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Application of conductive polymer analysis for wood and woody plant identifications

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Abstract

An electronic aroma detection (EAD) technology known as conductive polymer analysis (CPA) was evaluated as a means of identifying and discriminating woody samples of angiosperms and gymnosperms using an analytical instrument (electronic nose) that characterizes the aroma profiles of volatiles released from excised wood into sampled headspace. The instrument measures electrical-resistance changes generated by adsorption of volatiles to the surface of electroactive, polymer-coated sensors. Unique digital electronic fingerprints of wood aromas, derived from multisensor-responses to distinct mixtures of wood volatiles, were obtained from woods of individual tree species. A reference library containing aroma signature patterns for 23 tree species was constructed for identifications of unknown samples using pattern-recognition algorithms. The 32-sensor array used with an Aromascan A32S instrument was sensitive to a wide diversity of organic compounds and produced outputs of distinct electronic aroma signature patterns in response to wood volatiles that effectively identified unknown samples from individual tree species included in the reference library. Some potential applications of CPA methods for research in ecology, forestry, plant taxonomy, and related disciplines were identified with some significant advantages and limitations. Other applications of this technology were discovered for the management of forested stands and ecosystems based on the identification of roles that wood-inhabiting organisms play in stand dynamics and long-term ecosystem functions. Results pertaining to tree systematics and phylogeny are discussed in the context of prevailing opinions of oak taxonomy.
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1. Introduction

Analytical methods are needed in forest ecology research to provide reliable means of identifying woody samples from a variety of sources and locations that are not readily identifiable by conventional means. Traditional chemical and microscopic methods

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used for wood identification hitherto are cumbersome because they often require extensive sample preparation and analysis. New methods yielding rapid woody sample identifications could facilitate determinations of biological activities, interactions, and ecological roles of microorganisms, insects, and other invertebrates that interact with wood in living and dead trees or coarse woody debris on the forest floor. Organisms associated with wood in forest ecosystems are involved in such activities as wood decomposition, nutrient cycling, and other functional niches that affect forest stand structure, dynamics, and ecosystem processes (Andrews and Harris, 2000; Hansen and Goheen, 2000; Whipps, 2001). Many microbes have effects on forest health and ecosystem functions because they include causal agents of tree mortality, forest diseases, wood decay, and lumber defects of importance in ecosystem and timber management, and in the manufacture of forest products. Also, certain types of ecological studies in forest science and related research disciplines have need of specialized analytical equipment capable of quickly recognizing and discriminating between various types of woody samples with minimal destructiveness during sampling. Such instruments and methods are needed with the capability of providing these determinations based on a rapid chemical means of detection. Elaborate analytical methods such as gas chromatography–mass spectroscopy are only capable of identifying the chemical compounds present in wood, but this information does not necessarily provide the information needed for wood identifications.

There are numerous instances where woody samples collected for research purposes are not readily identifiable because of the difficulty in determining individual source trees from which woody samples were derived, or because samples were taken from woody plants during times of the year when identifying characters (flowers, fruits, and leaves, etc.) are absent. Analytical tools and methods capable of identifying woody samples within these limitations are essential for characterizing and determining the roles, damaging effects, and interrelationships among wood-feeding and wood-inhabiting organisms that interact with trees in forest stands and ultimately affect forest health, stand structure, composition, stability, ecosystem processes, and forest dynamics over time. Such capabilities were made possible with the invention of instruments called "electronic noses" that were designed to produce digital electronic signatures of volatiles released from any organic source (Dodd and Persaud, 1982; Pelosi and Persaud, 1988; Shirley and Persaud, 1990; Persaud, 1992; Persaud et al., 1993). Unlike other analytical instruments, these devices allow the identification of organic samples without having to identify individual chemical components within the volatile mixture (Gardner, 1991; Davide et al., 1995; Lonergan et al., 1996), and avoid operator fatigue (Shurmer, 1990; Gardner and Shurmer, 1992). Agricultural and food industries have utilized conductive polymer analysis (CPA), a type of electronic aroma detection (EAD) technology, to measure product and food quality (Aishima, 1991; Hanaki et al., 1996; Bartlett et al., 1997), storage life (Di Natale et al., 1995, 1996), freshness (Egashira, 1997), agricultural waste detection (Hobbs et al., 1995; Persaud et al., 1996), recognition of organic chemicals (Kowaiski and Bender, 1972), diagnosis of plant diseases (Wilson et al., 2004), and many other applications (Ouellette, 1999; Yea et al., 1994). Within the field of forest pest management, CPA has proven useful in the detection of bacterial wetwood infections in cottonwood, the detection and identification of fungal forest pathogens (e.g. *Ceratocystis fagacearum*), and the discrimination of wood decay fungi in woody samples (Wilson and Lester, 1999; Wilson et al., 2004). A variety of different sensor types have been developed for these various applications including optical sensors (White et al., 1996), metal oxides (Shurmer et al., 1989; Egashira and Shimizu, 1993; Nanto et al., 1993), semiconductive polymers (Meyerhoff, 1993; Yim et al., 1993; Pisanelli et al., 1994), and conductive polymers (Hatfield et al., 1994; Freund and Lewis, 1995; Lonergan et al., 1996).

An electronic nose typically consists of a multisensor array, an information-processing system such as an artificial neural network (ANN), software with digital pattern-recognition algorithms, and reference-library databases (Abe et al., 1988; Freund and Lewis, 1995; Gardner, 1991; Gardner and Shurmer, 1992; Kowaiski and Bender, 1972). The sensor array consists of incrementally different sensors that respond to a wide range of chemical classes and discriminate diverse mixtures of possible analytes. The output from

individual sensors are assembled and integrated to produce a distinct digital pattern of responses (aroma fingerprint) called an electronic aroma signature pattern (EASP), allowing classification and identification of the analyte. Using EASPs, representing unique aroma signature patterns for complex mixtures of distinctive aromas in the sample mixture, allows recognition of the sample as a whole without requiring the mixture to be separated into its individual components prior to or during analysis. A reference library of EASPs for known samples is constructed prior to analysis of unknowns by assigning descriptor names (identifiers) to patterns of known origin. The ANN is configured through a learning process (neural net training) using pattern-recognition algorithms that look for differences between the patterns of all the descriptor types included in the reference library. This process continues until a previously selected level of discrimination is met. The results are validated and assembled into the reference library to which unknown samples can be compared. Identification of unknowns is based on the distribution of aroma attributes or elements that the analyte pattern has in common with patterns present in databases of the reference library.

This investigation focuses on the potential application of conductive polymers for the identification of woody samples for forest ecology and related research. The objectives of this study were to: (i) evaluate the feasibility of using CPA technology as a means of identifying and discriminating woods of angiosperms and gymnosperms based on electronic signatures of volatiles released from excised wood cores, (ii) develop CPA methods and applications useful for forest ecology through woody plant identifications, and (iii) examine the potential applicability of these methods to plant taxonomy and for determining chemical relatedness between plant species. Some preliminary results of this work were reported previously (Wilson and Lester, 1999).

2. Materials and methods

2.1. Collection and storage of woody samples

Increment cores of standard dimensions (5 mm diameter × 5 cm length) were collected in winter and early spring from sapwood tissues of plants representing 23 species of trees and shrubs from 14 plant families typical of bottomland and upland forest types in the southern United States (Table 1). Two tree cores were extracted from the boles of at least 10 individual living trees of each species from various locations (stands) using a Haglöf tree increment borer (Forest Suppliers, Inc., Jackson, MS) and placed into 14.8 mm glass vials. Increment cores used in the analysis were collected primarily from healthy trees, although some cores were collected from diseased tissues of trees for comparison with healthy cores of the same tree species to investigate limitations on applications of the methods. Woody cores in all cases were frozen at −20 °C in long-term storage and thawed immediately prior to sample analysis. Cores that became desiccated due to sublimation during 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 analysis.

2.2. Sample preparation and prerun procedures

Woody core samples in 14.8 ml glass vials were uncapped and placed into a 500 ml glass 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 just above the bottom of the sampling bottle. The sampling bottle was held in the sampling chamber within the instrument at a constant air temperature of 25 °C. The sampling bottle was purged with filtered, moisture-conditioned reference air for 2 min prior to building headspace. The sampling bottle was sealed and volatiles from the sample 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. Reference air was set at 4% RH for most runs and adjusted to within 2% below sample air at 25 °C. The sampling bottle cap and exhaust port were opened between runs to purge the previous sample with conditioned reference air.

2.3. Instrument configuration and run parameters

All analyses were conducted with an Aromascan A32S (Osmetech, Inc., Wobum, MA) instrument fitted

Table 1
Types and sources of tree species from which woody samples were collected for use in constructing reference libraries of electronic aroma signatures by conductive polymer analysis

Tree species	Common name	Plant family	Stand type[a]	Collection site
Acer rubrum	Red maple	Aceraceae	Upland	Union Co., AR
Carpinus caroliniana	American hornbeam	Betulaceae	Bottomland	Washington Co., MS
Carya illinoensis	Pecan	Juglandaceae	Upland	Washington Co., MS
Carya tomentosa	Mockernut hickory	Juglandaceae	Upland	Union Co., AR
Celtis laevigata	Sugarberry	Ulmaceae	Bottomland	Washington Co., MS
Cornus florida	Flowering dogwood	Cornaceae	Upland	Union Co., AR
Diospyras virginiana	Common persimmon	Ebenaceae	Upland	Washington Co., MS
Ilex opaca	American holly	Aquifoliaceae	Upland	Union Co., AR
Liquidambar styraciflua	Sweetgum	Hamamelidaceae	Upland	Washington Co., MS
Platanus occidentalis	Sycamore	Platanaceae	Upland	Washington Co., MS
Populus deltoides	Eastern cottonwood	Salicaceae	Bottomland	Washington Co., MS
Prosopis glandulosa	Honey mesquite	Fabaceae	Upland	Travis Co., TX
Quercus alba	White oak	Fagaceae	Upland	Union Co., AR
Quercus buckleyi	Spanish oak	Fagaceae	Upland	Travis Co., TX
Quercus falcata	Southern red oak	Fagaceae	Upland	Union Co., AR
Quercus marilandica	Blackjack oak	Fagaceae	Upland	Union Co., AR
Quercus nigra	Water oak	Fagaceae	Bottomland	Washington Co., MS
Quercus stellata	Post oak	Fagaceae	Upland	Travis Co., TX
Quercus virginiana	Coastal live oak	Fagaceae	Upland	Travis Co., TX
Salix nigra	Black willow	Salicaceae	Bottomland	Union Co., AR
Sassafras albidum	Sassafras	Lauraceae	Upland	Union Co., AR
Taxodium distichum	Baldcypress	Taxodiaceae	Bottomland	Washington Co., MS
Ulmus crassifolia	Cedar elm	Ulmaceae	Upland	Travis Co., MS

[a] Type of forest stand based on position of collection site relative to surrounding topography. Upland sites were generally close to the tops of ridges or at positions above drainage areas, and bottomland sites were close to water courses or within drainage areas.

with a conventional 32-sensor array designed for general-use applications with 15 V across sensor paths. Prior to analysis of known and unknown volatiles from woody plant tissues in this study, individual sensors in the sensor array were characterized and calibrated by testing sensitivity responses to representative compounds from different classes of organic compounds potentially relevant to wood identifications. Among compounds present in wood volatiles, sensors were more sensitive to long-chain alcohols, long-chain esters, and aromatic hydrocarbons, and slightly less sensitive to short-chain esters and aliphatic ketones. However, sensors were most sensitive to amines and sulfur-containing compounds not normally found in wood volatiles. The response sensitivities of individual sensors, measured as percent changes in resistance response across sensor paths relative to base resistance ($\%\Delta R/R_{base}$), varied with the type of plastic polymer used in the sensor matrix coating, the type of ring-substitutions used to modify its conductive properties, and the type of metal ions used to dope the matrix to improve and modulate sensor response. Detailed results of analyses that provided prior characterization and calibration of the sensor array were reported previously (Wilson et al., 2004).

The block temperature of the sensor array was maintained at a constant 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, particulates, and microbes, respectively, prior to humidity control and introduction into the sampling bottle. The flow rate (suction) of sample air at the sampling port 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 altered from conventional architecture and specifically configured for static sampling of the

headspace by allowing air flow, maintained at 605 ml/min flow rate, coming out of the external vent (bag-fill) port of the instrument during analytical runs, 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 parameters and run schedules

Data from the sensor array were collected at 1 s intervals using a 0.2 detection threshold (y-units), a 15–20 y-max graph scale, and with a pattern average of five data samples taken per run during data acquisition. A uniform run schedule (1 valve sequence) was used and 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. A 2 min reference air purge followed by a 30 min equilibration period was allowed between runs.

2.5. Construction of reference libraries and validation

An aroma signature reference library was constructed from wood samples of all known reference woods of angiosperm and gymnosperm 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 ($P \leq 0.01$), hidden nodes = 5, maximum iterations (epochs) = 10,000, using normalized input data, not actual intensity data. Some of these parameters were modified as described in the results for specific applications or 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.

2.6. Identification of unknowns using recognition files

A reference library, constructed from electronic aroma signature patterns (EASPs) of headspace volatiles from 23 known woods, was used for comparison and identification of unknown samples. This was accomplished either in real time or by using off-line analysis using a recognition file (containing databases) created from the reference library. In each case, the neural net compared the response pattern of the unknown sample with databases found in the recognition file. The pattern-recognition algorithms quickly determined a best match that most closely fit the aroma elements found in the unknown sample. The closeness of the match was expressed as a percentage value allocated to different global classes (individual tree species) represented in the sample. A value greater than 90% was considered to be a good match. Global class distributions of major and minor identification elements were used for determinations of relatedness between sample types. The neural net software had settings that allow for training to any level of significance that was desired in discriminating samples. A significance level of 0.05 or lower was used depending on the level of specificity desired in the discrimination.

2.7. Instrument reliability in identifications

The reliability of the instrument and methods to accurately identify sample unknowns was tested by collecting two cores per tree from 13 to 30 individual trees for each of 12 tree species. Analyzed samples were determined to be either correctly identified, not identified (indeterminate), incorrectly identified, or ambiguously identified based on recognition results obtained from pattern-recognition software using the tree reference library. Determinations falling outside of the domain of defined global classes were recorded as unknown. Ambiguous determinations were indicated when samples were identified in different global classes from separate runs.

2.8. Data processing, manipulations, and statistical evaluations

Data slices for processing and analysis were taken from a 20 s sampling interval (85–105 s) near the end

of the sampling segment of each run before the sampling-valve closed. The data slice from the raw data file was used to create a representative descriptor database file. A minimum of 10 descriptor database files were created from separate specimens of each sample type. Aroma signature patterns of individual aroma classes (descriptors) were reported from calculated means ± S.E.M. (standard errors of the mean) of raw relative resistance sensor values from runs of at least 10 different samples of each odor class. Real time determinations of unknowns utilized recognition files with normalized sensor intensity responses and pattern-recognition algorithms and matrices.

2.9. Principal component analysis

Detailed comparisons of relatedness of odor classes were determined using principal component analysis (PCA) algorithms provided by Aromascan Version 3.51 software. Three-dimensional PCA was used to distinguish between headspace volatiles released from seven *Quercus* species, including *Q. alba*, *Q. buckleyi*, *Q. falcata*, *Q. marilandica*, *Q. nigra*, *Q. stellata*, and *Q. virginiana*. The mapping parameters for three-dimensional PCA were: iterations = 30, units in Eigen values (%), and with normalized input data.

3. Results

3.1. Sensor responses to headspace volatiles

The detection limits and sensitivity of the A32S instrument were dependent on the classes of organic components present in the sample and the combined sensitivities of the sensor array. Highly polar compounds like carboxylic acids tended to bind and accumulate on some sensors causing negative responses in some cases at higher concentrations. This was observed here when cores were taken from oaks that were infected with wetwood bacteria. The bacterial species responsible for causing wetwood in eastern hardwoods, particularly in *Quercus* species, commonly produce carboxylic acids as fermentation products released from anaerobic respiration. Sensor 7 was most sensitive to carboxylic acids and amines, although other sensors (17, 18, 22–24, 28) also were strongly sensitive to carbonyl compounds (carboxylic acids, aldehydes, esters, and ketones), alcohols, aromatics, and chlorinated hydrocarbons.

The sensor array was highly responsive to water vapor present in the sample headspace. This strong response to water necessitated controlling the RH of the sample air to 4% to assure that the sensor response was positive, with the exception of some sensors that responded negatively to the presence of carboxylic acids. Thus, conditioning of reference air to 4% RH, controlled by a prerun setting on the instrument control panel, was necessary before building headspace prior to analysis.

A number of factors affected quality, stability, and uniformity of runs during data acquisition. Sensor output was sensitive to sample size. Control of sample size provided standardized quantities of headspace volatiles being analyzed. Sampling methods also had a large impact on uniformity of signal output from the sensor array. Static sampling used here provided more uniform and stable data output than dynamic stripping and equilibration sampling because it avoided the dilution of headspace volatiles (increasing sensitivity) and precluded perturbations of sampling air that caused temporal variability in sample concentration during the run. The instrument architecture used here was modified so that sample air could be vented during sample introduction to avoid dilution effects. Samples were introduced from a closed sampling bottle, without reference air introduction, to maintain uniform sample concentrations during data acquisition.

3.2. Reliability of unknown sample identifications

The reliability of CPA methods to correctly identify unknown samples was evaluated with blind tests using 13–30 unknowns from 12 tree species included in the reference library. All unknown samples from 9 of the 12 species tested were identified correctly (Table 2). Approximately 92% of unknown samples from two additional species, *Carpinus caroliniana* and *Platanus occidentalis*, were identified correctly, and the remaining samples (8%) were determined to be unidentified or unknown. The lowest level of correct identification (86%) occurred with unknown samples of *Liquidambar styraciflua*, and the remaining samples (14%) remained unidentified. This was the only species in which less than 90% of unknown

Table 2
Tests of the reliability of tree identifications for 12 species determined by CPA with the Aromascan A32S using recognition files constructed from a 23-species tree reference library

Sample unknowns[a]	*n*	Correctly identified[b] (%)	Indeterminate not identified[c] (%)
Carya illinoensis	13	100.0	0.0
Carya tomentosa	13	100.0	0.0
Carpinus caroliniana	13	92.3	7.7
Celtis laevigata	17	100.0	0.0
Cornus florida	13	100.0	0.0
Liquidambar styraciflua	14	85.7	14.3
Platanus occidentalis	13	92.3	7.7
Quercus virginiana	30	100.0	0.0
Quercus stellata	13	100.0	0.0
Salix nigra	13	100.0	0.0
Sassafras albidum	13	100.0	0.0
Taxodium distichum	13	100.0	0.0

[a] Tree identifications of sample unknowns were determined from two sapwood tree core samples from each of 13 to 30 replicate trees as indicated for each species.
[b] Percentage of unknown samples identified correctly using application-specific reference library.
[c] Unidentified samples resulted from a global class distribution with less than 70% ownership in any one global class. None of the sample unknowns were incorrectly or ambiguously identified.

samples were correctly identified. None of the determinations resulted in incorrect or ambiguous identifications. These results with the lower-performing tree species were subsequently improved up to 5% in a second test (using the same unknowns) by adjusting the specificity and sensitivity parameters used during neural net training to enhance sample recognition.

3.3. Comparison of EASPs of tree cores

The run characteristics of observed multisensor outputs produced during data acquisition for all tree species analyzed with the A32S instrument produced relatively compressed low-resistance response curves (<5% above baseline resistance) for all 32 sensors in the array. The sensor responses were so tightly clustered in some cases that curves representing individual sensors were not resolved completely in the data acquisition window without reducing the range of the *y*-axis display scale. Nevertheless, sufficient differences in individual sensor responses were produced to allow discrimination among tree species. Individual sensor response outputs within signature patterns of individual tree species ranged from 0.1 to 2.6%, and were rarely greater than 2.5%. However, sensor responses less than 1.0% were common. Statistical analysis showed high precision and low variability of individual sensor responses between analytical runs for any one sample type. No standard errors of means for individual sensors were greater than 0.2 intensity units, and most standard errors of means were 0.1 or less.

A diversity of electronic aroma signatures were derived from CPA of headspace volatiles from sapwood cores of the 23 tree species examined. Comparisons of compiled and statistically analyzed EASPs resulting from CPA tests indicated that unique electronic signature patterns were identified for each species (Table 3). No combined outputs from the sensor array were identical for any two tree species, although identical intensity responses for any one sensor were common among species. Widely varying signature patterns were observed in most cases among tree species from different genera. However, examinations of EASPs of volatiles from closely related tree species yielded mixed results. Large differences were observed in signature pattern comparisons between two *Carya* species, *C. illinoensis* and *C. tomentosa*, whereas great similarities were shared in the signature patterns among *Quercus* species, with the exception of *Q. alba* and *Q. stellata*. Analysis of volatiles from *Q. alba* (white oak) and *Q. stellata* (post oak), both in the white oak group (subgenus *Quercus* section *Quercus*) of oaks, consistently produced relatively low sensor response curves. By comparison, the other five oaks tested were from the red/black oak group (subgenus *Quercus* section *Lobatae* Loudon). All of these red oak species produced consistently high sensor response curves across the sensor array relative to the white oak species tested.

Analyses of signature pattern differences among angiosperm species and among species within the genus *Quercus* were easily observed using simultaneous display mode of sensor outputs with non-normalized data in line-graph format. Simultaneous comparisons of seven angiosperm species indicated different sensor-response patterns (Fig. 1A). CPA of American hornbeam or ironwood (*C. caroliniana*) volatiles produced the widest-ranging EASP with at least 11 sensor responses that exceeded those of the

Table 3
Electronic aroma signature patterns derived from conductive polymer analyses of headspace volatiles released from sapwood cores of selected upland and bottomland hardwoods and conifers

Tree species	Sensor number[a]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A. rubrum	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
C. caroliniana	0.5 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.1 ± 0.0	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.5 ± 0.0	1.1 ± 0.1	1.2 ± 0.1
C. florida	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
C. illinoensis	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
C. laevigata	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
C. tomentosa	0.5 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.8 ± 0.1	0.8 ± 0.1
D. virginiana	0.6 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.2	1.7 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	0.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
I. opaca	1.0 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
L. styraciflua	2.4 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
P. deltoides	2.3 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.1	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	2.0 ± 0.2
P. glandulosa	1.1 ± 0.0	1.2 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.9 ± 0.0	1.3 ± 0.1	1.3 ± 0.1
P. occidentalis	1.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Q. alba	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.2 ± 0.2	2.2 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Q. buckleyi	2.3 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Q. falcata	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Q. marilandica	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Q. nigra	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
Q. stellata	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
Q. virginiana	2.1 ± 0.0	1.8 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	2.1 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	2.0 ± 0.0	1.8 ± 0.0	1.9 ± 0.0
S. albidum	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
S. nigra	2.1 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
T. distichum	2.3 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
U. crassifolia	1.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1

Tree species	Sensor number															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
A. rubrum	1.1 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
C. caroliniana	0.9 ± 0.1	1.1 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
C. florida	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	1.9 ± 0.1	2.4 ± 0.2
C. illinoensis	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
C. laevigata	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	1.8 ± 0.1	2.2 ± 0.1
C. tomentosa	0.7 ± 0.0	0.8 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	0.8 ± 0.1
D. virginiana	1.1 ± 0.1	1.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1
I. opaca	1.2 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.2 ± 0.1	1.5 ± 0.1
L. styraciflua	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.1 ± 0.1	2.5 ± 0.1
P. deltoides	1.9 ± 0.2	1.8 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	2.3 ± 0.2
P. glandulosa	1.3 ± 0.1	1.3 ± 0.1	0.9 ± 0.0	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1
P. occidentalis	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	1.7 ± 0.1	2.0 ± 0.1
Q. alba	1.7 ± 0.1	1.8 ± 0.1	1.3 ± 0.0	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.2	2.3 ± 0.2	1.7 ± 0.1	2.2 ± 0.2
Q. buckleyi	2.0 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	
Q. falcata	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	1.8 ± 0.1	2.3 ± 0.1
Q. marilandica	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.8 ± 0.1	2.2 ± 0.1
Q. nigra	1.8 ± 0.1	1.9 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	1.8 ± 0.1	2.3 ± 0.1

Q. stellata	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1							
Q. virginiana	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	2.0 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	1.7 ± 0.0	2.0 ± 0.0								
S. albidum	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.2 ± 0.1								
S. nigra	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.8 ± 0.1	2.2 ± 0.1								
T. distichum	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.0 ± 0.1	2.5 ± 0.1								
U. crassifolia	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.7 ± 0.1	2.1 ± 0.1								

[a] Mean sensor responses (mean ± S.E.M.) to headspace volatiles were determined from percent changes in electrical resistance relative to base resistance ($\Delta R/R_{base} \times 100$) for each of 32 individual sensors based on two cores per tree and a minimum of 10 replicate trees cores analyzed for each tree species. Each sensor in the array was coated with a different conductive polymer produced by electro-polymerization of either polypyrrole, polyanaline, or polythiophene derivatives which have been modified with ring-substitutions of different functional groups that impart unique conductive properties (resistance responses to different classes of organic compounds). All conductive polymers were doped with specific metal ions to improve and modulate polymer conductivity and sensor sensitivity.

other angiosperm species, and at least 5 sensor responses that were lower than those of the other angiosperm species. Volatiles from sycamore (*P. occidentalis*) yielded the narrowest range of sensor responses. The EASP of southern red oak (*Q. falcata*) produced the highest mean sensor responses among these seven angiosperms, and eastern cottonwood (*P. deltoides*) exhibited the lowest mean sensor responses in the group. Comparisons of response patterns for *Quercus* species also indicated differences in signature patterns, but *Quercus* EASPs were more clustered within a narrower range and different from the EASPs of other angiosperms (Fig. 1B). Volatiles from white oak (*Q. alba*) produced a sensor-response pattern that was strikingly different from those of the red oak species, including water oak (*Q. nigra*), blackjack oak (*Q. marilandica*), and southern red oak, but coastal live oak (*Q. virginiana*) produced an EASP that was intermediate in intensity between these two pattern types, although closer to the patterns of red oaks than to the white oak pattern.

A closer analysis comparing pairs of signature patterns for individual species within a genus using difference display mode revealed more precise differences in individual sensor responses within signature patterns. Large differences were found in sensor responses to volatiles of southern red oak (*Q. falcata*) relative to white oak (*Q. alba*) using normalized data (Fig. 2A). The majority (56%) of sensor responses for southern red oak were greater than those for white oak. The greatest differences (>0.5%) occurred in seven sensors, three positive differences and four negative differences. Sensor-response differences were skewed to the positive side within the sensor range of 21–32, whereas response differences appeared more random in the sensor range of 1–20. Differences in sensor responses to volatiles of mockernut hickory (*C. tomentosa*) relative to sweet pecan (*C. illinoensis*) were much greater than for the *Quercus* species (Fig. 2B). The number of differences of individual sensor responses was evenly distributed in the positive and negative, but the top set of sensors (21–32) was skewed to the negative side in *Carya* species. The greatest differences (>0.5%) occurred in eight sensors, four with positive differences and four with negative differences. Similar differences in signature patterns were obtained for the comparison of *Quercus* species using nonnormalized data, except that the differences were accentuated, and the curve was

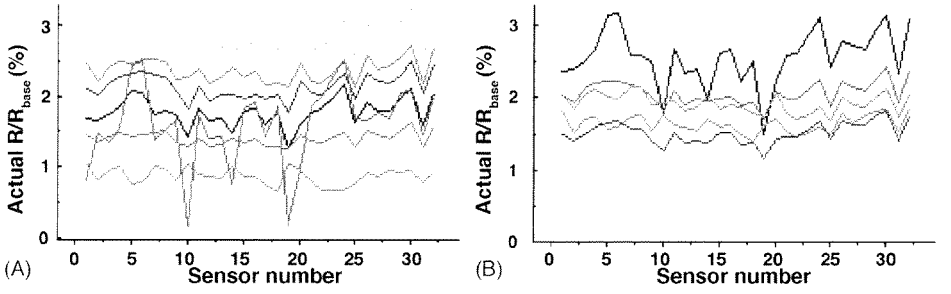

Fig. 1. Multiple comparisons of electronic aroma signature patterns for sapwood cores of seven hardwoods and five oak species using superimpose mode (display function) following conductive polymer analysis. Simultaneous colored line graphs of actual (nonnormalized) sensor array response [$\Delta R/R_{base}$] percentages for: (A) *A. rubrum* (black), *C. caroliniana* (red), *C. laevigata* (green), *L. styraciflua* (blue), *P. deltoides* (cyan), *P. occidentalis* (purple), *Q. falcata* (yellow); and (B) *Q. alba* (black), *Q. falcata* (red), *Q. marilandica* (green), *Q. nigra* (blue), and *Q. virginiana* (cyan).

shifted more positive with most (90.6%) of the difference in sensor responses above the zero baseline on the *y*-axis (Fig. 2C). A similar shift to the positive occurred for *Carya* volatiles using nonnormalized data (Fig. 2D).

3.4. Global class distributions of identification elements

The recognition file created by neural net training using sensor responses derived from CPA provided

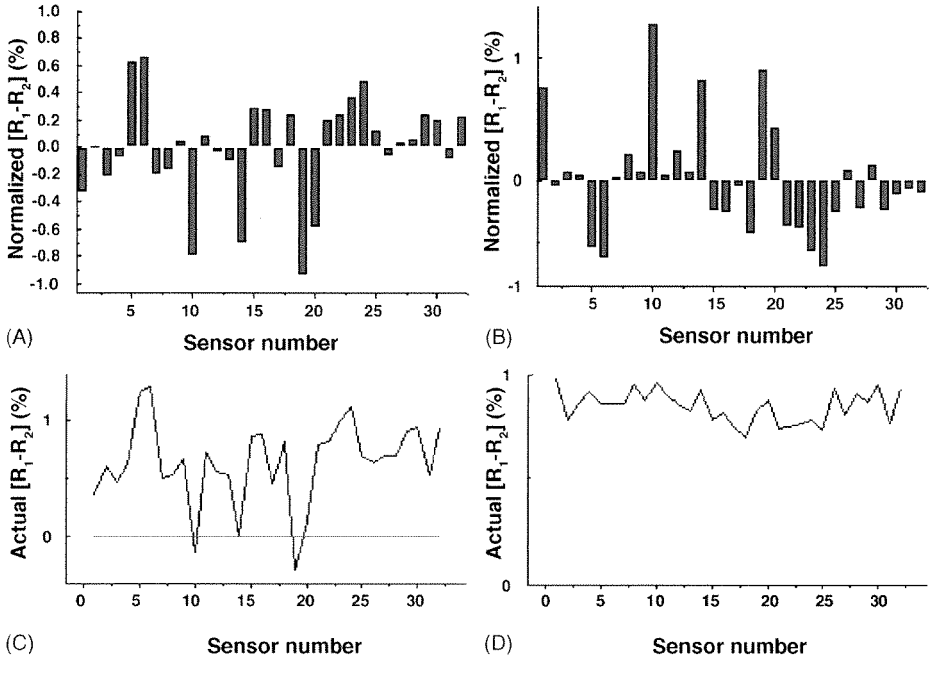

Fig. 2. Pairwise comparisons of differences in electronic aroma signature patterns for sapwood volatiles from *Quercus* and *Carya* species using difference mode (display function) following conductive polymer analysis. Normalized sensor array response-difference [$R_1 - R_2$] percentages, indicated with bar graphs, between volatiles from: (A) southern red oak (*Q. falcata*) relative to white oak (*Q. alba*); (B) mockernut hickory (*C. tomentosa*) relative to sweet pecan (*C. illinoensis*), and actual (nonnormalized) response-difference percentages for the same comparisons; (C) southern red oak relative to white oak; and (D) mockernut hickory relative to sweet pecan, indicated by line grafts with the baseline at zero on the *y*-axis.

Table 4
Mean global class distributions (%)[a] of identification elements determined from headspace volatiles, released from 21 species of upland and bottomland hardwoods and conifers, using conductive polymer analysis with neural net training

Tree species	Tree species (abbrev.)[b]																			
	Ar	Cc	Cf	Ci	Cl	Ct	Dv	Io	Ls	Pd	Pg	Po	Qa	Qf	Qm	Qn	Qv	Sa	Sn	Td	Uc
A. rubrum	98.7	–	0.3	0.2	–	–	–	0.2	–	0.1	–	–	–	0.2	–	0.6	–	0.6	–	–	0.1
C. caroliniana	–	99.1	–	–	–	0.3	0.2	0.8	–	–	0.1	–	–	–	–	–	–	–	–	–	–
C. florida	0.1	–	96.7	0.1	1.0	0.1	–	–	–	–	–	0.7	–	1.0	–	0.9	–	0.2	0.1	0.1	0.2
C. illinoensis	0.4	–	–	97.7	–	0.1	–	1.2	–	0.1	–	0.1	0.2	0.2	–	–	–	0.5	–	–	–
C. laevigata	–	–	0.4	–	96.3	–	–	–	1.5	–	–	1.0	–	0.1	0.2	–	–	–	0.1	0.6	–
C. tomentosa	–	0.4	0.2	0.3	–	99.4	–	0.1	–	–	–	0.1	–	–	–	–	–	–	–	–	–
D. virginiana	–	0.4	–	–	–	–	98.4	–	0.1	0.1	0.2	0.1	0.3	–	–	–	–	0.2	–	0.1	–
I. opaca	0.2	–	–	1.4	–	0.2	0.1	97.7	0.1	0.1	–	–	0.1	–	–	–	–	0.4	–	–	–
L. styraciflua	–	–	0.2	–	1.7	–	–	–	94.1	0.4	–	0.9	–	–	–	–	–	–	2.2	0.3	0.1
P. deltoides	–	–	–	–	–	–	0.4	–	0.4	98.2	0.1	0.2	–	0.1	1.6	–	0.1	0.2	–	0.3	–
P. glandulosa	0.1	0.1	–	–	–	–	0.2	–	–	0.2	98.9	–	–	0.1	0.2	–	0.2	0.2	–	–	–
P. occidentalis	–	–	0.8	–	0.3	–	0.2	–	–	0.1	0.1	96.0	–	0.5	–	0.1	0.3	–	–	0.2	3.8
Q. alba	–	0.1	–	0.1	–	–	0.3	0.2	–	–	–	–	98.9	–	–	0.6	–	0.1	–	–	–
Q. falcata	–	–	1.1	–	0.2	–	–	–	–	–	–	0.7	–	96.4	–	0.1	–	–	–	0.1	2.0
Q. marilandica	–	–	0.1	0.1	–	–	0.1	–	0.1	1.1	0.3	0.4	–	–	97.5	–	–	–	0.3	0.6	0.1
Q. nigra	0.3	–	1.0	–	0.1	–	–	–	0.2	–	–	–	0.5	0.7	–	98.8	–	0.2	–	–	0.3
Q. virginiana	–	–	–	–	–	–	–	–	–	–	0.5	0.6	–	–	–	–	99.2	–	–	–	–
S. albidum	0.5	–	–	0.9	–	–	0.2	0.4	–	–	0.2	–	–	0.1	–	–	–	98.3	–	–	0.2
S. nigra	–	–	0.2	–	0.1	–	–	–	1.1	–	–	0.5	–	0.2	0.4	–	–	–	98.2	–	–
T. distichum	–	–	–	–	0.2	–	0.2	–	0.4	0.6	–	0.9	–	0.5	0.5	–	–	–	–	95.9	0.9
U. crassifolia	–	–	0.1	–	–	–	–	–	–	–	–	2.0	–	0.1	–	–	–	–	0.4	0.9	97.4

[a] Mean global class distributions indicated for each tree species must be read from left to right (by rows) only, not top to bottom (by columns) in the table. Values were determined from a minimum of 10 replicate samples of each tree species. Primary identification elements are indicated by distribution values greater than 90% found in a diagonal pattern across the table. Secondary elements are all other identification elements in the table with distribution values less than 5.0%.

[b] Tree species abbreviations are listed in the same order as given for the specific epithets in the far left column.

useful information about the overlap in identification elements, and thus relatedness, among trees included in the reference database. Mean global class distributions of identification elements present in the validated recognition file, determined from headspace volatiles of 23 tree species, indicated that the majority of the recognition capabilities for a given tree species in the reference library were attributed to primary identification elements found only in each respective species. Well over 90% (range 94.1–99.4%) of the global class distribution of identification elements for each species were accounted for by these primary identification elements unique to individual tree species (Table 4).

Secondary identification elements, defined as those having distribution values less than 5% with any one other species, also were found for all 23 tree species tested. These values indicate relative amounts of shared elements between individual pairs and thus a measure of relatedness based on the quantitative distribution of these shared characters. Major secondary identification elements were defined as those secondary elements with global class distributions of $\geq$0.5% (but <5%) which were shared with at least one other tree species. The numbers of major secondary identification elements shared with other species varied considerably in different tree species from the highest number of elements shared in *Taxodium distichum* (5) and *Cornus florida* (4), down to one shared element in *Carpinus caroliniana*, *Ilex opaca*, *Populus deltoides*, and *Q. alba*; and no major secondary identification elements shared with other species by *Carya tomentosa*, *Diospyros virginiana*, and *Prosopis glandulosa*. The highest recorded distribution of 3.8% was found in sycamore (*P.*

Table 5
Incidence and distribution of major and minor secondary identification elements among 21 species of upland and bottomland hardwoods and conifers

Tree species	Major secondary identification elements[a]				Minor secondary identification elements[b]			
	Specific incidence (%)	Mean distribution (%)	Nonspecific incidence (%)	Mean distribution (%)	Specific incidence (%)	Mean distribution (%)	Nonspecific incidence (%)	Mean distribution (%)
A. rubrum	10	0.6	5	0.5	30	0.2	25	0.2
C. caroliniana	5	0.8	0	0.0	15	0.2	20	0.3
C. florida	20	0.9	15	1.0	35	0.1	35	0.2
C. illinoensis	10	0.9	10	1.2	30	0.2	25	0.2
C. laevigata	15	1.0	10	1.4	20	0.2	25	0.2
C. tomentosa	0	0.0	0	0.0	25	0.2	20	0.2
D. virginiana	0	0.0	0	0.0	40	0.2	45	0.2
I. opaca	5	1.4	10	1.0	35	0.2	20	0.2
L. styraciflua	15	1.6	10	1.3	20	0.3	30	0.2
P. deltoides	5	1.6	10	0.9	40	0.2	35	0.2
P. glandulosa	0	0.0	5	0.5	40	0.2	30	0.2
P. occidentalis	15	1.7	40	0.9	35	0.2	25	0.2
Q. alba	5	0.6	5	0.5	25	0.2	15	0.2
Q. falcata	15	1.3	20	0.7	15	0.1	40	0.1
Q. marilandica	10	0.9	10	1.1	40	0.2	15	0.3
Q. nigra	15	0.7	15	0.7	25	0.2	10	0.1
Q. virginiana	10	0.6	0	0.0	0	0.0	15	0.2
S. albidum	10	0.7	10	0.6	25	0.2	35	0.2
S. nigra	10	0.8	5	2.2	20	0.2	20	0.2
T. distichum	50	0.7	15	0.7	15	0.3	30	0.2
U. crassifolia	10	1.5	15	2.2	15	0.2	30	0.2

[a] Major secondary identification elements account for $\geq$0.5% of global class distributions for any one tree species. Specific incidence indicates the proportion of all species included in the comparison that shared unique identification elements specific to the indicated tree species. Nonspecific incidence refers to identification elements that are not specific to just a relatively few species, but may be common among many other species. Mean distributions are the actual average distribution percentage of elements shared between the indicated tree species and other species.

[b] Minor secondary identification elements account for <0.5% of global class distributions for any one tree species.

occidentalis) which were shared with cedar elm (*Ulmus crassifolia*). Eight major secondary elements identified from other species also were found for *P. deltoides*, making cottonwood the species having the most secondary elements that were specific to other species. Volatiles from southern red oak (*Q. falcata*) shared four secondary elements that were specific to other species. By contrast, only one major secondary identification element specific to cottonwood was shared with another species, *Q. marilandica*. Only four species including *C. caroliniana*, *C. tomentosa*, *D. virginiana*, and *Q. virginiana* totally lacked major secondary identification elements that were specific to other species. Minor secondary identification elements were considered those with global class distributions less than 0.5% which were shared with at least one other tree species.

A more detailed analysis of the distribution of secondary identification elements, among species included in the recognition file, provided frequencies of incidence and mean distributions that indicated measures of relatedness among species (Table 5). Baldcypress had the highest incidence (50%) of specific major secondary identification elements that were found in other species. Incidence of major secondary elements was considerably lower (range 0–20%) in other species, with mean distributions up to 1.7%. However, the incidence of minor secondary elements was generally higher (range 15–40%) than major elements for all species except coastal live oak, with mean distributions up to 2.2%. The highest incidences of minor secondary elements occurred in common persimmon, eastern cottonwood, honey mesquite, and blackjack oak.

Nonspecific incidence refers to identification elements that are not specific to just a relatively few species, but may be common among many other species. The incidence of nonspecific secondary elements was generally lower for the major identification elements than for minor identification elements. The highest incidence of major nonspecific elements was determined for sycamore (40%), but the incidence was lower (range 0–20%) in all other species. Mean distributions of major nonspecific elements (range 0–2.2%) were slightly greater than distributions (0–1.7%). Minor nonspecific elements occurred at a higher range of incidence (10–45%) than major nonspecific elements, with the highest incidence found in common persimmon. Mean distributions of all minor secondary elements were uniform in a narrow range (0.1–0.3%) for both specific and nonspecific identification elements.

3.5. Principal component analysis (PCA) of oak species

A detailed pairwise comparison of relatedness between sapwood volatiles of seven oaks species using

Table 6
Pairwise comparisons of the relatedness of seven oak species using three-dimensional principal component analysis of headspace volatiles

Analyte 1	Analyte 2	QF significance[a]
Q. alba	*Quercus buckleyi*	47.02****
	Quercus falcata	19.21***
	Q. marilandica	26.11***
	Q. nigra	9.38**
	Q. stellata	25.68***
	Q. virginiana	55.01****
Q. buckleyi	*Q. falcata*	41.73****
	Q. marilandica	23.17***
	Q. nigra	22.31***
	Q. stellata	24.12***
	Q. virginiana	4.96*
Q. falcata	*Q. marilandica*	10.28**
	Q. nigra	5.88*
	Q. stellata	26.01***
	Q. virginiana	44.19****
Q. marilandica	*Q. nigra*	11.61**
	Q. stellata	20.62***
	Q. virginiana	25.25***
Q. nigra	*Q. stellata*	25.24***
	Q. virginiana	25.70***
Q. stellata	*Q. virginiana*	23.12***

[a] QF = quality factor. A quality factor value of 2.0 indicates a significant discrimination at approximately $P = 0.10$. The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1 = 95.26%; PC 2 = 4.27%; and PC 3 = 0.43%, representing the x, y, and z axis of the aroma map, respectively.

* Discrimination between global aroma classes was significant at $P < 0.05$.

** Discrimination between global aroma classes was significant at $P < 0.01$.

*** Discrimination between global aroma classes was significant at $P < 0.001$.

**** Discrimination between global aroma classes was significant at $P < 0.0001$.

three-dimensional PCA yielded a principal component model that explained over 99% of the differences in identification elements with only two principal components (Table 6). Quality factors (QFs), determined for each pairwise comparison, indicated quantitative measures of relatedness or the distance of relatedness between species. A QF of at least 2 is generally a significant discrimination at about the 0.10 level of significance. None of the seven oak species included in the analysis were closely related as indicated by QFs > 4 for all pairwise comparisons. Only two comparisons resulted in QFs < 6, but these were still significantly different ($P < 0.05$). Nevertheless, the degree of difference provided good indications of relative groupings of oak species. The lowest QFs (range 4.96–25.70) were determined between oaks in the red/black oak group (subgenus *Quercus* section *Lobatae* Loudon), with the exception of the *Q. alba*–*Q. nigra* comparison. The highest level of difference occurred between white oak *Q. alba* and two red oaks, *Q. buckleyi* and *Q. virginiana* with QFs of 47 and 55, respectively. However, a high level of significant difference also was found in comparisons between red oaks, including *Q. buckleyi*–*Q. falcata* and *Q. falcata*–*Q. virginiana*.

The production of an aroma map of the seven oaks based on three-dimensional PCA data provided a visual measure of relatedness between oak species by the clustering, separation, and spatial distribution of each species (Fig. 3). PCA data were run through an algorithm that identified principal components in the volatiles and separated oak species using Eigen values on three axes. Eigen values describe the amount of variance captured in the data for each individual principal component isolated into individual axes. The values were calculated by decomposing the covariance or correlation matrix generated from the data. The correlation matrix was a scaled version of the covariance matrix such that every individual element in the covariance matrix is divided by the product of the standard deviations of the two co-varying quantities to obtain the percentage Eigen values. The principal component value for each axis indicated the proportion (%) of the difference explained by that principal component. The clustering of samples from the two white oak species, *Q. alba* and *Q. stellata*, were well separated on the *x*-axis (representing PC 1), and generally lower than the majority of sample

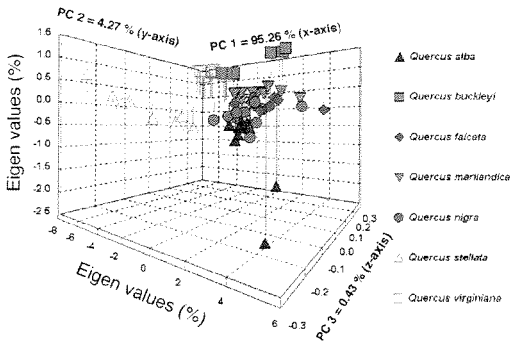

Fig. 3. Three-dimensional principal component analysis of electronic aroma signature patterns differentiating sapwood volatiles of seven oak species, including *Q. alba*, *Q. buckleyi*, *Q. falcata*, *Q. marilandica*, *Q. nigra*, *Q. stellata*, and *Q. virginiana*. Eigen values, describing the amount of variance captured in the data of each individual principal component axis, were calculated by decomposing the covariance or correlation matrix representing the data. The correlation matrix was a scaled version of the covariance matrix such that every individual element in the covariance matrix is divided by the product of the standard deviations of the two co-varying quantities to obtain the percentage Eigen values. The PC value for each axis indicates the proportion (%) of the difference explained by that principal component.

clusters of the six red oak species on the *y*-axis (representing PC 2). This was consistent with observations of sensor response patterns for white oak species being lower for most sensors than the red/black oak species. Some samples of the two white oaks also were well forward of the red/black oak species on the *z*-axis, representing PC 3. By contrast, three red oak/black oaks, *Q. falcata*, *Q. marilandica*, *Q. nigra* were adjacent and tightly clustered. However, the other two red/black oaks, *Q. buckleyi* and *Q. virginiana*, were separated above the other red/black oak clusters.

4. Discussion and conclusion

Electronic noses are electrical-resistance modulated, chemical-sensing devices containing a sensor array capable of producing a digital fingerprint of volatile organic compounds released from any source. Conductive polymer sensor arrays take advantage of differential responses of different conducting plastics (within each sensor) to various chemical species in the sample headspace, by producing a unique EASP

specific to the analyte mixture. The multisensor array provides an output response pattern analogous to a combination lock that reflects the collective responses of all sensors in the array, but sensor outputs are in continuous values. The pattern-recognition algorithms in the analysis software compare signature patterns stored in the reference library to those of unknown samples to look for similarities and differences in these patterns. The differences are expressed digitally as numerical values that are compared in matrix format. The algorithms assign distributions of similar elements found in principal components of the sample that are in common with known patterns in the reference library and make a determination of identity based on that distribution. The response of each sensor is based on the collective effect of the entire mixture of compounds in the headspace on electrical-resistance changes generated by adsorption of analytes to the sensor. Sensor adsorption is determined by the specific affinity of unique polymers in each sensor, the specificity of chemical types, quantities, and molar ratios of chemicals present in the sample mixture.

Conductive polymer analysis is a versatile new electronic aroma detection (EAD) technology that has been useful for numerous commercial applications in industrial production, processing, and manufacturing (Ouellette, 1999; Persaud et al., 1994; Pisanelli et al., 1994). This paper has demonstrated the potential use of CPA as a relatively nondestructive research tool for identifying woody samples from diagnostic EASPs derived from unique mixtures of volatile metabolites released into sampled headspace. The main strengths of CPA methods are the capability of identifying, characterizing, and categorizing mixtures of volatiles as a whole sample, without having to identify individual chemical compounds present in the sample mixture. Furthermore, utilizing application-specific reference libraries (specific to sample types), and tweaking discrimination-parameter values and recognition specificity (confidence level) during neural net training provided means of obtaining accurate identifications without false positives, ambiguous identifications, or misidentifications. Unsuccessful determinations were reduced by increasing the number of elements allowed in error before unknown identity was declared. The absence of false positives and ambiguous determinations with CPA assures that a sample will either be identified correctly or unsuccessfully identified. Sample discrimination also was improved by taking more than one sample from each plant or woody part, collecting multiple samples over time, increasing sample size to improve representation, and using known samples from the same geographical area from which unknown samples were collected.

Primary identification elements, recognized by global class distributions >95%, were useful for identifying wood samples of tree species. Primary elements represent species-specific mixtures of compounds found in wood volatiles, such as essential oils, that are unique to individual species. However, secondary identification elements were more useful for determining the relatedness between tree species because secondary elements are shared between different species and can be quantified using incidence and distribution percentages. These quantitative measures of secondary identification elements help to characterize levels of relatedness between species in terms of shared characters (production of similar or related mixtures of volatile compounds in this case), and thus may provide indications of relatedness in biosynthetic pathways utilized and volatile metabolites produced. Such information may be useful in chemotaxonomic studies by facilitating establishment of phylogenetic, biochemical, or genetic relationships between tree species. In this way, CPA data may compliment genetic homology data by providing indications of expressed chemical relatedness between species.

The high incidence of secondary identification elements among angiosperm species suggests that there are a large number of similar metabolites that are shared between individual species. Many shared secondary elements occur across family lines, but secondary elements also are common between species within individual plant families. Minor secondary elements, both species-specific and nonspecific, generally occurred at higher levels of incidence than major secondary elements. Minor secondary elements probably represent very common metabolites shared among a wide range of plants that utilize similar metabolic pathways for biosynthesis. By contrast, major secondary elements are likely indicative of more specific metabolites such as volatile oils, lignin and suberin derivatives, resins, bark exudates, terpenes, alkaloids, and other secondary metabolites

that yield unique mixtures of compounds for specific functions in individual species.

The high QF values obtained from pairwise comparisons of seven oak species using three-dimensional PCA indicated that none of these oak species were closely related. QF values greater that 2 generally are required to determine that volatiles from two samples represent distinct species. All comparisons of volatiles from oak species in this study indicated high levels of difference among the species. This was an expected result, although the apparent closer relationship between certain species was not expected. The limited analyses of EASPs and three-dimensional PCA for these oak species provide evidence indicating that distinct differences in headspace volatiles of oaks could distinguish between species in the white oak group (subgenus *Quercus* section *Quercus*) from those in the red/black oak group (subgenus *Quercus* section *Lobatae* Loudon) as defined by Nixon (1993). The significant differences demonstrated here in EASPs between *Quercus* species in the white oak and red/black oak groups provide strong indication that there are unique mixtures of volatiles, containing organic compounds representing common identification elements specific to each *Quercus* group, that may be used to distinguish, characterize, and categorize oak species within one of these major groups. The results are consistent with delineations based on morphological and ontological criteria that are currently used to define oaks within the two groups.

The lowest QF value was determined for the comparison between *Q. buckleyi* and *Q. virginiana* providing evidence that these species are the two most closely related oaks of the seven species included in the analysis. The highest QF value was determined between *Q. alba* and *Q. virginiana* (coastal live oak), suggesting that these were the most distantly related oak species. These data indicate that coastal live oak is most closely related to a red oak species and least related to a white oak species. Coastal live oak is considered by some authorities to be a member of the white oak group due to macroscopic leaf and acorn characters that are shared with white oak species. However, *Q. virginiana* is intermediate between white oaks and red oaks in the microscopic structure of its vessel elements. Unlike white oaks which have large pores containing abundant tyloses with a ring porous structure, coastal live oaks have semi-ring porous wood structure and tyloses are sparse in their vessels. This unique ring porosity with relatively small pores scattered across the entire ring, is a structural reason that partially explains why live oaks are more susceptible to oak wilt disease than white oaks. The biochemistry of host-defense in live oak also is more similar to red oaks than white oaks, indicated by the high susceptibility of live oak to infection by the oak wilt pathogen, *Ceratocystis fagacearum* (T.W. Bretz) J. Hunt (Wilson, 2001; Wilson and Lester, 2002). Most white oaks such as post oak (*Q. stellata* Wangenh.) and overcup oak (*Q. lyrata* Walter) are highly resistant to this vascular wilt disease (Wilson, 2001), providing further evidence that the host-defense metabolism of coastal live oak, with its associated volatiles, is more closely related to those of red oaks. The results in this study tend to support this conclusion.

Some important limitations of CPA for wood identifications should be noted. For example, the sources of woody material used in constructing recognition files and reference libraries have a significant effect on the ability to identify unknown samples. For best results, the sources of reference materials used in building recognition files should be obtained from the same geographical area where future unknown samples of that type are to be collected. Considerable variability in aroma profiles can result from wood of the same species collected from widely separated regions. If a comprehensive reference library for a wider geographical region is desired, reference samples should be collected throughout the region so that they are representative of the entire region for any tree species established in the reference library. A general rule is to collect a minimum of 10 distinct samples per species from each sampling area within the collecting region to define known species entered into the reference library. Specialized reference libraries for very specific applications generally are more useful and effective than libraries developed for broad applications because they provide more accurate determinations, shorter neural net training times, and lower instances of nonrecognition and incorrect (false positive) results. Other limitations of CPA include the inability to identify samples not represented in the reference library (such as woody samples contaminated with microorganisms or samples with excess moisture or

desiccation), the inability to identify individual chemical species within sample mixtures or make reliable quantitative determinations, and the time requirement for building head space prior to sample analyses. However, subsequent research has indicated that CPA can be used to identify aged wood specimens up to 40 years old (that release greatly attenuated volatiles), when these specimens are properly rehydrated (Wilson and Oberle, unpublished data).

This paper demonstrates the usefulness of CPA for woody plant and wood sample identifications. CPA technology is a potentially useful new research tool for forest biologists, ecologists, pathologists, research foresters and other scientists studying the processes, functions, interactions, and stability of forests and forested ecosystems. CPA methods are most useful for identifying biotic components (particularly woody plants and small organisms) in forested stands that interact and contribute to ecosystem functions. For example, forest biologists and ecologists could use CPA to study forest ecosystem processes and functions such as nutrient cycling, biotic decomposition of woody plant materials, symbiotic associations, biological interactions and interrelationships between organisms, forest structure and stand composition, and the roles that organisms play in forest ecosystem dynamics. CPA also might be useful to forest pathologists and entomologists to study biologically active species such as insect pests and pathogenic microbes that interact with woody materials in forest ecosystems and cause changes in stand structure and ecosystem functions over time that ultimately affect forest health, biodiversity, sustainability of future productivity, forest stability, and succession. Early detection is useful in the forest products industry for mitigating losses associated with lumber defects caused by differential shrinkage when hardwoods are infected by wetwood bacteria (Verkasalo et al., 1993). Many hardwood species are susceptible to infections by wetwood bacteria that cause lumber defects (Carpenter et al., 1989). Disruptive forest pests that cause adverse effects on forest stands are often difficult to identify because they are hidden inside of internal galleries (insects) or rarely produce fruiting structures (fungi) on the external surfaces of trees or detached tree parts. Thus, studies of the growth and development of microbial communities within wood tissues could be investigated more effectively with CPA than with genetic tools such as molecular markers and primers, because these hidden organisms can be detected and identified from the unique volatile compounds they release from wood. Information acquired from such studies ultimately could be used to facilitate management of forested stands and ecosystems.

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