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PLANT PATHOLOGY & NEMATOLOGY

Detecting Cotton Boll Rot with an Electronic Nose

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ABSTRACT

A non-traditional disease of cotton, *Gossypium hirsutum* L., was first reported in South Carolina in 1999 and can be caused by opportunistic strains of the bacterium *Pantoea agglomerans* (Ewing and Fife). Unlike typical fungal diseases, bolls infected with *P. agglomerans* continue to appear normal externally, complicating early and rapid detection of diseased bolls. We examined the use of a commercially-available electronic nose (e-nose) to distinguish between liquid Luria Bertani (LB) cultures of *P. agglomerans* and another non-traditional, opportunistic bacterial boll pathogen, *Klebsiella pneumoniae* (Schroeter). We also examined whether the e-nose could accurately discriminate between *P. agglomerans* infected and non-infected bolls. The e-nose was trained to recognize headspace collections of volatiles emitted from treatments established in each experiment. Cross-validation of the training data sets indicated the smell prints of the LB medium and each species of bacteria cultured in the medium could be discriminated with 69% accuracy. However, upon testing samples of each treatment solution, only 49% of the samples were correctly identified. In the second experiment, cross-validation of the training set indicated the smell prints of *P. agglomerans*-infected and non-infected bolls at one and two weeks post-infection could be distinguished with 62.5% accuracy. However, upon testing the discrimination accuracy of the e-nose, < 30% of the test bolls were correctly classified. In light of the marginal performance of the Cyranose 320 in our experiments, vast improvements in the discriminatory accuracy of this particular e-nose is needed before it can be recommended and adopted as a cotton crop disease management tool.

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Boll or seed rot are general terms used to describe a number of diseases that affect seed and lint development of cotton plants, *Gossypium hirsutum* L. Approximately 170 different microorganisms, many of which are fungi, have been implicated as causative agents of boll rot diseases (Guthrie et al., 1994). Medrano and Bell (2007) identified opportunistic strains of the bacterium, *Pantoea agglomerans* (Ewing and Fife), as the primary causative agent of "South Carolina boll rot". This disease of cotton was first observed in South Carolina cotton fields during the summer of 1999 (Hudson, 2000; Jones et al., 2000), but has since been detected in fields throughout the southeastern Cotton Belt (Hudson, 2000; Toews et al., 2010).

Boll rot symptoms include brown necrotic coloration of developing lint and seed tissue which can be readily observed when infected bolls are cross-sectioned or when mature bolls open (Jones and Edmisten, 2001; Medrano et al., 2007). As the disease progresses, necrosis intensifies and death of tissue is imminent. Entirely diseased bolls may be aborted, particularly if bolls are infected during the early stages of development. However, both infected and non-infected locules may also occur within the same boll. In such cases, non-infected locules continue to develop and produce healthy lint and seed, whereas infected locules contain matted, discolored fiber and deteriorated seed (Mauney and Stewart, 2003).

In contrast to typical fungal boll-rot diseases, those infected with *P. agglomerans* continue to appear normal externally, complicating early and rapid detection of diseased bolls. Currently, standard pathogen isolation and culturing techniques are required to positively identify bolls infected with *P. agglomerans*. Early detection of infected bolls is deemed critical for minimizing the spread of this disease within and among cotton fields, but current detection methods are tedious, time-consuming, and relatively expensive. Thus, there is an urgent need to develop or identify alternative methods for rapid determination of whether bolls in fields are infected with *P. agglomerans* or other bacterial boll pathogens.

During the past decade, electronic nose (e-nose) technology has received considerable attention as a tool for rapidly detecting and discriminating volatile organic compounds (VOCs). Unlike gas chromatography or mass spectrometry, this technology is designed to characterize the odor profile of VOCs rather than quantify or identify individual compounds (Laothawornkitkul et al., 2008; Rock and et al., 2008). Instruments are typically equipped with an array of chemical sensors that act as odor receptors. Each sensor generates an electrical signal when exposed to an odor, and the overall composition of individual signals is regarded as the smell print or signature for that odor. Sensors are typically designed to detect a range of volatile compounds including alcohols, aldehydes, ketones, amines, terpenes, and hydrocarbons. Because microbes commonly release volatiles as metabolites, initial e-nose studies generally explored the potential of using e-noses to rapidly detect the presence of microbial agents responsible for food spoilage or contamination (Bastos and Magan, 2006; Canhoto et al., 2004; Magan and Evans, 2001; Tothill and Magan, 2003). E-noses also have been used with success in the bio-medical arena for microbial detection and identification (Dutta et al., 2002; Gardner et al., 1998).

Given that plants emit a bouquet of volatiles in response to herbivory (Paré and Tumlinson, 1999; Rodriguez-Saona et al., 2002), e-noses have recently received attention as tools for monitoring insect feeding damage in several cropping systems, including cotton (Degenhardt et al., 2012; Henderson et., 2010). As plants also emit volatiles in response to microbial infection (Cardoza et al., 2002; Croft et al., 1993), e-nose technology may provide an alternative and rapid means for detecting diseased cotton bolls. However, this particular use of an e-nose has not been explored extensively, and the utility of e-noses in crop disease management remains unclear. The primary objective of this study was to determine whether a commercially-available e-nose could be used to discriminate between *P. agglomerans*-infected and non-infected bolls. As part of that objective, we also examined the ability of the e-nose to discriminate between liquid cultures of *P. agglomerans* and *Klebsiella pneumoniae* (Schroeter), another known opportunistic bacterial pathogen of bolls (Medrano et al., 2009b).

MATERIALS AND METHODS

A portable e-nose (Cyranose 320, Cyrano Sciences Inc., Pasadena, CA, USA) equipped with 32 carbon composite sensors was used in the study. Each sensor is constructed of an alumina substrate coated with a thin film of conductive carbon composite material. Two electrical leads are connected to each sensor film, essentially creating a chemiresistor. When exposed to chemical vapors, the film on each sensor swells causing an increase in resistance. The change in resistance of each sensor is measured and the overall composition of individual sensor responses represents the smell print or signature for that odor. Before the Cyranose 320 can be used to detect or identify an odor, it has to be trained to recognize the odor(s) of interest. Operation of the Cyranose 320 during the training and subsequent identification processes is simplified by onboard data processing software and proprietary pattern recognition algorithms. Additionally, the e-nose can be interfaced with a computer using the supplied PCnose software to set up instrument sampling and analytical parameters, and to view sensor data and analytical results.

The *P. agglomerans* isolate originated from diseased locules of cotton bolls collected from South Carolina, USA (Medrano and Bell, 2007). The isolate was classified by those authors as *P. agglomerans* based on standard carbon utilization and enzyme production testing, fatty acid profiling, and 16S ribosomal DNA sequence phylogenic analysis (Medrano and Bell, 2012). Currently, there is debate regarding the proper species designation of the isolate based on gyrB sequence identity; however, the original classification of the isolate as *P. agglomerans* is maintained in this study. The *K. pneumoniae* isolate, identified by the Laboratory of Healthcare Associated Infection at the Centre for Infections (HPA ref. no. H0 5186 0092), was recovered from a feral southern green stink bug collected near College Station (Medrano et al., 2009a). Derivatives of each isolate were spontaneously generated to be resistant to rifampicin (Rif) (Medrano and Bell, 2007). The Rif-resistant strains of *P. agglomerans* and *K. pneumoniae* were designated as SC 1-R and Kp 5-1R, respectively (Medrano et al., 2007), and were maintained on Luria Bertani (LB) medium (Difco, Detroit, MI) amended with Rif (100 µg/ml). The Rif-resistant derivatives of each bacterial strain were used in all subsequent experiments.

The e-nose was trained on five separate occasions to recognize the VOCs emitted from *P. agglomerans* and *K. pneumoniae* strains cultured in LB liquid medium. For each training session, suspensions of each bacterium were prepared from 18-h cultures in 100 ml of LB medium amended with Rif (100 µg/ml). Each suspension was adjusted spectrophotometrically (A_{600}=1.0) to yield a final concentration of 10^{11} colony forming units (CFU) per ml. The respective growth medium served as a control to account for volatiles emitted by the amended LB medium. Aliquots (2 ml) of each bacterial and control solution were pipetted into separate 20-ml glass scintillation vials to yield a total of 31 to 41 vials for each solution. Eleven vials of each solution were randomly selected and used to train the e-nose. The remaining 20 to 30 vials were set aside to test the discrimination accuracy of the e-nose. Each training vial was sealed with Parafilm® tape for 4 h prior to taking sample readings. One sample reading was taken from each vial by inserting the snout of the e-nose through a small puncture in the Parafilm® tape and positioning the end of the snout ≈1 cm above the surface of the solution. The first sampled vial of each treatment solution was used to initially "exercise" the e-nose and the remaining10 vials of each solution were used to train the e-nose using the settings detailed in Fig. 1. All of the treatment solutions and subsequent vials were prepared and sampled in a class II type biological safety cabinet (Nuare, Plymouth, MN) or sterile laminar airflow hood (Baker Co., Inc, Sanford, ME) maintained at 23 ± 2°C.

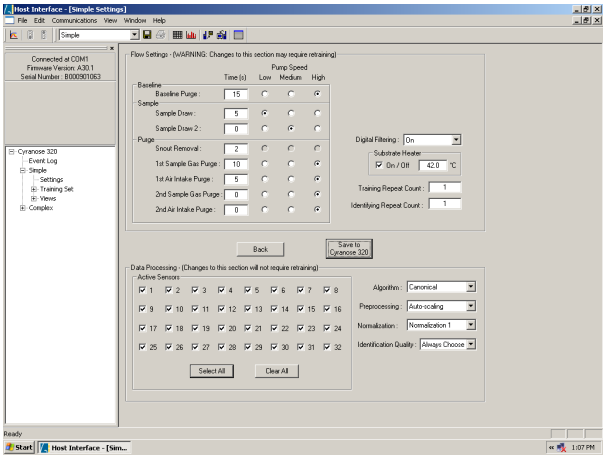

Figure 1. Cyranose 320 detection and processing settings used to train and test the accuracy of the e-nose in distinguishing Luria Bertani liquid medium (control) and two opportunistic strains of bacteria, *Pantoea agglomerans* and *Klebsiella pneumoniae,* cultured in the medium.

Data generated from each training session were analyzed using the built-in signal and data processing software in conjunction with the PCnose software loaded on a computer. The software uses Principal Component Analysis (PCA) to diagnose outliers and convert the useful information into several principal components. A supervised algorithm, canonical discriminant analysis (CDA), was selected to build a model and predict unknowns. Upon cross-validation of the data, two tables were generated to estimate the robustness of the training set and to determine the accuracy of the e-nose in discriminating among the classes of odors. The cross validation table provided the overall classification accuracy and frequency of samples that were correctly and incorrectly identified within each class. The interclass Mahalanobis distance (M-distance) table indicated the degree of separation of smell prints among treatment classes. Additionally, a PCA projection plot was generated to help visualize the degree of separation between smell prints within and among classes.

After each training session, the accuracy of the e-nose in discriminating between the control and two bacterial strains was confirmed on the remaining 20 to 30 vials of each treatment solution. Thus, the training and test samples originated from the same stock suspensions and a total of five test trials were conducted. As in the training sessions, test vials were sealed with Parafilm® tape for 4 h before sampling the headspace of test vials. The identification quality parameter of the e-nose was set to "always choose" and the e-nose classification was recorded after each sample exposure. The percentage of correctly identified vials within each treatment class was used to determine the overall classification accuracy of the e-nose.

Based on the hypothesis that bolls infected with *P. agglomerans* emit a unique odor in response to infection, or from the progression of disease, the ability of the e-nose to detect infected bolls was examined under greenhouse conditions. The e-nose was trained on two separate occasions to recognize the volatiles emitted from *P. agglomerans*- and non-infected bolls. For each training session, suspensions of *P. agglomerans* in a PO_4 buffer solution (0.1 mol l^{-1}, pH of 7.1) were prepared from 18-h cultures and adjusted spectrophotometrically (A_{600} = 0.5). Twenty bolls on greenhouse-grown plants (Deltapine 0935 B2RF) were inoculated 13-15 days post-anthesis with the bacterial suspension. A control group of 20 bolls were inoculated with PO_4 buffer solution. Solutions were injected, using a 31 gauge needle (ca. 5 mm depth), into the center of the

suture of two opposing locules (2 x 20 µl) that had previously been surface-sterilized with 95% ethanol. Thus, each boll injected with the bacterial solution received a final dose of ≈$2x10^3$ CFUs and each control boll received 40 µl of the PO^4 buffer solution.

One week after inoculation, *P. agglomerans*-inoculated and control bolls were encased in 4-oz Whirl-Pak® sample bags (Nasco, Ft. Atkinson, WI) for 1 h to collect released volatiles. The snout of the e-nose was inserted into each bag through a small slit made with a razor blade to characterize the smell print of volatiles released into the headspace of bags. One boll from each treatment group was used to initially "exercise" the e-nose and 10 randomly-selected bolls from each treatment group were used to train the e-nose. One sample reading was taken from each boll using the same e-nose analytical parameter previously described (Fig. 1), except sensors 5, 6, 23, and 31 were deactivated due to their sensitivity to moisture. After all of the bolls were sampled with the e-nose, the bags were immediately removed from bolls. Headspace sample readings were taken again with the e-nose two weeks post-inoculation using the same bolls and e-nose sampling procedures described above. Thus, the e-nose was trained to recognize the volatiles emitted from 10 infected and 10 non-infected bolls at one and two weeks post-inoculation. Upon completion of each training session, sensor data were cross-validated as described in the laboratory experiment.

Once trained, the accuracy of the e-nose in discriminating between *P. agglomerans*-infected and non-infected bolls at one and two weeks post-inoculation was tested on another set of bolls (10 to 13 bolls per treatment) using the exact same inoculation and e-nose sampling procedures as in the training sessions. The identification quality parameter of the e-nose was set to "always choose", and the e-nose classification was recorded after each sample exposure. The percentage of correctly identified bolls within each treatment class was used to determine the overall classification accuracy of the e-nose. Three individual tests were conducted using different sets of bolls and newly prepared bacterial/buffer solutions each time. Upon completion of each test, all sampled bolls were dissected to confirm the presence or absence of disease symptoms. Additionally, the air temperature in the greenhouse was monitored hourly with a HOBO datalogger (Onset Corp., Pocasset, MA) during the training and testing phases of the experiment to ensure environmental conditions were similar between experimental phases and among experimental trials. Hourly air temperatures in the greenhouse ranged from 24° to 41°, which is reflective of air temperatures encountered in Texas during the respective time of year.

RESULTS AND DISCUSSION

Based on internal cross-validation of sensor data, the classification accuracy obtained during each of the five independent laboratory training sessions ranged from 40 to 97%, with an average of 69% (Table 1). On average, the e-nose demonstrated the capacity to discriminate the smell print of the LB medium (control) from the smell prints produced by each of the two bacteria species cultured in the medium with 90% accuracy (Table 1). Correspondingly, 60 and 72% of the *P. agglomerans* and *K. pneumoniae* samples, respectively, were correctly classified. The majority of *P. agglomerans* samples that were misidentified by the e-nose were classified as *K. pneumoniae*. In comparison, the misidentified *K. pneumoniae* samples were equally classified as the control or *P. agglomerans* treatment. Notably, all of the misidentified control samples were classified as *K. pneumoniae* (Table 1).

Table 1. Cross-validation average of sensor data obtained by training the Cyranose 320 to recognize the smell print of volatiles emitted from 2-ml aliquots of Luria Bertani (LB) liquid medium (control) and two opportunistic species of bacteria cultured in the medium (10 samples per treatment). Values in parentheses indicate the range of accuracy over the five training sessions.

Trained as	Identified as		
	Control	*Pantoea agglomerans*	*Klebsiella pneumoniae*
Control	9.0 (7-9)	0.0 (0)	1.0 (1-3)
Pantoea agglomerans	0.4 (0-2)	6.0 (3-9)	3.6 (1-7)
Klebsiella pneumoniae	1.4 (0-3)	1.4 (0-3)	7.2 (5-10)

Overall cross-validation average over the five training sessions: correct = 69% (40-97%); incorrect =31% (3-60%).

Observation of the PCA projection plots generally revealed distinct grouping of smell prints with some overlap among treatment classes (Fig. 2). Inspection of the interclass M-distance values also indicated modest overlap of smell prints among treatments (Table 2). In general, values > 6 signify good separation of smell prints between paired classes with larger values indicating greater separation, while values < 5 imply overlap of classes (Anonymous 2000). Based on these criteria and the observed

range of interclass M-distance values (Table 2), there was good separation among paired treatments in some of the training sessions, particularly between the control and each species of bacteria.

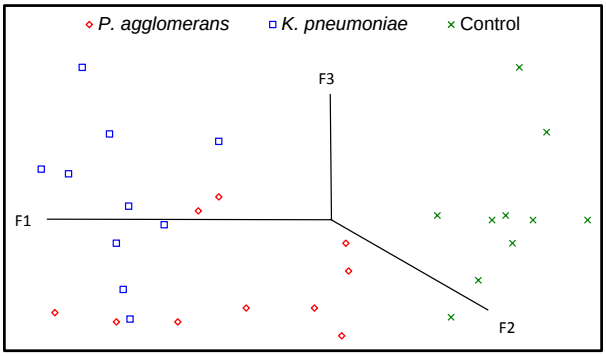

Figure 2. Reconstructed Principal Component Analysis projection plot of smell prints obtained by training the Cyranose 320 to recognize and differentiate headspace collections of volatiles emitted from vials containing 2-ml aliquots of Luria Bertani (LB) liquid medium (control) and two opportunistic species of bacteria, *Pantoea agglomerans* and *Klebsiella pneumoniae*, cultured in the medium (10 samples per treatment). Bacterial suspensions were initially prepared from 18-h cultures in 100 ml of LB medium amended with Rifampicin (100 µg/ml), and each suspension was adjusted spectrophotometrically (A_{600}=1.0) to yield a final concentration of 10^{11} colony forming units per ml.

Table 2. Average Interclass Mahalanobis distance (M-distance) values indicating the degree of separation between smell prints of paired classes of odors emitted from 2-ml aliquots of Luria Bertani (LB) liquid medium (control) and two opportunistic species of bacterial cultured in the same medium.

Paired classes of odors	M-distance values (range of values)[z]
Control – *Pantoea agglomerans*	3.5 (1.5 – 5.4)
Control – *Klebsiella pneumoniae*	3.4 (0.7 – 7.9)
P. agglomerans – *K. pneumoniae*	2.1 (1.1 – 3.6)

[z] Values > 6 indicate good separation between classes with larger values indicating greater separation; values < 5 indicate overlap of classes.

Considering the variable and modest level of classification accuracy obtained during the training sessions, we anticipated the actual discrimination accuracy would also be marginal and vary among test trials. Indeed, the discrimination accuracy obtained over the five test trials averaged 49% with a range of 37 to 80% (Table 3). On average, the e-nose correctly identified 59% of the control samples whereas only 37 and 51% of the *P. agglomerans* and *K. pneumoniae* samples, respectively, were accurately classified (Table 3). Thus, as indicated in the training sessions, the e-nose was able to discriminate between the control and each species of bacteria with greater accuracy than between species of bacteria.

Based on the training and test results, our findings indicate both species of bacteria cultured in LB medium emit distinct odors, which the Cyranose 320 can be trained to recognize and distinguish with a marginal level of accuracy. Although the overall discrimination accuracy observed during each test trial was considerably lower than the cross-validation accuracy predicted in the respective training session, the levels of accuracy in the test and respective training trials followed similar patterns. However, given that each of the test and training sessions were performed identically on the same respective stock solutions in our experiment, explanations for the discrepancy in discrimination accuracy and variability among trials cannot be provided at this time. Interestingly, nearly identical results were obtained using a simple growth medium (M-9) as the culture and test medium (C. Suh, unpublished data). Furthermore, similar reductions in discrimination accuracy with the Cyranose 320 between the training and testing results have been reported (Degenhardt et al., 2012; Suh et al., 2011).

Given that the discrimination accuracy of the e-nose is dependent on the training process,

Table 3. Accuracy of the Cyranose 320 in distinguishing headspace collections of volatiles emitted from 2-ml aliquots of Luria Bertani (LB) liquid medium (control) and two opportunistic species of bacteria, *Pantoea agglomerans* and *Klebsiella pneumoniae*, cultured in the medium.

Treatment	% of samples (*n*) identified correctly					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
P. agglomerans	37 (*30*)	55 (*20*)	50 (*20*)	5 (*20*)	40 (*20*)	37
K. pneumoniae	33 (*30*)	58 (*19*)	40 (*20*)	25 (*20*)	100 (*20*)	51
Control	40 (*30*)	25 (*20*)	30 (*20*)	100 (*20*)	100 (*20*)	59
Overall accuracy	37	46	40	43	80	50

modifications to the e-nose parameter settings and sampling procedures during the training phase may have resulted in improved separation of smell prints among treatments. Additionally, incorporation of external data clustering methods and analysis techniques during the training phase also may have improved the classification accuracy of the e-nose. For instance, Dutta et al. (2002) examined the ability of the Cyranose 320 to distinguish six species of bacteria commonly responsible for eye infections. Those authors reported the e-nose was able to discriminate among bacterial species in culture with 74% accuracy using the standard linear PCA method. However, with the addition of complex nonlinear data analysis techniques, the e-nose was capable of discriminating the six species of bacteria with 98% accuracy. However, it should be noted this was the predicted level of accuracy obtained during a one time classification exercise and tests to confirm the actual discrimination accuracy of the e-nose were not conducted. In our study five separate training and test sessions were performed identically, yet the discrimination accuracy of the e-nose varied considerably among sessions. Based on the variable and marginal performance of the Cyranose 320 in our experiment, it is apparent that the discrimination accuracy of this e-nose needs to be greatly improved before it can be recommended as a tool for discriminating species of opportunistic bacterial cotton pathogens in culture.

Although the results from our laboratory study suggested the e-nose could not accurately discriminate among two pure cultures of bacteria in growth media, the profile of volatiles or odor emitted by *P. agglomerans*-infected bolls may be completely different than those emitted from healthy bolls. Based on the overall cross-validation accuracy of the first (62.5%) and second training session (57.5%), the first training session was retained and used to discriminate boll samples in subsequent tests. As such, only results of the first training session are presented and discussed. Upon completion of the training session, dissection of bolls confirmed that all of the *P. agglomerans*-infected bolls used to train the e-nose displayed advanced disease symptoms (Fig. 3a). Correspondingly, all of the control bolls appeared healthy and free of infection; only a slight discoloration of lint immediately surrounding each inoculation site was observed (Fig. 3b).

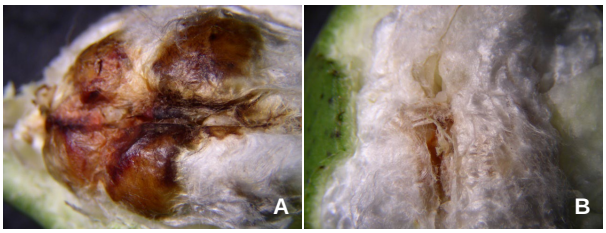

Figure 3. Typical internal effects observed in cotton bolls two weeks after inoculation with a *Pantoea agglomerans* suspension (A) or a PO_4 (0.1 mol l^{-1}, pH=7.1) control solution (B). Suspensions of *P. agglomerans* in a PO_4 buffer solution were initially prepared from 18-h cultures and adjusted spectrophotometrically (A_{600} = 0.5). Each boll injected with the bacterial solution received a final dose of ≈$2x10^3$ colony forming units and each control boll received 40 µl of the PO^4 buffer solution.

Cross-validation of the training data indicated the e-nose was capable of discriminating the smell prints of *P. agglomerans*- and non-infected bolls at one- and two-weeks post-inoculation with 62.5% accuracy (Table 4). However, only 30 and 50% of infected and non-infected bolls, respectively, at one week post-inoculation were correctly classified (Table 4). In comparison, 80 and 90% of respective bolls were correctly classified at two weeks post-inoculation. Thus, the predicted discrimination accuracy of the e-nose appeared to improve as the duration of time between infection and sampling increased. However, observation of the Interclass M-distance values and PCA projection plot indicated considerable overlap of smell prints among all four treatment classes (Table 5; Fig. 4).

Table 4. Cross-validation of sensor data obtained by training the Cyranose 320 to recognize the smell print of volatiles emitted from 10 *Pantoea agglomerans*- and 10 non-infected (control) cotton bolls one and two weeks after inoculation.

Trained as	Identified As			
	Control wk1	Bacteria wk1	Control wk2	Bacteria wk2
Control wk1	5	1	3	1
Bacteria wk1	5	3	1	1
Control wk2	0	0	9	1
Bacteria wk2	0	0	2	8

Overall cross validation accuracy: correct=62.5%; incorrect=37.5%.

Table 5. Interclass Mahalanobis distance (M-distance) values indicating the degree of separation between paired classes of smell prints obtained from cotton bolls one and two weeks after being inoculated with a suspension of *Pantoea agglomerans* or a control PO_4 buffer solution.

Paired classes of odors	M-distance values[z]
Control week 1 – bacteria week 1	2.6
Control week 1 – control week 2	1.6
Control week 1 – bacteria week 2	1.4
Bacteria week 1 – control week 2	3.4
Bacteria week 1 – bacteria week 2	2.9
Control week 2 – bacteria week 2	1.2

[z] Values > 6 indicate good separation between classes with larger values indicating greater separation; values < 5 indicate overlap of classes.

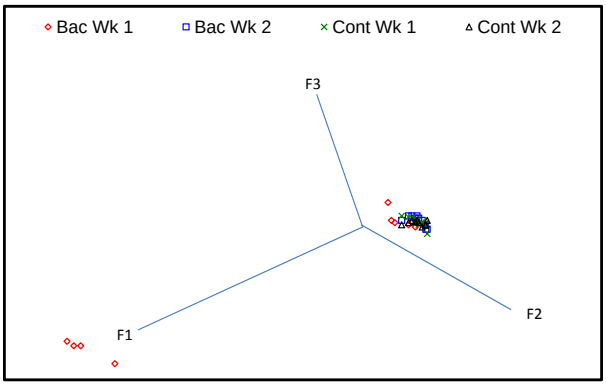

Figure 4. Reconstructed Principal Component Analysis projection plot of the smell prints obtained while training the Cyranose 320 to recognize volatiles emitted from 10 *Pantoea agglomerans*- and 10 non-infected cotton bolls (control) one and two weeks after inoculation. Suspensions of *P. agglomerans* in a PO_4 buffer solution (0.1 mol l^{-1}, pH=7.1) were initially prepared from 18-h cultures and adjusted spectrophotometrically (A_{600} = 0.5). Each boll injected with the bacterial solution received a final dose of ≈2x10^3 colony forming units and each control boll received 40 µl of the PO^4 buffer solution.

Upon testing the accuracy of the e-nose in discriminating *P. agglomerans*-infected and healthy bolls at one and two weeks post-inoculation, <30% of the bolls sampled in each test trial were correctly identified (Table 6). Although none of the control bolls at one week post-inoculation were correctly classified, it should be noted that all of these bolls were misclassified as control bolls two weeks post-inoculation. Also, none of the *P. agglomerans*-infected bolls at one week post-inoculation were correctly classified (Table 6). However, as observed in the training session, there was a considerable improvement in the discrimination accuracy of the e-nose when bolls were sampled at two weeks post-inoculation. On average, 90% of the control bolls were correctly classified; however, only 20% of the infected bolls were correctly classified. The improved discrimination accuracy at two weeks post-inoculation may be related to the progression of the disease over time. Medrano et al. (2009a) examined the seeds and lint of bolls of various ages (one, two, and three weeks after flowering) infected with *P. agglomerans* strain SC 1-R, and concluded that the expression of disease symptoms largely depended on the age of bolls at time of infection and the elapsed period of time following infection. Those authors showed that bolls examined one week after infection, regardless of boll age, had discolored seed but the fiber appeared asymptomatic; whereas bolls inspected two and three weeks after infection displayed brown necrotic coloration of developing lint and seed tissue. Thus, it is likely that volatiles released from *P. agglomerans*-infected and non-infected bolls were too similar for the e-nose to distinguish at one week post-infection. Additionally, it is unclear whether the volatiles detected by the e-nose were directly emitted from the pathogen or related to emissions from the plant in response to infection and/or progression of disease. Perhaps, identification of the VOCs emitted from diseased and healthy bolls over time and subsequent incorporations of sensors specific for those volatiles would greatly enhance the performance of the e-nose.

Table 6. Accuracy of the Cyranose 320 in distinguishing headspace collections of volatiles emitted from *Pantoea agglomerans*- and non-infected cotton bolls (control) one and two weeks after inoculation.

Treatment	% of samples identified correctly			
	Trial 1	Trial 2	Trial 3	Average
Control wk 1	0	0	0	0
Control wk 2	100	100	70	90
Bacteria wk 1	0	0	0	0
Bacteria wk 2	15	8	36	20
Overall average	29	27	27	28

While the performance of the e-nose in discriminating among treatment odors in both of our experiments was marginal at best, advancements in sensor technology have greatly improved the sensitivity and discrimination accuracy of e-noses. Although the VOCs emitted from cultured bacteria or from healthy and diseased bolls were not identified in

our study, it may be possible to develop an e-nose with sensors specifically designed to detect VOCs of interest. Perhaps the development and evaluation of e-noses with sensors specific for volatiles emitted by boll rot pathogens may yield more promising results. As such, the ability to rapidly and accurately identify diseased plants or distinguish species of microorganisms based solely on emissions of volatiles makes e-nose technology an attractive tool for crop disease management. Our results demonstrate that the Cyranose 320 can be trained to recognize and discriminate the odors emitted from diseased bolls and bacteria in culture. However, the discrimination accuracy of the Cyranose 320 needs to be greatly improved before this particular e-nose can be recommended and adopted as a crop disease management tool.

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DISCLAIMER

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