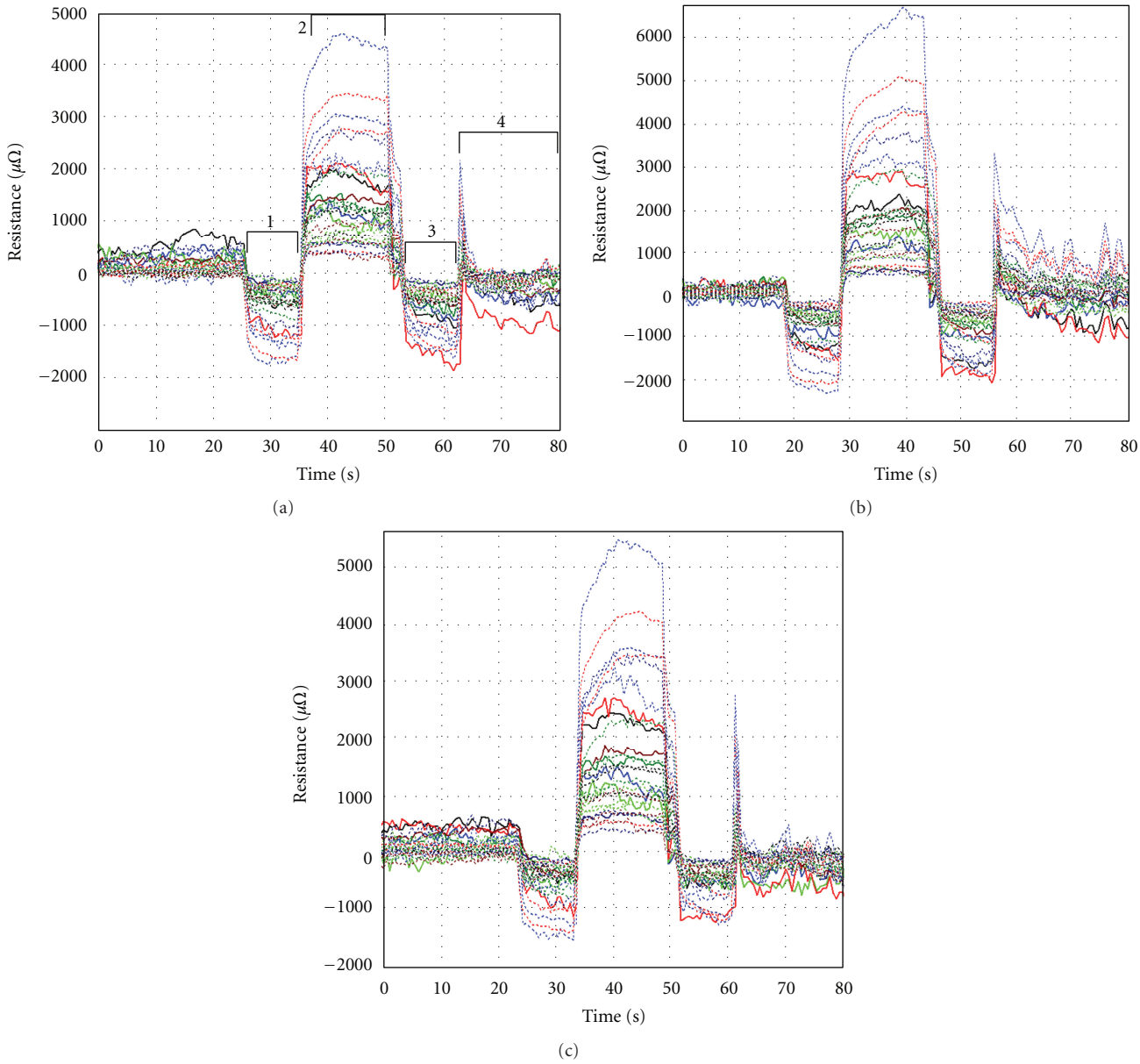

FIGURE 4: Representative E-nose sensor response patterns (smellprints) based on a 15 sec sample of headspace VOC emissions from unexposed (control) cotton bolls (a), or bolls exposed to *Euschistus servus* (b), and *Nezara viridula* (c) following 3 d of feeding damage. 1: baseline purge; 2: sample draw; 3: air purge; 4: sample purge.

defensive secretions released by *N. viridula* and green stink bug, *Acrosternum hilare* (Say) [24]. While E-nose technology was sufficiently sensitive to discriminate species-specific stink bug odors, the results presented here suggest that E-nose is not capable of accurately differentiating sources of damage based on plant VOCs induced by different stink bug species. This is most likely due to the lack of differences in VOC emissions from bolls damaged by *N. viridula* and *E. servus* detected in this study. These results are similar to those reported in a previous study, which indicated that VOC emissions were similar in response to feeding by three hemipteran species [17]. It has been suggested that sufficient specificity in VOC emissions may enable E-nose to detect particular host plant-pest interactions [23]. In a study investigating different types of damage to rice plants, it was demonstrated that E-nose could discriminate between damage by striped stem borer, *Chilo suppressalis* (Walker), and the rice brown plant hopper, *Nilaparvata lugens* (Stäl) [30]; however, these herbivores are from different feeding guilds (leaf chewer versus piercing sucking) and likely cause significant differences in VOC emissions due to differences in elicitors and physical damage inflicted during feeding [28, 31]. The results presented here indicate that feeding by different species of stink bugs does not result in sufficient

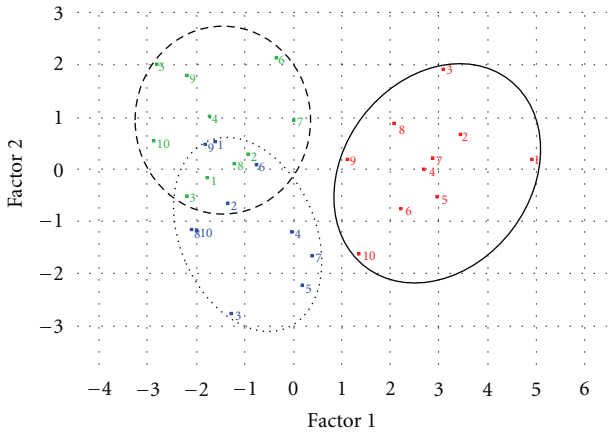

Figure 5: Canonical projection plot of E-nose sensor responses after training the E-nose to recognize VOC emissions from 10 unexposed (control) bolls (solid ellipsoid) and 10 bolls exposed to *Euschistus servus* (fine-dashed ellipsoid) and *Nezara viridula* (broad-dashed ellipsoid) following a 3 d exposure.

specificity in VOC emissions to allow E-nose to discriminate between species-specific sources of damage when the attacking herbivores have similar feeding modes.

While E-nose could not accurately differentiate between plant VOCs released in response to feeding by different species of stink bug, it was highly accurate when discriminating between damaged and undamaged bolls. Prolonged feeding exposure led to greater separation between treatments based on E-nose training sets. Training set data indicated that E-nose was capable of separating damaged and undamaged bolls with 100% accuracy. Identification of known (damaged or undamaged) samples based on E-nose training sets revealed that identification accuracy was consistently 10–15% less than training set accuracy over the course of the experiment. This is likely due to the modest separation of treatment groups as indicated by the low interclass M-distances. In previous research, it was demonstrated that E-nose was capable of identifying damaged bolls with 90% accuracy using bolls excised from plants and measured under laboratory conditions [24]. In this study, all tests were performed on intact plants under field conditions to more accurately demonstrate the potential of this technology as a nondestructive, in-field assessment tool for stink bug damage. In several cases, E-nose technology has been successfully applied to discriminate between healthy and pest-damaged plants [23, 24, 30], and, to our knowledge, this is the first study to demonstrate that E-nose technology is capable of accurately distinguishing between stink bug-damaged and undamaged bolls under field conditions based on a rapid sample of headspace VOC emissions.

While the results of this study are promising, it remains to be determined how the temporal dynamics in VOC emissions (and subsequently E-nose detection) observed from these feeding assays relate to natural variation in stink bug feeding dynamics. Under field conditions, cotton bolls are likely not injured by a single individual over 96 hr, but, rather, visited by one or multiple foraging stink bugs over time. As a result, VOC emissions under broader spatiotemporal variation in feeding dynamics may inhibit the ability to accurately distinguish damaged and healthy bolls using a predetermined training set. Whether induced VOC emissions reach significantly different levels from bolls exposed to much broader (and variable) spatiotemporal feeding dynamics from stink bugs and, subsequently, whether E-nose training sets are capable of differentiating those emissions remain to be determined. Preliminary data suggest that this may not be a major complication, as training sets based on 96 hr of feeding damage were successful in identifying stink bug damage in naturally infested fields (unpublished data); however, this is still under investigation.

The ability to differentiate pest injury based on brief samples of VOC emissions makes E-nose an attractive technology for monitoring stink bug feeding damage in cotton because it could potentially serve as a non-destructive monitoring tool, increase the accuracy of monitoring, and reduce the time and effort associated with current techniques. Further separation of treatment groups by E-nose could be achieved by optimizing sensor technology for the detection of stink bug induced VOCs. For example, sensor chemistries have been designed to respond specifically to VOCs induced by bark beetle attack [32]. In addition to specific sensor chemistries, research also suggests that longer durations of absorption and desorption cycles from E-nose may increase the ability to differentiate among treatments [23]. In this study, E-nose discrimination accuracy was achieved using standard sampling protocols that were not optimized specifically for detecting stink bug damage. Further sampling modifications and/or incorporation of VOC-specific sensor technology would likely improve the discrimination accuracy of E-nose. Nevertheless, the results of this study support the conclusion that E-nose is a promising technology for development of a rapid, nondestructive monitoring tool for stink bug feeding damage in cotton.

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