

Detection and diagnosis of bacterial wetwood in *Tilia americana* and *Ulmus americana* sapwood using a CP electronic-nose

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Abstract

Electronic-nose (e-nose) methods for detecting bacterial wetwood were developed and tested using a conducting polymer (CP)-type electronic nose, the Aromascan A32S, to diagnose the disease in two hardwood species (*Tilia americana* and *Ulmus americana*) based on headspace volatile metabolites in sapwood. An aroma library was developed using diagnostic aroma signature patterns (profile databases) derived from e-nose analysis of known healthy and wetwood-infected sapwood cores of each tree species. The library was used to screen sapwood cores for the presence of wetwood in unknown samples. The A32S e-nose effectively distinguished between differences in headspace volatiles from tree cores of different sample types, correctly identifying them at frequencies ranging from 88.1-100%. The distribution of aroma class components, based on artificial neural net training and principal component analysis (PCA), indicated the relatedness and differences in headspace volatiles of aroma classes represented by each sample type. Significant differences were found between the aroma profiles of healthy vs. wetwood-infected sapwood of basswood and American elm, and even greater differences between the headspace wood volatiles released from the two wood types.

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

1. Introduction

Bacterial wetwood, caused by various soil-inhabiting anaerobic bacteria found in periodically-flooded bottomland forests, is a disease common primarily to bottomland hardwood species, but also some upland conifer tree species. Wetwood bacteria attack the middle lamellae between wood cells and fibers (in both sapwood and heartwood) using various pectolytic enzymes (pectolases) that cause radial and lateral separations of wood fibers, resulting in cracks and splits in processed lumber during kiln drying. The defect damage to processed lumber results in economic losses as a consequence of a reduction in lumber grade quality that lowers the value of the lumber for commercial sale. An electronic-nose (e-nose) device was first utilized to detect bacterial wetwood in the sapwood of *Populus deltoides* (cottonwood) plantation trees and to identify host plants of the disease [1-3]. The current research focused on the detection of wetwood in two hardwood species, *Tilia americana* (Basswood) and *Ulmus americana* (American elm), 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 determine the capability of the Aromascan A32S electronic nose (e-nose) to 1) detect bacterial wetwood disease in the sapwood of two hardwood species, basswood and American elm, 2) discriminate between healthy and wetwood-infected sapwood samples based on differences in headspace volatiles, and 3) determine the relatedness between the aroma signatures of all sample types using principal component analysis (PCA) of volatile organic compounds (VOCs) present in headspace volatiles.

2. Materials and methods

2.1 Collection and storage of sapwood core samples

Sapwood increment core samples (5 mm diameter × 5 cm length) were collected in spring from freshly-harvested *T. americana* (basswood) and *U. americana* (American elm) logs deposited in piles within the Andersen Tully log and lumber yard at Vicksburg, Mississippi. A minimum of two core samples were

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extracted from the bores 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. 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 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.

2.2 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 $\leq 4\%$) 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.

2.3 Instrument configuration and run parameters

The Aromascan A32S 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 ($\% \Delta R/R_{base}$), 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. 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 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 a 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.

2.4 Data acquisition 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.

2.5 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.

3. Results

3.1 Discrimination between e-nose aroma patterns of sapwood types

The A32S conductive polymer e-nose correctly identified the majority of sapwood types tested based on differences in the aroma profiles of headspace wood volatiles. Correct identifications of unknown sapwood cores were determined at rates above 90% (range 91.7-100%) for samples of *T. americana*, and 88.1-99.0% for samples of *U. americana*. Only one sample of *U. americana* could not be identified and was determined to be unknown due to the inability of the e-nose to assign the aroma profile to a specific aroma class.

The sensor array of the Aromascan A32S electronic nose provided unique and significantly different aroma profiles for the four sapwood core types, representing both healthy and wetwood-infected samples for each species (Table 1). Large statistical differences in sensor outputs were recorded between species, although smaller significant differences were found between healthy and wetwood-infected sapwood cores.

Differences in individual sensor intensities, using the difference-mode software option, provided indications of the degree of differences in VOCs present in headspace volatile mixtures for the different sapwood sample types (Fig. 1). Significant differences in sensor patterns occurred for *T. americana* (Fig. 1A) and *U. americana* (Fig. 1B). Differences were negative for sensors 7, 9, 10, and 27 in *T. americana* and sensor 20 in *U. americana*.

The consistent, relatively strong negative differences in sensor responses (sensors 7, 9, and 10) to healthy vs. wetwood-infected sapwood cores of both *T. americana* and *U. americana* is a characteristic in common between the two hardwood species. This result suggests a possible specific chemical component in common in the headspace volatile mixture of wetwood cores of these two species that may contribute to the strong differences in responses of these specific sensors.

3.2 Principal component analysis

PCA showed significant differences between the aroma profiles of healthy vs. wetwood-infected sapwood of basswood and American elm, and much higher differences between different volatiles released from the two wood types (Fig. 2). PCA generated precise statistical numerical values (quality factors of significance) that provided some precise indications of relatedness between aroma profiles of the four sample types.

Table 1. Sensor outputs from the A32S electronic-nose sensor array comparing headspace volatiles released from healthy and wetwood-infected sapwood cores of basswood and American elm based on conducting polymer (CP) analyses.

Wood type	Status	Sensor number ¹								
		1	2	3	4	5	6	7	8	9
Basswood	healthy	7.04	6.34	7.09	3.57	3.56	3.58	6.88	7.05	5.43
	wetwood	6.79	6.13	6.93	3.58	3.56	3.61	7.06	7.02	5.62
American elm	healthy	3.92	3.57	4.03	1.98	1.97	1.97	4.38	4.36	3.70
	wetwood	3.90	3.53	4.02	1.99	1.97	1.98	4.40	4.36	3.81

Wood type	Status	Sensor number ¹								
		10	13	14	15	16	17	18	19	27
Basswood	healthy	4.98	5.02	4.66	5.22	4.85	5.63	5.85	5.70	7.55
	wetwood	5.48	4.91	4.48	5.21	4.79	5.51	5.73	5.58	8.02
American elm	healthy	3.38	2.95	2.72	3.06	2.81	3.88	4.00	3.83	5.72
	wetwood	3.44	2.95	2.70	3.07	2.81	3.87	3.98	3.78	5.56

¹ Each sensor in the array was coated with a different conducting polymer composed of polypyrrole, polyaniline, or polythiophene derivatives. Values are normalized and standard deviations of all means were ≤ 0.03 , indicating high precision and a high level of significant difference ($P < 0.01$) between means.

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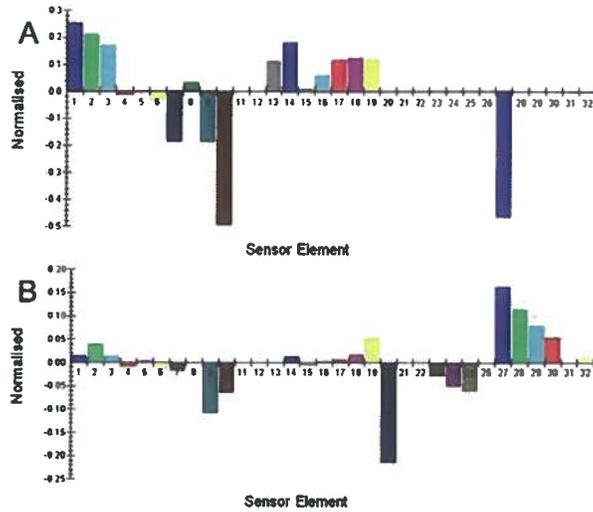


Fig. 1. Aroma sensor responses of the A32S e-nose sensor array in difference mode. Sensor response percentage differences in e-nose sensor output intensities of individual numbered sensors are indicated for headspace volatiles of healthy minus wetwood-infected sapwood samples of A) *Tilia americana* (basswood), and B) *Ulmus americana* (American elm), indicating percent changes in sensor resistance responses relative to baseline resistance. Sensor elements represent individual sensor numbers in the e-nose sensor array.

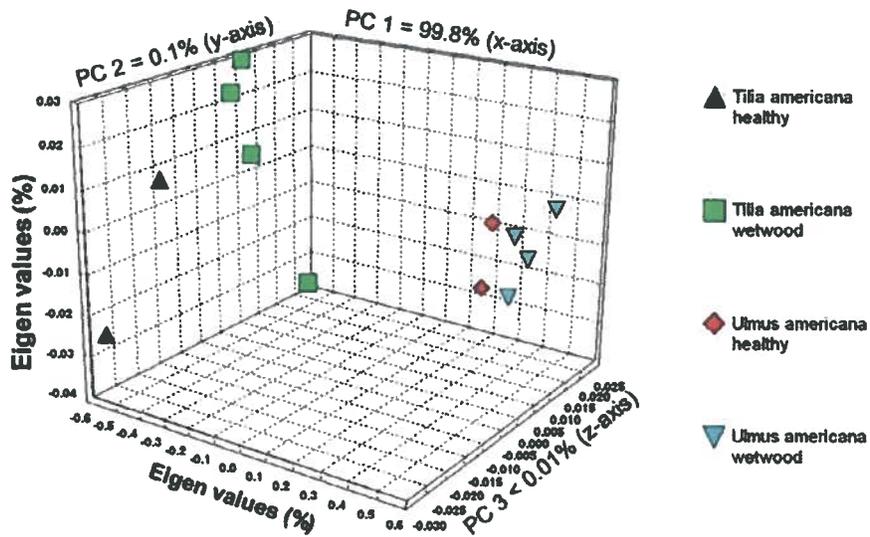


Fig. 2. Aroma map of headspace volatiles from *Tilia americana* (basswood) and *Ulmus americana* (American elm), sapwood samples (aroma classes) based on principal component analysis (PCA). The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1=99.8%, PC 2=0.1%, and PC 3<0.01%.

Pairwise comparisons of sapwood aroma classes (different sample types), using Quality Factor (QF) significance values based on PCA data, provided indications of levels of relatedness between the aroma profiles of healthy and wetwood-infected core types of the two wood species. The results indicated that

the e-nose aroma profiles of healthy vs. wetwood-infected sapwood cores of basswood (QF=26.07) and healthy vs. wetwood-infected sapwood cores of American elm (QF=16.45), were significantly different at ($P<0.01$). However, the differences in e-nose aroma profiles of sapwood headspace volatiles between healthy cores of basswood and American elm (QF=133.28), between healthy *T. americana* and wetwood-infected *U. americana* (QF=244.91), between wetwood-infected *T. americana* and healthy *U. americana* (QF=331.96), and between wetwood-infected *T. americana* and *U. americana* (QF=385.77) indicated much higher levels of statistical difference ($P<0.001$) between aroma classes of the different wood types, and even greater differences when one or both of the sapwood cores were infected with wetwood bacteria.

4. Discussion and conclusions

The common occurrence of negative difference values, resulting from pairwise comparisons with sensor output data displayed in difference mode for sensors 7, 9, and 10 (in response to healthy vs. wetwood-infected sapwood cores for both *T. americana* and *U. americana*), suggested a commonality of VOC chemical components in the headspace mixture derived from wetwood samples that might be produced by wetwood bacteria in the wood of both tree species. These three sensors in the A32S e-nose sensor array are particularly sensitive to carboxylic acids, such as acetic, propionic and butyric acids that are commonly produced by wetwood bacteria and may account for the similarities of sensor responses to headspace VOCs in wetwood-infected cores from both hardwood species.

The combined results of significant differences in electronic aroma signature patterns (aroma profiles) of healthy and wetwood infected *T. americana* and *U. americana* sapwood cores, and PCA data clearly indicate that the Aromascan A32s e-nose is capable of detecting the presence of wetwood bacteria in sapwood cores taken from infected logs. The high levels of significant differences between the aroma profiles of healthy vs. wetwood-infected sapwood cores of both species show the e-nose capability of discrimination between sapwood cores of all four sapwood sample types (aroma classes) based on differences in VOCs present in headspace volatile mixtures. The capability of the A32S e-nose to diagnose wetwood in sapwood cores from raw logs of basswood and American elm was clearly demonstrated.

The potential applications of the A32S CP e-nose for the detection and identification of wetwood in hardwood logs and lumber were demonstrated. The diagnostic methods developed here are potentially useful for screening and tagging raw logs of basswood and American elm (brought into the lumber mill) for the presence of wetwood disease based on e-nose detection using diagnostic headspace aroma profiles. An accurate diagnosis of wetwood in logs (prior to lumber processing) allows for the mitigation of economic losses, due to lumber degrades, by providing opportunities for changes or adjustments in lumber kiln-drying schedules in order to slow the drying process for wetwood lumber and prevent wetwood-associated damage to lumber cut from wetwood-infected logs.

Previous studies have shown the capability of e-nose instruments to detect plant diseases [1,4-11], wood decay fungi [12-15], and other phytopathogenic and human-pathogenic microbes [16-19]. Many new e-nose applications for the diagnosis of diseases caused by other pathogenic microbe groups are no doubt forthcoming.

Acknowledgements

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References

- [1] Wilson AD, Lester DG, Oberle CS. Development of conductive polymer analysis for the rapid detection and identification of phytopathogenic microbes. *Phytopathology*. 2004;94:419-431.
- [2] Wilson AD, Lester DG, Oberle CS. Application of conductive polymer analysis for wood and woody plant identifications. *For. Ecol. & Managem.* 2005;209:207-224.
- [3] Wilson AD. Application of a conductive polymer electronic-nose device to identify aged woody samples. In: Yurish S, Chilimon I, Carvalho V, Gervais-Ducouret S, eds. *Proceedings of The 3rd International IARIA Conference on Sensor Device Technologies and Applications*. Wilmington, DE: Xpert Publishing Services;2012:77-82.

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- [4] Prithiviraj B, Vikram A, Kushalappa AC, Yaylayan V. Volatile metabolite profiling for the discrimination of onion bulbs infected by *Erwinia carotovora* spp. *carotovora*, *Fusarium oxysporum*, and *Botrytis allii*. *Eur. J. Plant Pathol.* 2004;110:371-377.
- [5] Vikram A, Hamzehzarghani H, Kushalappa AC. Volatile metabolites from the headspace of onion bulbs inoculated with postharvest pathogens as a tool for disease discrimination. *Can. J. Plant Pathol.* 2005;27:194-203.
- [6] Laothawornkitkul J, Moore JP, Taylor JE, Possell M, Gibson TD, Hewitt CN, Paul ND. Discrimination of plant volatile signatures by an electronic nose: A potential technology for plant pest and disease monitoring. *Environ. Sci. Technol.* 2008;42:8433-8439.
- [7] Jansen RMC, Wildt J, Kappers IF, Bouwmeester HJ, Hofstee JW, van Henten EJ. Detection of diseased plants by analysis of volatile organic compound emission. *Annu. Rev. Phytopathol.* 2011;49:157-174.
- [8] Spinelli F, Cellini A, Vanneste, JL, Rodriguez-Estrada MT, Costa G, Savioli S, Harren FJM, Cristescu SM. Emission of volatile compounds by *Erwinia amylovora*: Biological activity *in vitro* and possible exploitation for bacterial identification. *Trees.* 2012;26:141-152.
- [9] Magan N, Evans P. Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. *J. Stored Prod. Res.* 2000;36:319-340.
- [10] Li Z, Wang N, Raghavan GSV, Vigneault C. Ripeness and rot evaluation of 'Tommy Atkins' mango fruit through volatiles detection. *J. Food Eng.* 2009;91:319-324.
- [11] Li C, Krewer GW, Ji P, Scherm H, Kays SJ. Gas sensor array for blueberry fruit disease detection and classification. *Postharvest Biol. Technol.* 2010;55:144-149.
- [12] Baietto M, Wilson AD, Bassi D, Ferrini F. Evaluation of three electronic noses for detecting incipient wood decay. *Sensors.* 2010;10:1062-1092.
- [13] Hamilton S, Hopher MJ, Sommerville J. Detection of *Serpula lacrymans* infestation with a polypyrrole sensor array. *Sens. Actuator B Chem.* 2006;113:989-997.
- [14] Markoma MA, Shakaff AY, Adom AH, Ahmad MN, Hidayat W, Abdullah AH, Fikri NA. Intelligent electronic nose system for basal stem rot disease detection. *Comput. Electron. Agric.* 2009;66:140-146.
- [15] Baietto M, Letizia P, Wilson AD, Bassi D. Evaluation of a portable MOS electronic nose to detect root rots in shade tree species. *Comput. Electron. Agric.* 2013;96:117-125.
- [16] Wilson AD, Baietto M. Applications and advances in electronic-nose technologies. *Sensors.* 2009;9:5099-5148.
- [17] Wilson AD, Baietto M. Advances in electronic-nose technologies developed for biomedical applications. *Sensors.* 2011;11:1105-1176.
- [18] Wilson AD. Future applications of electronic-nose technologies in healthcare and biomedicine. Chapter 15 In: Akyar I, ed. *Wide Spectra of Quality Control*. Rijeka, Croatia: InTech Publishing;2011:267-290.
- [19] Wilson AD. Diverse applications of electronic-nose technologies in agriculture and forestry. *Sensors.* 2013;13:2295-2348.