Bacterial Wetwood Detection in *Fagus grandifolia* and *Prunus serotina* Sapwood using a Conducting Polymer Electronic-nose Device

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***Abstract*— New electronic gas-detection methods were developed and tested for the diagnosis of bacterial wetwood disease in *Fagus grandifolia* (American beech) and *Prunus serotina* (black cherry) using a Conducting Polymer (CP)-type electronic nose (e-nose), the Aromascan A32S, based on detection of headspace volatile microbial and plant metabolites derived from sapwood. Diagnostic application-specific aroma signature patterns (profile databases), derived from e-nose analysis of known healthy and wetwood-infected sapwood cores of each hardwood tree species, were used to develop an aroma database library. The library was used as known references to screen aroma profiles of sapwood cores for the presence of wetwood in unknown samples. The Aromascan A32S e-nose effectively distinguished between headspace volatiles from tree cores of different wood types, correctly identifying them at frequencies ranging from 92.3-100%. Principal Component Analysis (PCA) and Quality Factor (QF) statistical values indicated the relatedness and significance of differences between headspace volatiles from aroma classes of each sample type. Significant differences were found between the aroma profiles of healthy vs. wetwood-infected sapwood of American beech and black cherry, and greater differences occurred between headspace wood volatiles released from healthy sapwoods of the two species.**

Keywords-artificial olfaction; disease diagnosis; electronic aroma detection; volatile organic compounds

I. INTRODUCTION

Bacterial wetwood is an important wood disease that is common primarily in bottomland hardwood tree species of seasonally-flooded forests of the lower Mississippi Delta region [1]. Wetwood bacteria are soil-inhabiting, facultative anaerobes that are taken up by trees via the roots in the water of the transpiration stream (xylem elements) where they attack the middle lamellae between wood cells and fibers in sapwood and heartwood in the main bole (trunk) by releasing pectolytic enzymes (pectolases) [2]. These enzymes damage the structural integrity of the wood and cause radial and lateral separations of wood fibers, resulting in cracks and splits (defects) in processed lumber during kiln drying [3]. The damage to processed kiln-dried lumber results in economic losses due to these defects that reduce lumber grade quality and the value of the lumber for commercial sale [4]. An electronic-nose (e-nose) instrument was first utilized to detect bacterial wetwood in the sapwood of *Populus deltoides* (cottonwood) plantation trees and to identify host plants of the disease [5]-[7]. The current research focused on the detection of wetwood in two hardwood species, *Fagus grandifolia* (American beech) and *Prunus serotina* (black cherry), that occasionally become infected with wetwood bacteria in low-lying, seasonally-flood bottomland forest sites with anaerobic or water-saturated soils.

The objectives of this study were to 1) determine if an electronic-nose (e-nose) device, the Conducting Polymer (CP)-type Aromascan A32S e-nose, has the capability of detecting bacterial wetwood disease in the sapwood of two hardwood species, including *Fagus grandifolia* and *Prunus serotina*, and to 2) evaluate the effectiveness of this e-nose method to distinguished between the wood types (either healthy or uninfected and wetwood-infected sapwood) of these two species based on aroma signatures of Volatile Organic Compounds (VOCs) present in sample headspace derived from sapwood cores.

This paper is composed of an introduction to the wetwood problem in section 1, followed by e-nose experimental methods used in section 2, describing the specific materials and methods used in associated with e-nose run and analytical procedures, followed by results in section 3 that provide details of experimental research results and findings for CP e-nose analyses of wetwood samples, including sensor outputs, aroma map, and QF analysis of PCA data. Discussion and conclusions, based on the e-nose experimental results, are presented in section 4 to summarize the significance of findings and new discoveries resulting from this research.

II. MATERIALS AND METHODS

A. Collection and storage of sapwood core samples

Sapwood increment core samples (5 mm diameter × 5 cm length) were collected in spring from freshly-harvested *Fagus grandifolia* (American beech) and *Prunus serotina* (black cherry) logs deposited in piles within the Andersen Tully log and lumber mill yard at Vicksburg, Mississippi. A minimum of two core samples were extracted from the boles of at least twenty logs of each species using a Haglöf tree increment borer (Forest Suppliers, Inc., Jackson, MS) and placed into 14.8 mm glass vials. Increment cores were collected from healthy and bacterial wetwood-infected logs.

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Wetwood logs were identified by the combined presence of water soaking, dark brown discoloration of sapwood tissue, and the acetic smell associated with this disease. Woody cores in all cases were frozen within 14.8 ml glass vials stored at -20 °C in long-term storage and thawed immediately prior to sample analysis. Cores that became desiccated in storage were rehydrated by soaking in sterile distilled water for 15 min followed by blotting on Chemwipe tissue paper to remove excess free moisture immediately prior to e-nose analysis.

B. Sample preparation and prerun procedures

Sapwood core samples in 14.8 ml glass vials were uncapped and placed into a 500 ml glass headspace sampling bottle fitted with reference air, sampling, and exhaust ports on a polypropylene bottle cap. Reference air entered the sampling bottle through a 3 mm polypropylene tube extending to the bottom of the sampling bottle. The sampling bottle was held at a constant air temperature of 25 ° C. The sampling bottle was purged with filtered, reference air (relative humidity ⩽ 4%) for 2 min prior to building headspace. The sampling bottle was sealed and volatiles from the samples were allowed to build headspace and equilibrate for 30 min prior to each run. Prerun tests were performed as needed to determine sample air Relative Humidity (RH) compared with that of reference air. The sampling bottle cap and exhaust port were opened between runs to purge the previous sample with preconditioned reference air.

C. Instrument configuration and run parameters

The Aromascan A32S Conducting Polymer (CP) e-nose (Osmetech Inc.,Wobum, MA) with a 32-sensor array and 15 V across sensor paths was used for all analyses. Fourteen sensors, (including sensors 11, 12, 20-26, 28-32) that did not respond or did not contribute to the discrimination of sapwood volatiles, were turned off. The response sensitivities of the 18 active sensors used, measured as percent changes in electrical resistance responses across sensor paths relative to base resistance (%ΔR/Rbase), varied with the type of polymer used in the sensor-matrix coating, the type of proprietary ring substitutions used, and the type of metal ions used to dope the matrix to improve and modulate sensor response. Detailed analyses containing calibration data for the sensor array were reported previously [5]. The block temperature of the sensor array was maintained at 30 °C. Reference air was preconditioned by passing room air sequentially through a carbon filter, silica gel beads, inline filter, and Hepa filter [5] to remove organic compounds, moisture, and particulates prior to humidity control and introduction into the sampling bottle. The flow rate (suction) was maintained at -702 ml/min using an air flow-calibrated ADM 3000 flow meter (Agilent Technologies,Wilmington, DE). Sensors were purged between runs using a 2% isopropanol wash solution. The instrument was interfaced with a personal computer via an RS232 cable and controlled with Aromascan Version 3.51 software. The instrument plumbing was configured for static sampling of the headspace by allowing air flow out of the external vent port and closing the exhaust port on the sampling bottle so that headspace volatiles were taken from a homogeneous static air mass within the sampling bottle.

D. Data acquisition parameters and run schedules

Data from the sensor array were recorded at 1 s intervals using a 0.2 detection threshold (y-units), a 15–20 y-max graph scale, and pattern average of five data samples taken per run during data acquisition. A uniform run schedule consisted of reference air 20 s, sampling time 90 s, and wash 20 s, followed by 90 s of reference air for a total run time of 220 s. Data slices for processing and analysis were taken from a 20 s sampling interval (85–105 s) near the end of the sampling segment just before the sampling-valve closed. A 2 min reference air purge followed by a 30 min equilibration period was allowed between runs.

E. Construction of reference libraries and validation

An aroma signature reference library was constructed from wood types of known reference woods of hardwoods (angiosperm) species included in this study. All database files were linked to specific (designated) aroma classes defining each sample type or category. The following recognition network options (neural net training parameters) were used for each training session: training threshold = 0.60, recognition threshold = 0.60, number of elements allowed in error = 5, learning rate = 0.10, momentum = 0.60, error goal = 0.010 (P = 0.01), hidden nodes = 5, maximum iterations (epochs) = 10,000, using normalized input data, not actual intensity data. Some parameters were modified for improvement of recognition accuracy. A typical training required 2–35 min, depending on the size of the database applied, using an IBM-compatible personal computer with a minimum of 64 mb of RAM and 350 MHz run speed. Neural net trainings were validated by examining training results that compare individual database files for compatibility or by similarity matches to each specific odor classes by test-assigned odor class distributions among related odor classes included in each library. The specific detailed analytical methods used for identification of unknowns, data processing, and statistical determinations followed the procedures and specifications indicated by Wilson et al. [5].

F. Principal component analysis

Three-dimensional PCA was used to distinguish between headspace volatiles of all sapwood samples and to determine the relatedness of the four aroma classes derived from sapwood types of the two hardwood species, either healthy or wetwood-infected, based on PCA algorithms available with the Aromascan 3.51 software. The mapping parameters for three-dimensional PCA were: iterations = 30, units in Eigen values (%), and use of normalized input data.

III. RESULTS

A. Discrimination between e-nose aroma patterns of sapwood types

The A32S CP e-nose provided correct identifications of the majority of sapwood types tested based on differences in

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the aroma profiles of headspace wood volatiles. Correct identifications of unknown sapwood cores were determined at rates above 90% (range 92.3-100%) for the four samples types of healthy and wetwood-infected sapwood of American beech and black cherry.

The sensor array of the Aromascan A32S electronic nose provided unique and significantly different aroma signature profiles for the four sapwood core types, representing healthy and wetwood-infected samples of the two hardwood species, including American beech (*F. grandifolia*) and Black Cherry (*P. serotina*) based on CP analysis of volatile organic compounds (Table 1). Sensor output values were statistically different ($P<0.01$) between sample types for each sensor with standard deviations for means generally <0.05 for all normalized sensor values of each sample type. Sensors 11, 12, 20-26, and 28-32 did not provide data values that significantly added to the discrimination of sample types in pretest runs, and therefore these sensors were turned off prior to analytical runs in order to exclude this data from the statistical analysis. Thus, 18 sensors were used in the data analysis of aroma profiles for each sensor type.

TABLE I. SENSOR OUTPUTS FROM THE A32S E-NOSE SENSOR ARRAY COMPARING HEALTHY AND WETWOOD-INFECTED SAPWOOD CORES OF TWO SPECIES

E-nose	***F. grandifolia***		***P. serotina***	
Sensor	**Healthy**	**Wetwood**	**Healthy**	**Wetwood**
1	6.83	6.74	7.45	7.15
2	6.15	6.06	6.74	6.46
3	6.94	6.86	7.54	7.31
4	3.48	3.41	3.81	3.69
5	3.46	3.39	3.78	3.67
6	3.48	3.42	3.83	3.70
7	3.84	6.88	7.20	7.13
8	3.99	6.99	7.26	7.15
9	5.46	5.53	5.57	5.51
10	5.09	5.16	5.28	5.20
13	4.93	4.91	5.05	4.89
14	4.52	4.53	4.74	4.51
15	5.09	5.15	5.36	5.14
16	4.77	4.81	4.95	4.75
17	6.09	6.09	4.47	5.12
18	6.32	6.28	4.77	5.40
19	6.10	6.09	4.68	5.24
27	7.47	7.68	7.53	7.98

Normalized sensor output values for wetwood cores were significantly lower than for healthy sapwood cores of both *F. grandifolia* and *P. serotina* species for sensors 1-6. Similar results were observed for sensors 7-27 in *P. serotina*, but these sensor output values were generally greater for wetwood cores than healthy cores of *F. grandifolia*. Sensor values 1-16 for *P. serotina* generally were greater than those of *F. grandifolia* for both wetwood and healthy cores, but the reverse was true for outputs of sensors 17-16. Normalized sensor output values for wetwood cores were significantly lower than for healthy sapwood cores of both *F. grandifolia* and *P. serotina* species for sensors 1-6.

Each sensor in the sensor array was coated with a different conducting polymer composed of polypyrrole, polyanaline, or polythiophene derivatives. Values for normalized and standard deviations of all means were $\leqslant$ 0.05, indicating high precision and a high level of statistical significant difference ($P<0.01$) between means of individual sensor outputs.

B. Principal component analysis

Principal Component Analysis (PCA) tests showed significant differences between wetwood-infected and healthy aroma profiles of beech and black cherry sapwood based on differences between headspace volatiles released from the two wood types of each species. PCA generated precise statistical numerical QF values (quality factors of significance) that provided precise indications of relatedness between aroma profiles of the four sapwood sample types. These QF values indicate the statistical differences between data clusters of all sapwood sample types, plotted as an aroma map in Figure 1. The aroma data plots of healthy and wetwood-infected beech (*F. grandifolia*) sapwood were closer together than healthy and wetwood-infected black cherry (*P. serotina*), although healthy sapwood was significantly different from wetwood-infected profiles for both species. These data plots for healthy black cherry were somewhat separated because one of the samples was considerably dryer and older that other samples of this species. The variability between samples likely indicates differences in the moisture content and amount of wood volatiles released from sapwood cores of healthy black cherry. PCA of this 3-dimensional aroma-map model, as indicated by the principal components in the sapwood volatiles, showed that principal component 1 (PC 1), represented by the x-axis, accounted for 96.4% of the sample variability in the data, while principal component 2 (PC 2), represented by the y-axis, accounted for only 3.5% of the sample variability. Thus, the percentages of the total variance, accounting for by PC 1 and PC 2, explained over 99% of the sample variability in these two orthogonal principal components. The percentage of the variance attributed to principal component 3 (PC 3), represented by the z axis, was negligible and explained less that 0.1% of the sample variance.

The clustering patterns of data points in the aroma for each sample type indicate the precision of the e-nose in

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providing consistent data for multiple sample replications. The greater the distance between data clusters of each sample type, the less related the aroma signatures of headspace volatiles associated with each sample type.

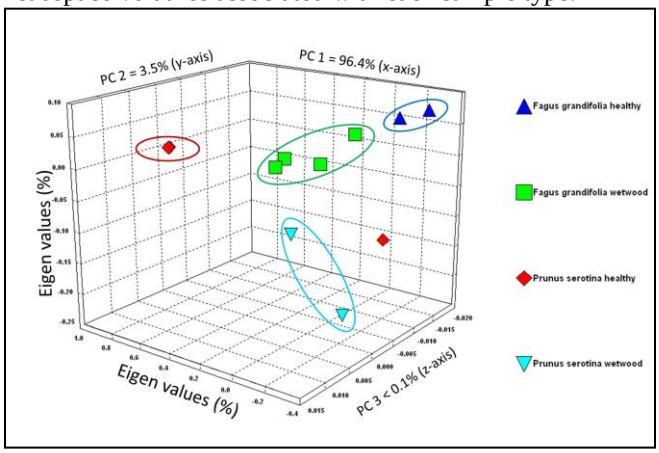

Figure 1. E-nose aroma map showing the differences in aroma profiles of headspace volatiles from healthy vs. wetwood-infected sapwood of *Fagus grandifolia* and *Prunus serotina* using Principal Component Analysis (PCA) of Volatile Organic Compounds (VOCs).

Pairwise comparisons of healthy and wetwood-infected sapwood aroma classes of the two wood types using Quality Factor (QF) significance values provided indications of levels of relatedness between the aroma profiles of healthy and wetwood-infected core types of the two wood species. The e-nose aroma profiles of healthy sapwood cores of American beech were highly significantly different from healthy black cherry (QF=1905.4) at P<0.0001 (Table II).

TABLE II. RELATEDNESS OF HEADSPACE VOLATILES RELEASED FROM HEALTHY AND WETWOOD-INFECTED SAPWOOD CORES OF TWO SPECIES

Aroma class	***Aroma class***	***QF value***
F. grandifolia healthy	*F. grandifolia* wetwood	5.7***
	P. serotina healthy	1905.4****
	P. serotina wetwood	4.5**
F. grandifolia wetwood	*P. serotina* healthy	32.3***
	P. serotina wetwood	12.3***
P. serotina healthy	*P. serotina* wetwood	256.4****

Statistical analysis symbols for quality factor (QF) significant difference levels between aroma classes were as follows: * = P < 0.05; ** = P < 0.01; *** = P < 0.001; **** = P < 0.0001. The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), were as follows: PC 1 = 96.40%; PC 2 = 3.51%; and PC 3 < 0.05%.

The aroma profile of healthy black cherry also was very significantly different from wetwood-infected black cherry (QF=256.4). However, differences in aroma profiles of sapwood headspace volatiles between healthy cores of American beech and wetwood-infected beech and black cherry were much less significantly different at P < 0.001 and P < 0.01, respectively. By comparison, aroma profiles of wetwood-infected beech sapwood were different than healthy and wetwood-infected black cherry sapwood volatiles at intermediate levels of statistical difference (P<0.001). The lowest level of difference between sapwood aroma profiles occurred between healthy American beech and wetwood-infected black cherry (QF=4.5) at P < 0.01.

The large significant differences between healthy and wetwood-infected sapwood of both species provide evidence that the headspace volatiles derived from wetwood bacteria have a large effect on the aroma signature pattern, but not as great as the differences in healthy sapwood volatiles between the two hardwood species.

IV. DISCUSSION AND CONCLUSIONS

Analyses of data indicating unique aroma signature patterns, based on output results from the sensor array of the Aromascan A32S CP electronic-nose, provided effective discriminations between headspace volatiles derived from sapwood core samples of American beech and black cherry. Discriminations and correct identification of samples types were determined at high levels of statistical confidence.

These results are similar to those found for previous studies involving detections of plant disease infections using comparable e-nose analyses for bacterial diseases of onion [8][9], blueberry diseases [10], grain spoilage [11], mango fruit rot [12], wood decay [13]-[16], and other diseases [17][18].

E-nose analysis results of sapwood types in the current study were similar to those obtained from sapwood cores in related studies using different types of e-nose gas-detection technologies based on several different gas-sensing principles as summarized previously [19]-[22]. Wilson et al. [6] was able to distinguished between angiosperm and gymnosperm sapwood types using fresh tree cores frozen at -20 C and thawed immediately prior to analysis with a A32S CP e-nose. Baietto et al. [13] compared the performance of three different e-noses, including the PEN3 metal-oxide (MOS) e-nose, the LibraNose Quartz Microbalance (QMB) e-nose, and the Aromascan A32S CP e-nose to discriminate between healthy and decayed wood of different wood types, decayed by various wood-decay fungi. Other potential agricultural applications of e-nose instruments have been found in pesticide-residue identifications [23][24], environmental monitoring for agricultural wastes and pollutants [25], and disease-detection in fish culture [26].

The large differences between healthy sapwood of American beech and black cherry provide evidence that the headspace volatiles derived from healthy sapwood of these two species have a larger effect on the aroma signature pattern than do differences between wetwood-infected and healthy sapwood of the two hardwood species.

The aromascan A32S CP e-nose has been shown here to have the capability of identifying and discriminating between sapwood types and between healthy and wetwood-infected

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sapwood of American beech and black cherry. These results provide additional corroborative evidence of the utility that e-nose devises provide for the diagnosis of bacterial wetwood in bottomland hardwood species. Previously, no consistently reliable methods for detecting the presence of wetwood in hardwoods have been developed [4]. The use of ultrasonic devices for wetwood detection [27] are unreliable and unfeasible due to the requirement for destructive sampling of sapwood logs to obtain measurements, the slow device recovery and setup time between log tests, and difficulties of distinguishing between wetwood and healthy sapwood, because wetwood does not significantly alter wood density unlike decayed wood that does physically soften woods, slowing the transmission rate of sound waves.

The diagnosis of bacterial wetwood in logs of hardwood species is an important first step for log and lumber processing in hardwood lumber mills. Wetwood can be effectively and rapidly detected with e-nose technologies in raw logs prior to cutting into lumber so that all boards derived from wetwood-infected logs can be treated with a different (slower) kiln-drying schedule to mitigate damage to wetwood lumber that results from damaged by shakes and splits during rapid kiln drying. This adjustment in the kiln drying schedule to a slower drying process is essential to preserve lumber value and minimize lumber defects to avoid commercial losses when the lumber is marketed.

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