DETECTING BOLL ROT OF COTTON WITH AN ELECTRONIC NOSE

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Abstract

Early and rapid detection of diseased cotton bolls is often complicated by the absence of external symptoms on infected bolls. A preliminary study was initiated in 2009 to examine the potential of using an electronic nose (E-nose) to detect volatiles emitted from bolls infected with the opportunistic bacterium *Pantoea agglomerans*. Bolls on greenhouse-grown plants were inoculated at 2 wk postanthesis with a suspension of *P. agglomerans* (2×10^3 colony forming units) or sterile water only (control group). An E-nose (Cyranose 320) was trained to recognize the "smell print" of volatiles emitted from bolls two weeks after inoculation. Canonical projection plots and principal component analysis of the smell prints obtained during the training session showed distinct separation between *P. agglomerans*- and non-infected bolls. Cross-validation of the sensor data indicated the E-nose was 90% accurate in discriminating between these treatment groups. However, upon testing the bolls used to train the E-nose, only 66% of the bolls were correctly identified. Despite this marginal performance, it is anticipated that a higher level of accuracy could be achieved with minor adjustments to the training procedures as well as E-nose detection and data processing settings. Based on these preliminary results, the E-nose shows promise as a non-destructive screening tool for rapidly detecting diseased bolls, and continued investigation of this technology is warranted.

Introduction

The bacterium *Pantoea agglomerans* has recently been identified as the causative agent of the boll disease commonly referred to as "South Carolina Boll Rot" (Medrano and Bell 2007). First observed in South Carolina cotton fields (Edmisten 1999), this disease has since been detected in fields throughout the southeastern Cotton Belt (Hudson 2000). Diagnostic symptoms include brown necrotic coloration of developing lint and seed tissue which can be readily observed when infected bolls are cross-sectioned or when bolls open. Early and rapid detection of this disease, however, is complicated by the absence of external symptoms on infected bolls.

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, this technology is designed to characterize the odor profile of VOCs rather than quantify individual compounds (Laothawornkitkul 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", "fingerprint", or "signature" for that odor.

Microbial agents such as bacteria and fungi emit a range of volatile compounds including alcohols, aldehydes, ketones, amines, terpenes, and hydrocarbons (Korpi et al. 1997). Several bacterial and fungal species have also been shown to produce a unique blend of volatiles which can be used as fingerprints to determine the identity of the microbe (Magan and Evans 2001, Tothill and Magan 2003, Canhoto et al. 2004). The objective of our study was to determine the potential of using an E-nose to rapidly discriminate between *P. agglomerans*-infected and non-infected cotton bolls. Presented herein are results from a preliminary trial conducted in 2009.

Materials and Methods

Bolls on greenhouse-grown plants (Fibermax 966) were inoculated 13-15 days postanthesis with either a suspension of *P. agglomerans* rifampicin (Rif)-resistant mutant (Sc 1-R) or sterile water (control group). Sc 1-R suspensions in sterile water were prepared from 18-h cultures and adjusted spectrophotometrically (A_{600} = 0.5). Bacterial

suspensions were injected with a 31 gauge needle (ca. 5 mm depth) at a final concentration of $2x10^3$ colony forming units (CFU) into the center of the suture of two opposing locules that had previously been surface-sterilized with 95% ethanol. Control bolls were injected with 10 µl of sterile water using the same technique. Two weeks after inoculation, bolls on plants were encased in 4-oz Whirl-Pak® sample bags (Nasco, Ft. Atkinson, WI) for 1 h to collect released volatiles. The snout of a commercially-available E-nose (Cyranose 320, Smiths Technology, Watford, UK) was inserted into each bag through a small slit made with a razor 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 five bolls from each treatment group were used to train the E-nose using the detection and processing parameters described below (Fig. 1.). Once the E-nose was trained to recognize the smell print of volatiles released from bolls inoculated with and without *P. agglomerans*, the original bolls used to exercise and train the E-nose were sampled again to test the accuracy of the E-nose in discriminating between infected and non-infected bolls.

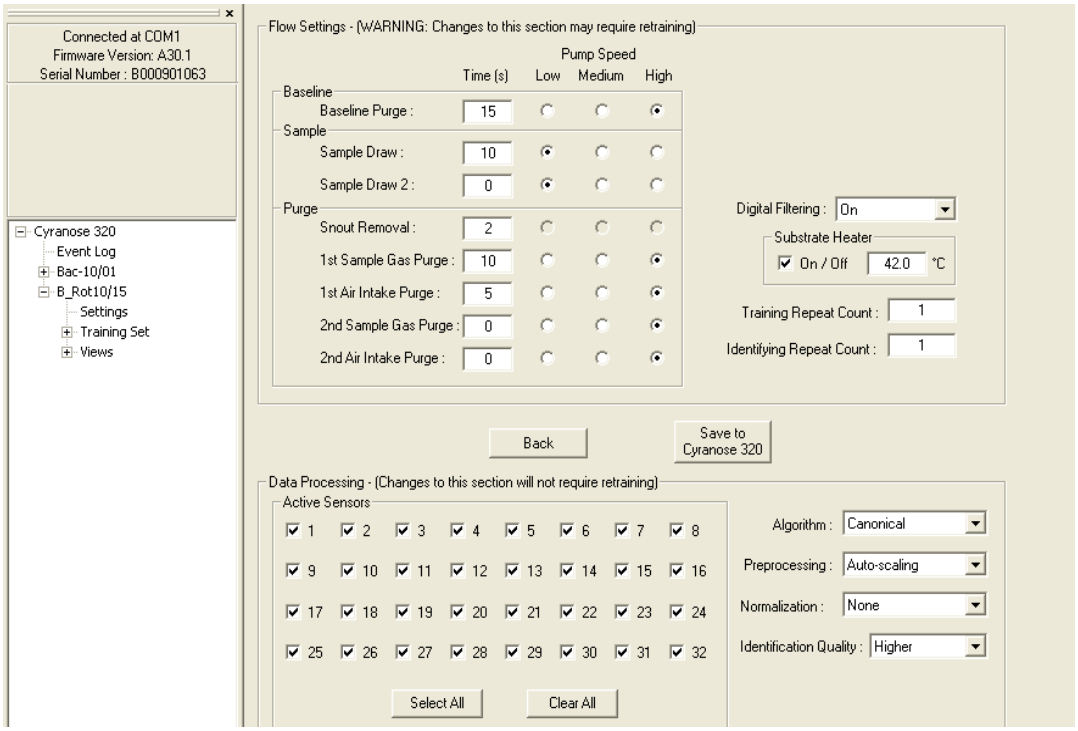

Figure 1. Detection and processing settings used on the Cyranose 320 while training and testing the E-nose to recognize volatiles emitted from cotton bolls inoculated with or without *P. agglomerans*.

Results and Discussion

Cross-validation of the sensor data obtained during the training session indicated the E-nose was 90% accurate in discriminating between the smell prints of *P. agglomerans*- and non-infected bolls (Table 1). Although the volatiles emitted from infected and non-infected bolls were not identified in this study, projection plots of the canonical and principal component analysis confirmed that the smell prints of infected and non-infected bolls were distinctly different (Fig. 2). These findings suggest diseased bolls emit a unique odor, or at least an odor that is considerably different than that produced by control bolls, and that the E-nose is capable of detecting these differences.

Table 1. Cross-validation of sensor data obtained while training the Cyranose 320 to recognize the smell print of volatiles emitted from *P. agglomerans*- and non-infected cotton bolls two weeks after infection.

		Identified as:	
		P. agglomerans-infected	Non-infected
Trained as:	*P. agglomerans*-infected	5	0
	Non-infected	1	4

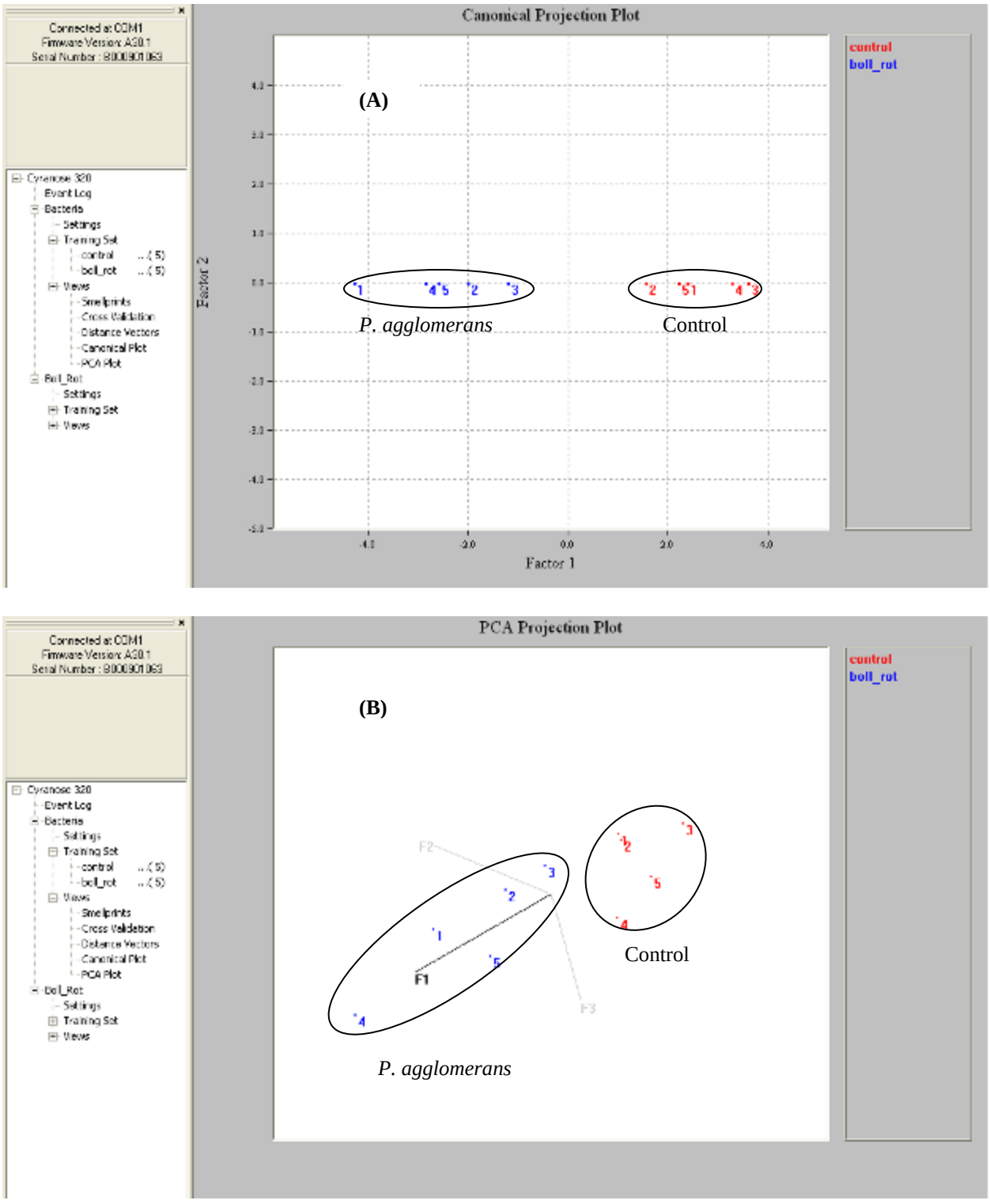

Figure 2. Canonical (A) and principle component analysis (B) projection plots of the smell prints obtained while training the Cyranose 320 to detect and recognize volatiles emitted from *P. agglomerans*- and non-infected cotton bolls two weeks after inoculation.

Upon testing the original bolls used to exercise and train the E-nose, five of the six *P. agglomerans*-infected bolls were correctly identified as “infected”, while only three of the six control bolls were correctly identified as “non-

infected". This lower than expected accuracy may have been attributed to the detection and processing settings used during the training and testing phases, as well as the methodologies used during the training and testing phases. Since this preliminary trial, tremendous knowledge has been gained on the use and limitations of the E-nose. Consequently, it is anticipated that a higher level of accuracy could be achieved with minor adjustments to the detection and data processing parameters as well as to the training and testing methodologies. Nevertheless, our results demonstrate the Cyranose 320 is capable of discriminating between *P. agglomerans*-infected and non-infected bolls, and continued investigation of this technology is warranted.

Summary

Although the volatiles emitted from *P. agglomerans*-infected and non-infected bolls were not identified in this study, our results suggest infected and non-infected bolls emit distinctly different odors. More importantly, our findings indicate the Cyranose 320 can be trained to detect and recognize these odors, and with some degree of accuracy, is capable of discriminating between *P. agglomerans*-infected and non-infected bolls. As such, this technology shows considerable potential as a non-destructive screening tool for rapidly detecting diseased bolls.

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