Evaluation of the diagnostic feasibility of the electronic nose in detecting incipient decay of artificially inoculated wood

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Abstract:
The tree stability-assessment methodology currently used in Italian cities initially follows a visual analysis of individual trees, followed by an evaluation of the internal state using different instruments that are often invasive, expensive, or cannot be effectively used in the urban environment. Moreover, many of these instruments do not provide an adequate evaluation of decay that occurs in the root system.
The aim of this research was to evaluate the possibility of integrating tools currently used for assessments of tree decay in the urban environment with innovative techniques used in other fields and industries for various applications, such as quality control, environmental monitoring, medical diagnoses, and perfumery. The electronic nose (e-nose) was tested for its capability of detecting volatiles released by wood decay fungi, healthy trees, and diseased trees. Two different types of e-noses, based on different technologies, also were compared to determine the feasibility of detecting incipient decays in artificially-inoculated wood. All e-nose devices were capable of discriminating between healthy and artificially-inoculated wood with very high levels of precision and confidence. The e-nose utilizing polypyrrole-coated quartz microbalances with acoustic wave sensor array provided better results than the other technology (metal-oxide gas sensors) in discriminating woody samples containing different agents of wood decay.

Introduction:
The early detection and diagnosis of decays by electronic noses is based on detecting changes in the release of volatile organic compounds (VOCs) emitted by either wood decay fungi or trees when wood decay is present. The composition of metabolites released by individual fungi is controlled largely by the types and combinations of metabolic pathways specific to microbial species. These metabolic pathways are regulated by genetic, substrate and environmental factors (Wilson *et al.*, 2004).
A range of VOCs has been reported to be emitted by fungi. Korpi *et al.* (1999) revealed the emission of pinenes, acrolein, a few ketones and acetylenes. Other investigations (Magan and Evans, 2000, de Lacy Costello *et al.*, 2005) have focused on the identification of VOCs from food spoilage fungi, whereas 1-octen-3-ol was detected in damp houses with various mold fungi present (Strom *et al.*, 1994). Numerous other chemical species have been reported as fungal metabolites, including complex acids, sesquiterpenes, methyl ketones and alcohols (Ewen *et al.*, 2004). Only a few recent studies have been done on VOCs emitted by healthy and diseased trees. The analysis of healthy *Populus* spp. and *Pinus* spp. have indicated the presence of mainly monoterpenes and acetone, together with a small amount of isoprene (Villanueva-Fierro *et al.*, 2004). Other studies have indicated increases in toluene and α-pinene emissions associated with *P. sylvestris* under

pathogen attack (Faldt *et al.*, 2006), and a decrease in isoprene emissions from diseased *Quercus fusiformis* L. and *Q. virginiana* L. (Anderson *et al.,* 2000). The bacteriostatic role of plant VOCs was studied by Gao *et al.* (2005) who found emissions of terpenoids, alcohols, aldehydes, acids, and esters released by five healthy coniferous species in which α-pinene, β-pinene, 2,(10)-pinene, myrcene and D-limonene represented more than 95% of total VOC emissions, whereas increased levels of α-pinene, limonene, nonaldehyde and benzaldehyde were found in artificially-inoculated wood shaves.

A decayed tree therefore emits a particular VOC bouquet which consists of fungal secondary metabolites, tree secondary metabolites, and fungus-induced tree antimicrobial defense compounds (e.g. phenolic metabolites and phytoalexins). In order to quickly detect and discriminate changes in VOCs that are released by trees attacked by wood decay fungi, an instrument is needed that can electronically sense these changes in VOC emissions without having to identify the individual chemical species present in the bouquet mixture. This study was aimed at evaluating the capability and feasibility of different electronic noses to discriminate between healthy and decayed (artificially inoculated) wood chips for the early detection of decays in standing trees within the urban environment.

Materials and methods:
This research was conducted in 2006 and 2007 using the Technobiochip "Libranose 2.1", a compact and semi-portable olfactory system, and the Airsense (Schwerin, Germany) PEN3, a portable electronic nose.

A single shade tree of the following ten species (including *Acer negundo* L., *Acer saccharinum* L., *Aesculus hippocastanum* L., *Castanea sativa* Mill., *Cedrus deodara* (D. Don) G. Don fil., *Celtis australis* L., *Platanus* x *acerifolia* Brot., *Quercus rubra* L., *Robinia pseudoacaci*a L., *Tili*a spp.) was cut down in the urban or peri-urban environment, peeled to remove the bark, and cut into wood chip parallelepipeds. A minimum of 96 wood chips were cut from each tree species, sterilized in an autoclave, dipped into a previously prepared liquid potato-dextrose (PD) broth culture of one of five major wood decay fungi chosen for the study (*Armillaria mellea* (Vahl) P. Kumm, *Armillaria ostoyae* (Romagn.) Herink, *Ganoderma lucidum* (Curtis) P. Karst, *Heterobasidion annosum* (Fr.) Bref, *Inonotus dryadeus* (Pers. ex Fr.) Murr), and finally incubated in the dark at room temperature for 6 and 12 months (decayed samples). Sterilized wood chips dipped into sterile liquid PD broth were used as controls (healthy samples). After the incubation period, the wood chips were rinsed with tap water to remove visible traces of fungal mycelium and blotted on tissue paper. A minimum of 8 replicate samples per analyte species (wood and fungus combinations plus controls) were prepared for this study, totalling 60 different analyte species and 480 total samples analyzed by each electronic nose.

All measurements were performed at 30°C (sensor array chamber temperature) and atmospheric air was used as the carrier gas. Reference air was preconditioned by passing room air sequentially through a series of filters to remove organic compounds, moisture, particulates and microbes. The flow rate (suction) of sample air at the sampling port was maintained at 600 ml/min by an automatic pump to aspirate the headspace and pass it over the sensors. Sensors were purged between runs using filtered and conditioned room air. The Libranose 2.1 instrument was controlled by E-nose software (Sigeda, Milan, Italy). The total measuring cycle for each sample was 30 min. A uniform run schedule was used. Data from the sensor array were collected at 1 s intervals and the averaged data of three repetitions per sample were taken per run during data acquisition. For the PEN3 e-nose, a conventional 10-s data sampling interval (from the 5th to 15th second) near the end of the sampling segment was utilized and controlled by the Winmuster 1.6.2.5 software.

Results and discussion:
Principal component analysis (PCA) was performed in order to identify the major identification elements (components) responsible for the discrimination between samples. The aroma map in Figure 1 shows how the controls (healthy samples, yellow labels) were clearly separated from artificially inoculated decayed wood chips (red labels, or decayed samples) by the Libranose 2.1 e-

nose. Principal component 1 and principal component 2 explained 90,98% and 7,88% respectively of the sample variance. The electronic nose could easily distinguish decayed samples from healthy or control samples.
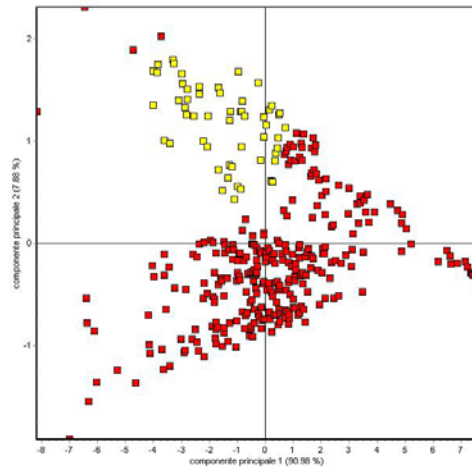

Fig. 1 - Discrimination of healthy (controls, yellow labels) from decayed (artificially inoculated, red labels) wood chip samples by principal component analysis of Δf averages.

Principal component statistical analysis of change in oscillating frequency (Δf) averages for all repetitions of samples from one tree species (*Tilia* spp.), decayed by different wood decay fungi, is shown in Figure 2.
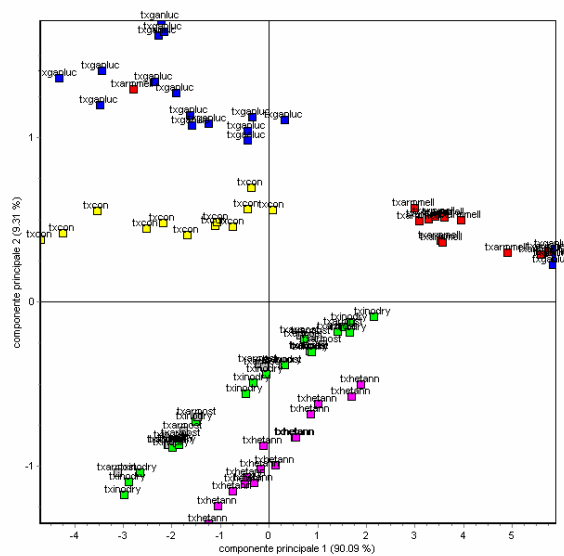

Fig. 2 – Discrimination of artificially inoculated samples of *Tilia* spp. by principal component analysis of Δf average statistical data. Different label colors indicate wood decayed by different fungi.

Healthy wood chips (yellow label) were clearly separated and clustered away from all other sample types. Moreover, different wood decay fungi-inoculated wood chips were generally separated from all others, suggesting that different wood decay fungi, causing different types of decay and releasing different VOCs, were recognized and discriminated by this electronic nose.

Ganoderma lucidum-infected samples were clearly distinguished from those decayed by other fungi, except for *R. pseudoacacia* samples (not shown) that were not separated from *Armillaria mellea*-decayed wood. *Heterobasidion annosum*–decayed wood samples also were discriminated

from all others, except for *Celtis australis* samples (not shown) that were not distinguished from *A. mellea* samples.

The feasibility of the PEN3 portable olfactory system in diagnosing the presence of incipient decay in artificially-inoculated wood chips was tested by means of statistical analysis of the averaged changes in conductivity ($\Delta\sigma$) of 10 metal oxide sensors reacting to headspace volatiles. Detailed comparisons of relatedness of aroma classes were determined using principal component analysis (PCA) algorithms provided in the Winmuster software.

Data illustrated in Figure 3 provide definitive evidence that controls or healthy samples (green labels) were clearly separated from artificially-inoculated wood chips (blue labels, decayed samples). Principal component 1 and principal component 2 explained 64,54% and 29,13% respectively of the sample variance in the data. Only the first of three repetitions are shown here.
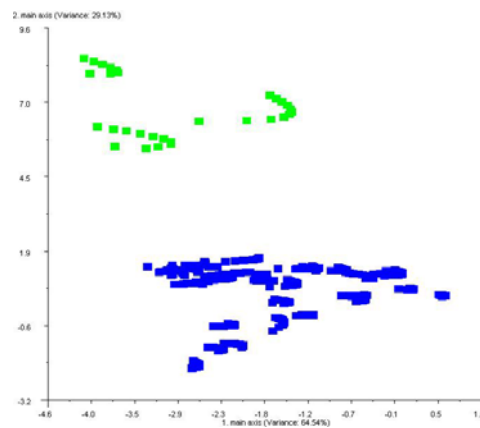

Fig. 3 – Discrimination of healthy (controls) and decayed (artificially inoculated wood chips) samples by principal component analysis of $\Delta\sigma$ averages. Green labels indicate controls, blue labels indicate decayed samples.

Conclusions:

The great quantity of data analyzed in the present study provides demonstrative evidence of the diagnostic feasibility of using electronic nose devices to assess the presence of incipient decay or wood decay fungi in wood colonized for relatively short time periods. These instruments potentially may serve as very effective and useful supporting tools for the diagnosis of decays and early detection of attacks by wood decay fungi in urban trees.

Commercial markets offer a wide variety of different electronic nose devices that may be used for this application. However, our experiments suggest that polypyrrole-coated quartz microbalances with acoustic wave sensor arrays provide better results than other technologies (organic matrix-coated polymer-type and metal-oxide gas sensors) for detecting decay in wood and recognizing the presence of different wood decay fungi and pathogens in wood.

One potential problem associated with the application of e-noses for decay diagnoses in urban trees is the variability of ambient air relative humidity and temperature in the urban environment which can greatly affect readings of electronic noses. Other factors that may affect the performance of electronic noses in the urban environment include the presence of air pollutants, such as in automobile exhaust, that could complicate data acquisition. For these reasons, further research is needed to evaluate the impact of these urban variables that may affect the usefulness and reliability of electronic noses as decay and disease diagnostic tools for assessing urban tree health as well as other potential applications in the field.

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